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

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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. 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. MSPFTC used the 3500xL in conjunction with the BSD600® Duet Series II Semi-automated Punch System for sampling of blood cards, two Janus™ Automated workstations for amplification and capillary electrophoresis setup, and the AmpFlSTR® Identifiler® Direct kit for direct amplification of autosomal STR loci from reference blood samples.

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 National DNA Index System (NDIS) contains DNA from individuals convicted of violent crimes, non-violent felonies, and felony arrestee profiles. Many forensic databasing laboratories have had an increasing number of samples that need processed and analyzed ("CODIS" 2010) based on increase in convicted offender samples and now arrestee samples. Direct amplification allows for high throughput processing while reducing the contamination risk due to less sample handling, time, labor, and costs. This can be easily automatable which can streamline the process to receive a quality profile for single source databasing samples (Applied Biosystems® AmpF*l*STR® Identifiler® Direct User Guide 2012). One way to automate this process is by using Identifiler® Direct (Applied Biosystems®, Foster City, CA) with an automated sample punch machine and a basic liquid handling system. The BSD600® Duet Series II Semi-automated Punch System (Applied Biosystems®, Foster City, CA) and the Janus™ automated workstation

(Perkin Elmer, Downers Grove, IL) were used for this validation. Identifiler® Direct, BSD600®, and the Janus™ were all previously validated and in use at MSPFTC prior to this internal validation

Validations are performed to authenticate a given process or instrument by performing studies that give corroboration. Developmental validations are completed first by the manufacturer to determine the conditions and limitations to a new methodology. An internal validation is completed within a laboratory to show that the method is efficient and performing as expected. It is completed to demonstrate and further confirm the conditions and limitations of the method in which it will obtain reliable and reproducible results (SWGDAM Validation Guidelines 2012). An internal validation of the Applied Biosystems® 3500xL Genetic Analyzer (Applied Biosystems®, Foster City, CA) was completed for the Massachusetts State Police Forensic and Technology Center (MSPFTC) for single source exemplar and convicted offenders' samples using Identifiler® Direct PCR amplification kit.

The AmpF*l*STR® Identifiler® Direct PCR Amplification kit is a short tandem repeat (STR) multiplex assay that allows for direct amplification of single source blood or buccal samples without DNA extraction, purification, or quantization (Wang 2009). Identifiler® Direct amplifies 16 loci in one PCR reaction: 15 autosomal STR markers (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA) and Amelogenin, the sex-determining marker (Applied Biosystems® AmpF*l*STR® Identifiler® Direct User Guide 2012). All loci can be accurately differentiated because of fluorescently labeled primers and non-nucleotide linkers for spacing. These primers attach to a

specific DNA sequence so that the CCD detector located in the 3500xL Genetic Analyzer can detect the DNA sequence (Park 2009).

The Applied Biosystems® 3500xL genetic analyzer is an automated 24 capillary instrument that uses fluorescence-based capillary electrophoresis for human identification analysis. Capillary electrophoresis separates DNA fragments based on their size to charge ratio. The cathode, negative electrode, is placed into the sample; an electrical pulse activates the migration and separation of the DNA through the capillary. The negatively charged DNA migrates from the cathode to the anode, (positive electrode), because the attraction of opposite charges. Smaller DNA fragments migrate faster than larger fragments thus reaching the detector sooner. The DNA fragments have fluorescently-labeled primers attached so that when the DNA goes past the detection window, a narrow beam of light from the laser excites the dyes. The excitation of the dyes give off an emission wavelength which is a longer wavelength of light than the laser's excitation wavelength in all directions, some of which pass through a diffraction grating which then sends the light to the CCD detector. The CCD detector can detect which color wavelength is coming off and the relative fluorescence units (RFU) are measured. Along with an internal size standard and allelic ladder, a software program takes these peaks that are detected and give it a specific allele designation in a given locus. The combinations of all of the fluorescent peaks give rise to an electropherogram. This electropherogram is an individual's DNA profile with his/her specific genotypes (Applied Biosystems® 3500xl User Guide 2010).

The 3500xL offers multiple advantages over the 3130xL genetic analyzers that are being used at the MSPFTC. These advantages include an increased dynamic range therefore off-scale peaks

and oversaturation will not occur until approximately 20,000-30,000 RFU, no lower pump block for less polymer waste, improved oven door sealing for better temperature control, easy to use reagents that are prepackaged for less variability and less analyst hands-on time, consumables with radio frequency identification (RFID) tags so expired reagents are not used, steady solid state laser requires less power, high signal intensity, and an increased number of capillaries for higher throughput (Applied Biosystems® 3500xL User Bulletin 2010).

The internal validation studies performed on the 3500xL, based on the Scientific Working Group for DNA Analysis Methods (SWGDAM) guidelines, included a LIZ comparison, LIZ optimization, injection time, analytical threshold, sensitivity, precision, contamination, concordance, reproducibility, stutter, and heterozygote balance study.

A LIZ comparison study between GeneScan™ LIZ 500 and LIZ 600 v2.0 was performed to evaluate any differences in peak sizing calculated from the two size standards at each allele in each locus. It was also performed to establish whether the Applied Biosystems'® recommended GeneScan™ LIZ 600 v2.0 is an acceptable replacement for GeneScan™ LIZ 500 when using Identifiler® Direct PCR Amplification kit on the 3500xL at MSPFTC. Applied Biosystems® recommends LIZ 600 v2.0 because it incorporates enhancements for improved lot to lot consistency and peak height balance. This study was also performed with two different genetic analyzers to determine if the results from the 3500xL would be concordant with the results obtained on the 3130xL The size standard figures and the size standard peaks can be seen in Appendix III: LIZ size standard comparison.

A LIZ 600 v2.0 optimization study was performed to determine the optimal amount of size standard to add to the Hi-Di-Formamide/LIZ master mix when setting up a plate with the Janus™ automated workstation, using Identifiler® Direct kit on the 3500xL Genetic Analyzer. An optimal amount should not create artifacts or other extraneous peaks, and will allow all size standard peaks to be consistently detected above analytical threshold while giving a clear, single-source profile. This study was also conducted by hand to determine if each method required similar amounts of size standard.

A DNA injection time study was performed to determine which injection time would lead to reliable data. The data should also have sharp, well-defined peaks, resolved baseline and limited artifacts. An analytical threshold study was performed to determine the RFU level that a true peak can be detected above noise levels. Two sensitivity studies were performed to determine the optimal range of DNA to amplify when using Identifiler® Direct kit on the 3500xL. This range should give accurate and reliable genotypes with full profiles detected above analytical threshold while limiting stochastic effects and artifacts.

The precision study was performed to determine if Identifiler® Direct would give accurate and reliable genotypes for each run on the 3500xL genetic analyzer. Three different sizing precision studies were conducted to demonstrate this; an allelic ladder precision study, amplification positive precision study, and 250 base pair migration study. The allelic ladder and amplification positive studies were performed to assess the variation in base pair size within each allele for each locus. The allelic ladder precision study also compared the precision between different concentrations of Identifiler® Direct allelic ladder and compare the precision between Identifiler®

Direct and Identifiler® ladder. The 250 base pair (bp) migration study was performed to assess the migration of the 250 bp peak that is in the LIZ 500 size standard. Migration of the 250 bp peak can vary from sample to sample throughout the run due to temperature fluctuations (Rosenblum 1997); therefore the peak was evaluated to assess the stability of instrument's oven temperature. The degree of precision at each allele can dictate the amount of measurable error at that given allele for the sizing method used. Precision should be less than 0.15 standard deviation (Wang 2011). Precision can be determined by calculating the standard deviation for each allele in a single capillary after multiple injections or across multiple wells on the sample plate.

A contamination study was performed to evaluate the level of contamination, if any, when using Identifiler® Direct kit on the 3500xL. Contamination could be due to; 1) BSD600® Duet Series II Semi-automated Punch System, 2) Janus™ automated workstations, 3) 3500xL Genetic Analyzer, or 4) analyst error when transferring or preparing the plate. Negative controls set up at each step were analyzed to assess contamination risk. A concordance study was performed to determine allele call consistency between two different genetic analyzers, the 3500xL and the 3130xL. Previously amplified and analyzed samples, that were ran on the 3130xL using Identifiler® Direct, would be compared to the same samples re-amplified with Identifiler® Direct and ran on the 3500xL Genetic Analyzer. A reproducibility study was performed to determine the ability of the 3500xL Genetic Analyzer to reproduce genotypic results across multiple runs on multiple days. The assessment of peak height reproducibility was also completed for each injection.

A stutter study was performed to determine the amount of stutter produced at each locus. Stutter within the four reproducibility and two sensitivity studies were evaluated to determine reasonable guidelines for the marker specific stutter ratios for Identifiler® Direct and assess whether internally generated stutter ratios differ from the manufactures' published values. A heterozygote allele balance study was conducted to determine if genotypes would consistently produce balanced peak heights in heterozygote loci. It was also conducted to establish MSPFTC's threshold for heterozygote peak height ratio.

These studies were conducted to set parameters and show the 3500xL performed as expected giving reliable, reproducible, and robust results for MSPFTC when using Identifiler® Direct on the 3500xL for single source exemplar and convicted offenders' samples after the completion of the validation.

Methods

LIZ Comparison

For the LIZ comparison study, four master mixes were prepared for two genetic analyzer runs.

The first was made by combining 8.7µL Hi-Di formamide with 0.3µL LIZ 500 per sample and the second was made by combining 8.5µL Hi-Di formamide with 0.5µL LIZ 500 per each sample. Processing two concentrations of LIZ size standard was a preliminary survey for the LIZ optimization study. The third and fourth master mixes were made of the same components but LIZ 600 v2.0 was used in place of LIZ 500 for the size standard. Two plates were set up; one was run on the 3130xL Genetic Analyzer and one on the 3500xL Genetic Analyzer.

The size standards were checked with the size match editor function in GeneMapper® ID-X (GMIDX) version 1.3 and all allelic ladders were checked to ensure proper allele calling. The samples that contained 8.7µL Hi-Di formamide with 0.3µL size standard, LIZ 500 or LIZ 600 v2.0, were used for calculations. The results obtained from each of the genetic analyzers were imported into an excel sheet and the average and standard deviation of the base pair sizes of allele peaks were calculated; minimum and maximum peak sizes were noted. The standard deviations of each of the samples using LIZ 500 were compared to the samples using LIZ 600 v2.0. An acceptable degree of precision for this would be 0.15 standard deviation.

LIZ Optimization

For the LIZ optimization study three concentrations of size standard were selected, 0.1µL, 0.3µL and 0.5µL. These selections were made because Applied Biosystems'® recommendation was 0.5µL, MSPFTC previously validated 0.3µL on the Janus™ for Identifiler® Direct, and 0.1µL was used to evaluate if a lower amount of LIZ could be used and still be detected.

Three master mixes were prepared. The first was made by combining 8.9µL Hi-Di formamide with 0.1µL LIZ 600 v2.0, the second was made by combining 8.7µL Hi-Di formamide with 0.3µL LIZ 600 v2.0, the third was made by combining 8.5µL Hi-Di formamide with 0.5µL LIZ 600 v2.0. Each LIZ 600 v2.0 concentration was evaluated by analyzing the average LIZ peak heights when used to size two amplification positives (9947A), two amplification negatives, one in house NIST-Traceable extraction positive, two ladders, and one formamide/LIZ blank. Two plates were created, one by hand and one by the Janus™ automated workstation. This was

conducted to see if the two methods were comparable. See Appendix II: Amplification Parameters for amplification master mix recipe.

The size standards were checked with GMIDX's size match editor function and all samples were checked to ensure proper allele calling. Extraneous artifact peaks were eliminated from the analysis and calculations. The size standard results obtained were imported into an excel sheet and the average and standard deviation of the peak heights were calculated; minimum and maximum peak heights were noted. The average was calculated in three ways, first just the samples then just the ladders and lastly all peaks in both the samples and ladders. This was conducted to see if the ladders and samples were comparable or if one had a large effect on the overall average peak height.

The injection parameters for the LIZ comparison and optimization studies were the recommended settings by Applied Biosystems®; 24 seconds at 1.2 kV. After data analysis for the concordance and reproducibility studies, another LIZ optimization study was conducted using 0.2µL LIZ 600 v2.0.

Injection Time, Analytical Threshold, and Sensitivity

The injection time, analytical threshold and first sensitivity study all were set up on the same run plate. Three previously extracted samples (14-1, 14-2, and 14-3) along with their 1:10 dilution, were quantified in duplicate. The samples were quantified using Quantifiler® Human kit on the Applied Biosystems® 7500 Real-time PCR system. The averaged quantization values for each sample were used to determine the sample amount needed for a 5 ng/10µL concentration (tube

A). A two-fold serial dilution was then completed for each of the samples to create tubes B-H, by adding 25µL of TE buffer in all tubes and then adding 25µL of the previous concentration tube. Tube I was created independently by taking a calculated amount of the 1:10 dilution for each of the samples that were quantified and adding TE to create a 10uL solution with a concentration of 2.0ng/10µL. TE blank (tube J) was also created for each set of samples. See Table 1. Ten microliters of each sample of the titration set for each of the samples were placed in the appropriate well of its 96 well plate and placed under a laminar fume hood to evaporate overnight.

Tube	Final Amplified Concentration	Starting Concentration
A	5.0 ng/10µL	0.5 ng/µL
B	2.5 ng/10µL	0.25 ng/µL
C	1.25 ng/10µL	0.125 ng/µL
D	0.62 ng/10µL	0.062 ng/µL
E	0.31 ng/10µL	0.031 ng/µL
F	0.15 ng/10µL	0.015 ng/µL
G	0.078 ng/10µL	0.0078 ng/µL
H	0.039 ng/10µL	0.0039 ng/µL
I	2.0 ng/10µL	0.2 ng/µL
J	TE blank	

Table 1: Titration set concentration values

The Janus™ automated workstation was used to set up the amplification and capillary electrophoresis plates. The master mixes for each were created manually before and placed into the designated slots. The tray was amplified on a GeneAmp® PCR System 9700 thermal cycler for 26 cycles. See Appendix II: Amplification Procedure. The capillary electrophoresis master mix contained 8.9µL Hi-Di formamide with 0.1µL LIZ 600 v2.0, per sample. The appropriate controls and ladders were also added. The samples were injected at 12, 18, 24, and 30 seconds at 1.2kV.

The size standards were checked with GMID-X's size match editor and all samples were checked to ensure proper allele calling. The analytical threshold was set to 50 RFU. The results obtained were imported into an excel sheet and the average peak height, baseline noise, artifacts, off-scale data, dropout, and peak height balance were analyzed and reported. Each concentration was analyzed separately. For homozygous loci, the peak height was divided in half and this value was used for the peak height calculations. Extraneous "OL Alleles" and other artifacts were noted and removed. A 15% stutter filter was utilized when analyzing the data (per current MSPFTC protocol).

After data analysis, another sensitivity study was conducted to confirm anomalies that were observed. Previously made sample series of 14-1 (Tubes A-I) from the first sensitivity study was re-setup in a 96 well plate alongside a remade titration set of 14-1. These samples were made as described above in the first sensitivity study. These were set to evaporate overnight. Amplification and capillary electrophoresis was completed as stated above, as well as data analysis.

The analytical threshold was calculated using two different methods. The first method used the Scientific Working Group DNA Analysis Method (SWGDAM) guidelines. The formula to calculate the analytical threshold (Figure 1) is in section 1.1 of the SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories (2010).

Figure 1: SWGDAM Analytical Threshold formula

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

Y_{max} is the highest peak within instrumental noise data
Y_{min} is the signal of the lowest trough
AT the analytical threshold calculated

The second method was from the International Union of Pure & Applied Chemists (IUPAC) (Figure 2). Kaiser believes that a value of k = 3 will result in an analytical threshold with 89% - 99.86% confidence that noise will be below this value. (Grgicak 2010)

Figure 2: IUPAC Analytical Threshold formula

$$AT = \bar{Y}_{bl} + ks_{bl}$$

$\bar{Y}_{bl}$ is the average blank RFU signal
s_{bl} the std deviation of the blank signal
AT the analytical signal calculated

These methods are used to determine at what amplitude one can no longer reliably separate signal from noise.

Precision

For the first precision study, 250 bp precision study, two master mixes were prepared for the genetic analyzer run. The first contained 8.7µL Hi-Di formamide with 0.3µL LIZ 500, per sample. This master mix was added to wells A01-D01, A03-D03, and A05-D05. The second contained 8.5µL Hi-Di formamide with 0.5µL LIZ 500, per sample. The master mix was added to wells E01-H01, E03-H03, and E05-H05. The ladders were not injected sequentially because this plate was also used for the LIZ comparison study. The two different master mixes were used to see if the concentration of the LIZ 500 made any difference in migration of the 250 bp peak.

For the second study, Allelic Ladder 1 and Amplification Positive Precision Study, a master mix was prepared for the genetic analyzer run which contained 8.9µL Hi-Di formamide with 0.1µL LIZ 600 v2.0, per sample. 1µL of allelic ladder was added to wells A01-H03 and A07-H09 along with the prepared master mix. Amplification positive was added to wells A04-H06 and A10-H12 along with the prepared master mix. Two injections of twenty-four ladders or amp positive were injected, one in each capillary.

For the third study, Allelic Ladder 2 Precision Study, a master mix was prepared which contained 8.8µL Hi-Di formamide with 0.2µL LIZ 600 v2.0, per sample. One microliter of Identifiler® Direct allelic ladder was added to wells A01-H03, 1µL of Identifiler® Direct Ladder diluted 1:2 with formamide (0.5µL) was added to wells A04-H06, and 1µL of Identifiler® ladder was added to wells A07-H09 along with the prepared master mix.

The size standards were checked, for all studies, with the size match editor and all samples were checked to ensure proper allele calling. Extraneous “OL Alleles” and other artifacts were noted and removed. A 15% filter was utilized when analyzing the data. The results obtained were imported into an excel sheet. For the allelic ladder and amplification positive precision studies, the average and standard deviation of each allele and locus were calculated and reported. For the 250 bp precision study; the average size, standard deviation of size, maximum size, minimum size, and maximum/minimum difference in size were calculated and reported.

Contamination

For the contamination study, a checkerboard pattern of blanks and extraction positive samples were set up in a tray to determine if contamination would occur across sample wells when setting up a plate or in the same capillary in multiple, sequential injections. The Janus™ automated workstation was used to set up the amplification and capillary electrophoresis plates. The master mixes for each were created manually before and placed into the designated slots. The tray was amplified on a GeneAmp® PCR System 9700 thermal cycler. See Appendix II: Amplification Procedure. After amplification, a master mix was prepared for the genetic analyzer run which contained 8.9µL Hi-Di formamide with 0.1µL LIZ 600 v2.0, per sample. One microliter of the appropriate controls and ladders were added.

The size standards were checked with the size match editor and all samples were checked to ensure proper allele calling. The negative samples were evaluated for peaks near or above the baseline to determine if it was contamination.

Concordance and Reproducibility

For the concordance and reproducibility studies, 8 saliva and 37 blood FTA® cards, that were previously analyzed by the 3130xl using Identifiler® Direct, were punched (1 punch, 1.2mm) using the BSD600® Duet Series II Semi-automated Punch System, into a 96 well plate in the assigned well. The Janus™ automated workstation was used to set up the amplification and capillary electrophoresis plates. The master mixes for each were created manually before and placed into the designated slots. The tray was amplified on a GeneAmp® PCR System 9700 thermal cycler. See Appendix II: Amplification Procedure. After amplification, a master mix was

prepared for the genetic analyzer run which contained 8.9µL Hi-Di formamide with 0.1µL LIZ 600 v2.0, per sample. The appropriate controls and ladders were added. The first plate was set up and ran on the 3500xL genetic analyzer on July 11 and then re-setup and re-injected on July 12, July 15, July 16, and July 17. The run completed on July 15 was the plate used for the Concordance study.

The size standards were checked with the size match editor and all samples were checked to ensure proper allele calling. Extraneous "OL Alleles" and artifacts were noted and removed. A comparison of the genotypes for each of the samples was completed. Non-concordant results were flagged. The reproducibility results were imported into an excel sheet and sample peak heights and allele call consistency was compared. An assessment of reproducibility of base pair sizes was completed in the LIZ comparison study. A 15% filter was utilized when analyzing the data.

Stutter

For the stutter study, 3307 alleles from samples in the reproducibility and sensitivity studies were evaluated for stutter. They were analyzed with no filter so all stutter would be called. Taking the stutter peak height and dividing it by the allele peak height that it corresponds with calculated the stutter ratio for each allele.

The size standards were checked with the size match editor and all samples were checked to ensure proper allele calling. Data from the studies was imported into excel. Average, standard deviation, minimum and maximum peak height ratios were calculated for each marker in each

locus. The average and standard deviation was entered into the equation shown in Figure 3 to determine the threshold for marker specific stutter.

Stutter = 3 x Standard Deviation + Average Stutter Ratio

Figure 3: Marker Specific Stutter Threshold equation

Heterozygous Balance

For the heterozygote balance study, samples from the reproducibility studies were evaluated and analyzed. Taking the smaller allele peak and dividing it by the taller allele peak height calculated the peak height ratio

The size standards were checked with the size match editor and all samples were checked to ensure proper allele calling. Data from the three studies were imported into excel. Average, minimum, maximum and peak height ratios were calculated for each marker in each locus. A 15% filter was utilized when analyzing the data.

The data for all studies were analyzed using GeneMapper® ID-*X* v1.3 with the Validation analysis method, with the exception of the analytical threshold study. See all analysis parameters in Appendix I: Analysis Methods, see amplification parameters in Appendix II: Amplification Parameters, and see expected cost in Appendix IV: Cost of Supplies and Reagents for 3500xL.

Results

LIZ comparison

Allele sizing variation across alleles and across loci is reduced when using GeneScan™ LIZ 600 Size Standard v2.0 compared to LIZ 500 at 0.3µL, as is illustrated in Figures 4- 35. When comparing the data obtained from just the 3500xL, overall the majority of the LIZ 600 v2.0 gave equal or more consistent base pair sizing than samples with LIZ 500. Exceptions are outlined in red in Figures 26 and 31; at the alleles that were exceptions there is minor differences between the LIZ 500 and LIZ 600 v2.0.

Figure 4: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at D8 on the 3130xl

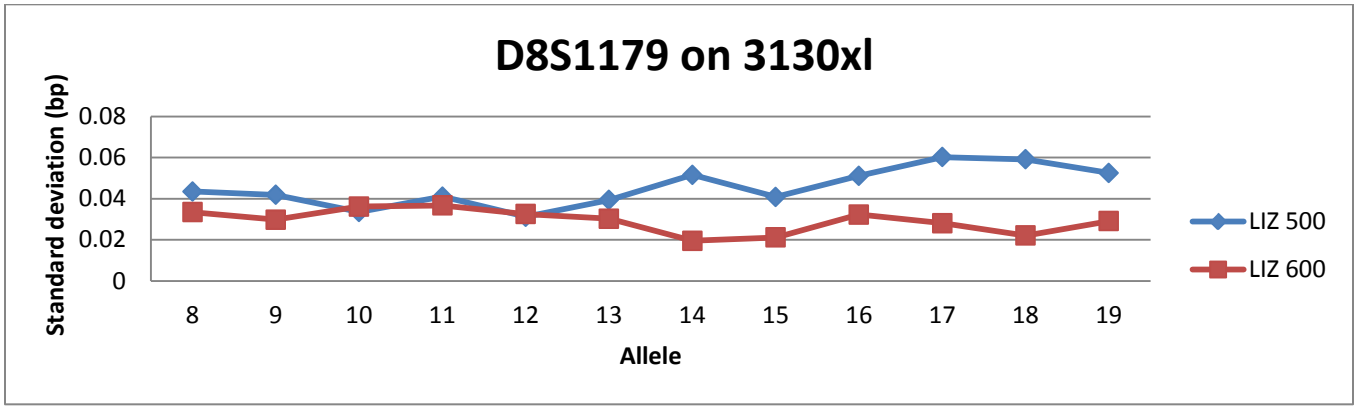


Figure 5: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at D21 on the 3130xl

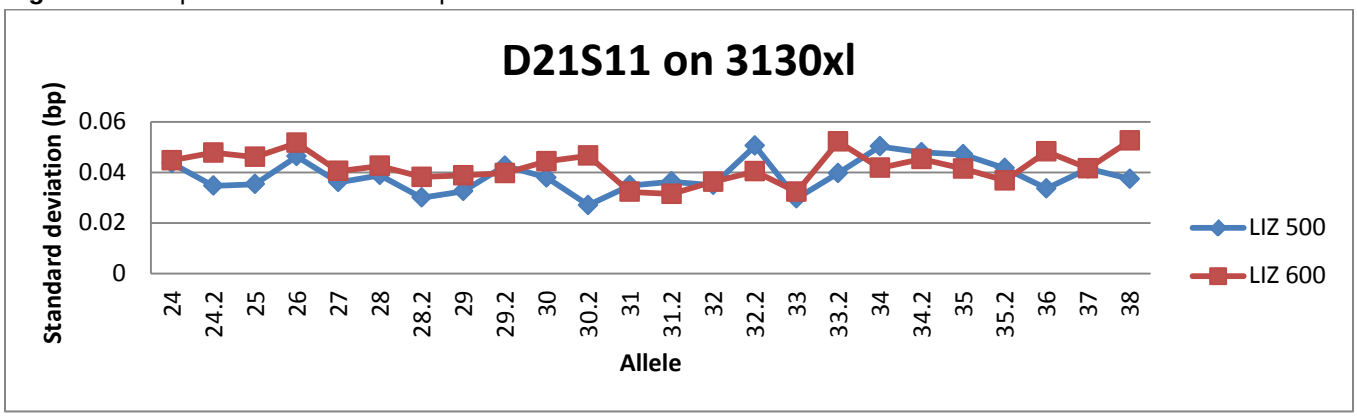


Figure 6: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at D7 on the 3130xl

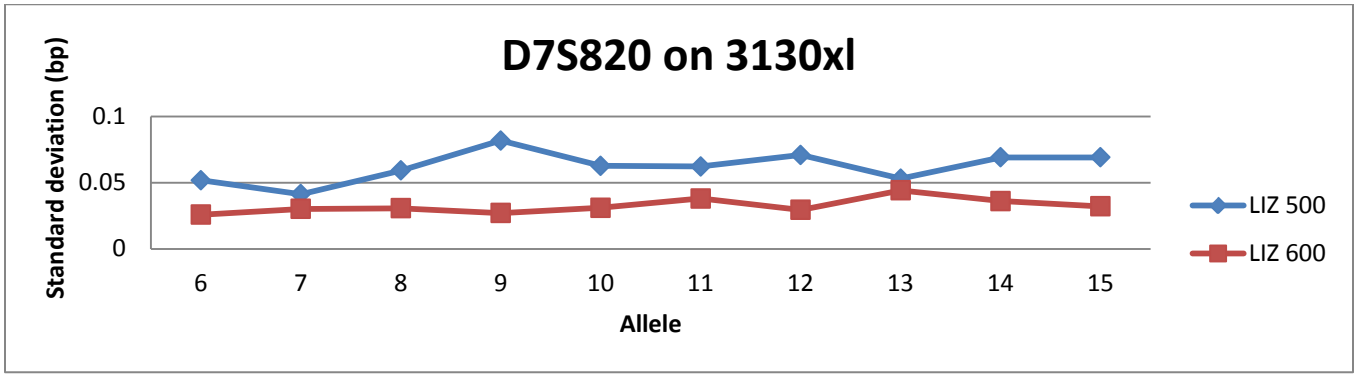


Figure 7: Comparison of allele base pair size between LIZ 500 & LIZ 600 at CSF1PO on the 3130xl

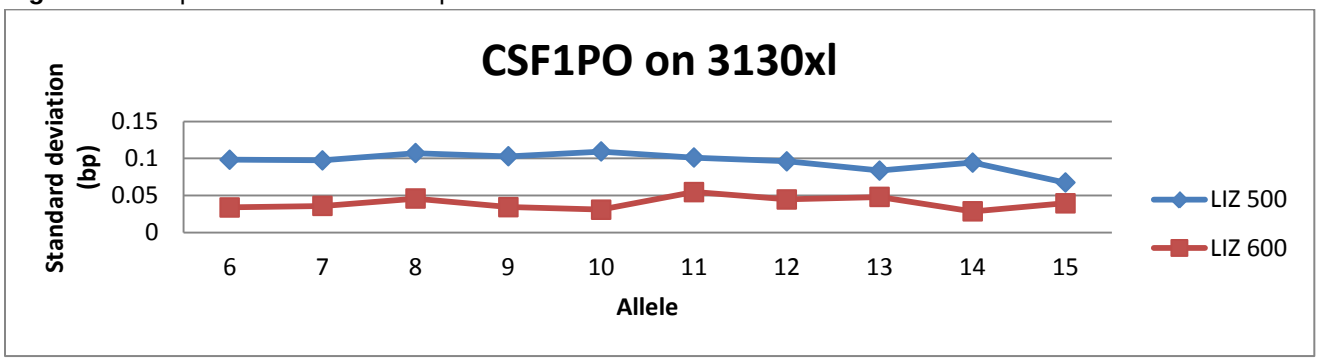


Figure 8: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at D3 on the 3130xl

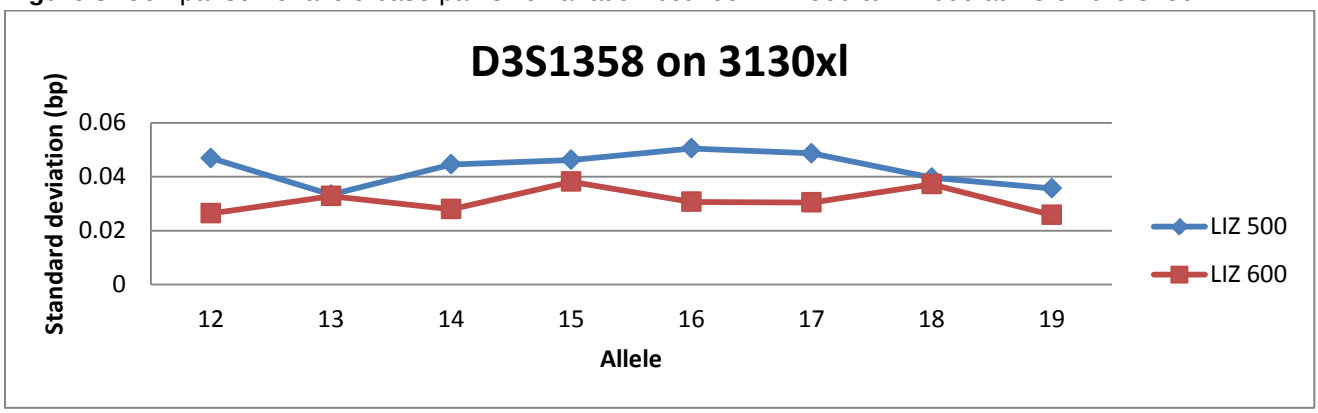


Figure 9: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at TH01 on the 3130xl

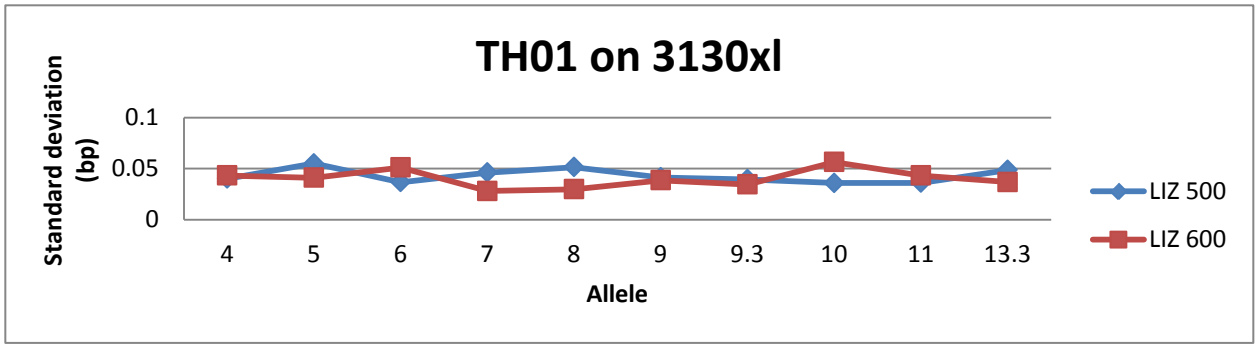


Figure 10: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at D13 on the 3130xl

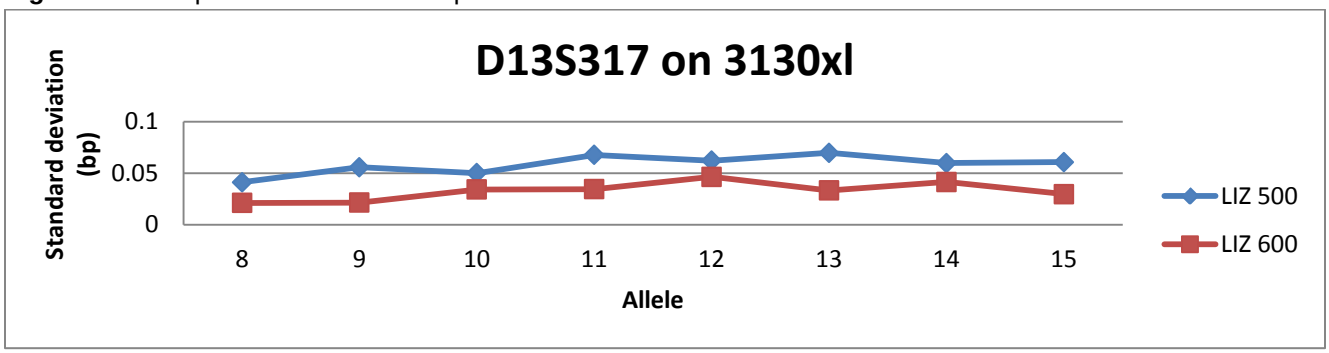


Figure 11: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at D16 on the 3130xl

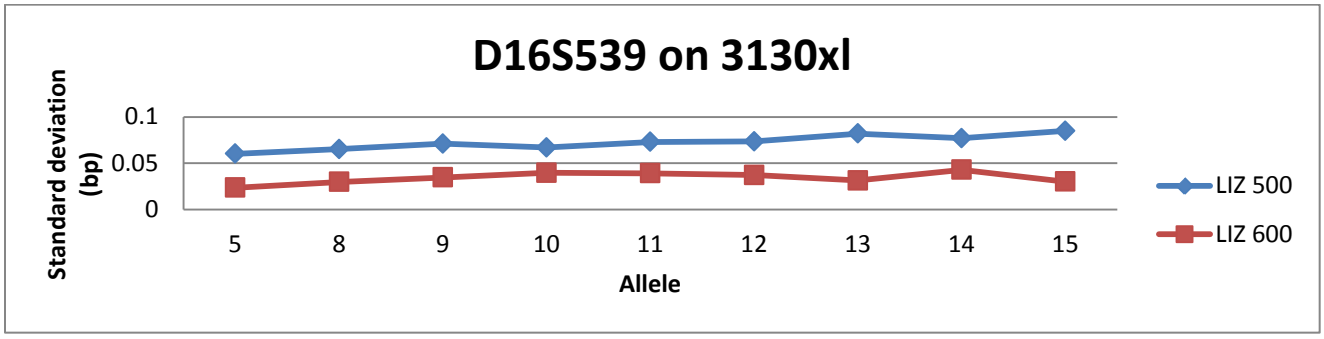


Figure 12: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at D2 on the 3130xl

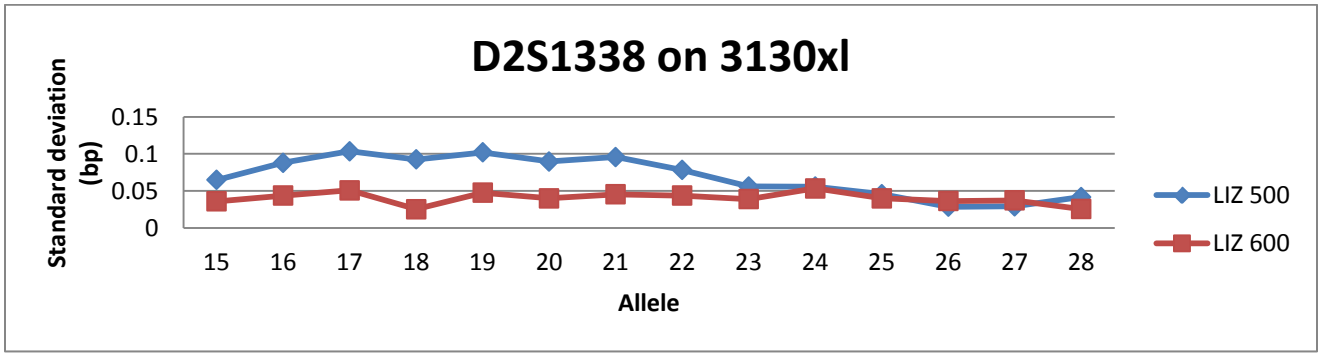


Figure 13: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at D19 on the 3130xl

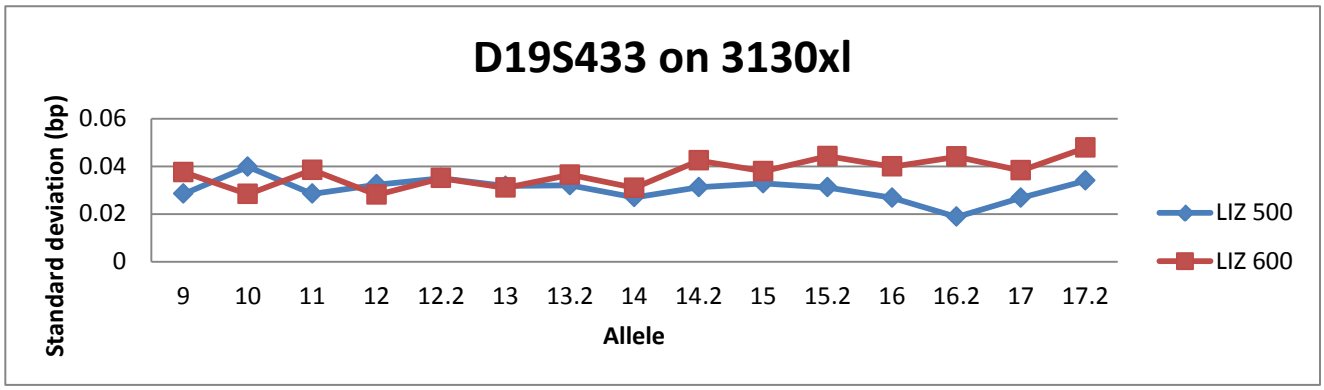


Figure 14: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at vWA on the 3130xl

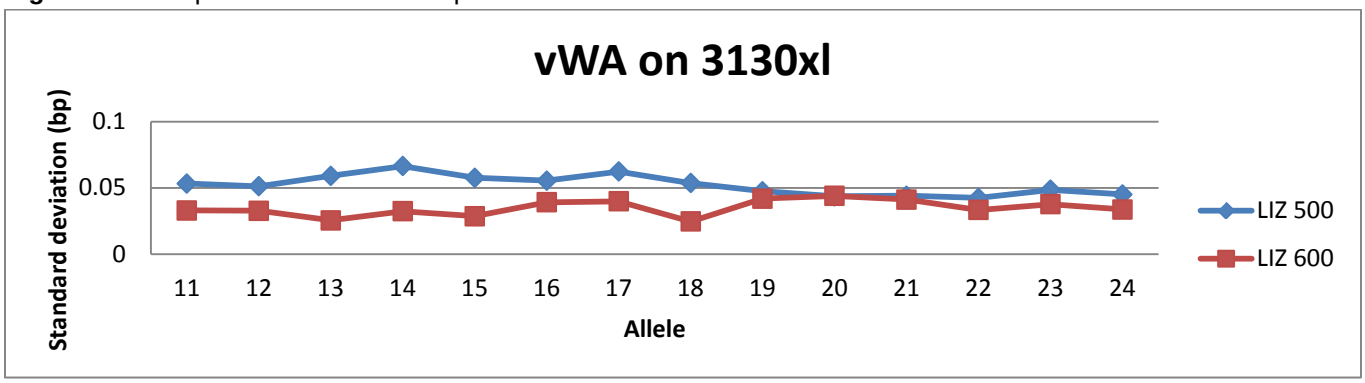


Figure 15: Comparison of allele base pair size between LIZ 500 & LIZ 600 at TPOX on the 3130xl

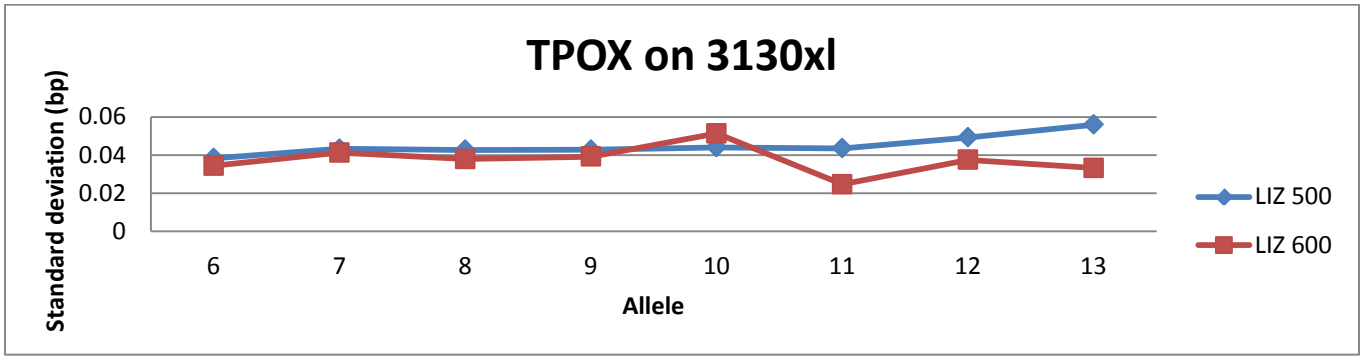


Figure 16: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at D18 on the 3130xl

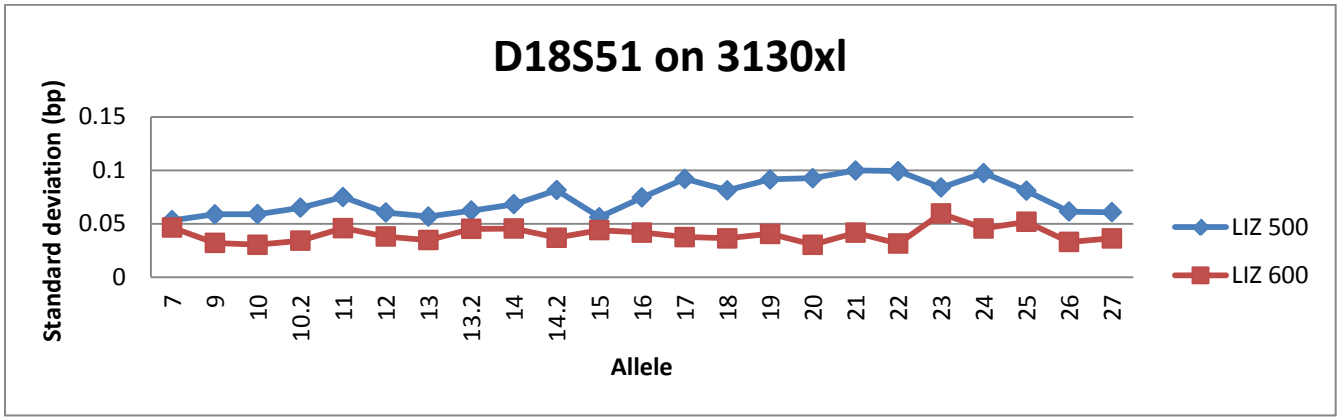


Figure 17: Comparison of allele base pair size between LIZ 500 & LIZ 600 at AMEL on the 3130xl

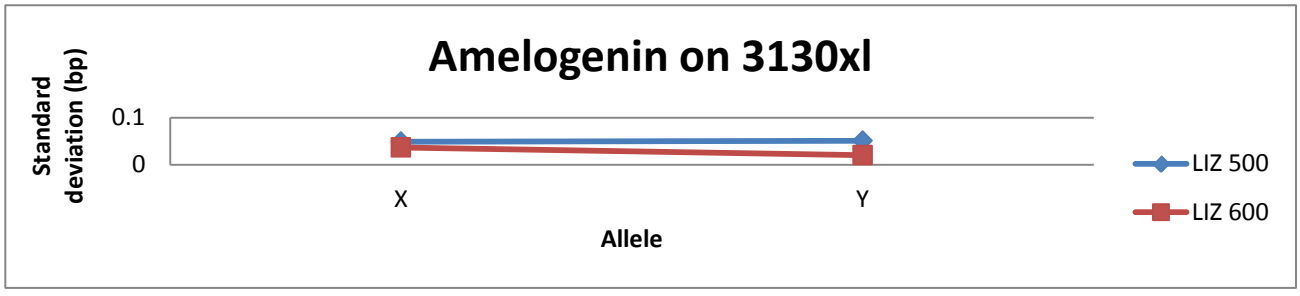


Figure 18: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at D5 on the 3130xl

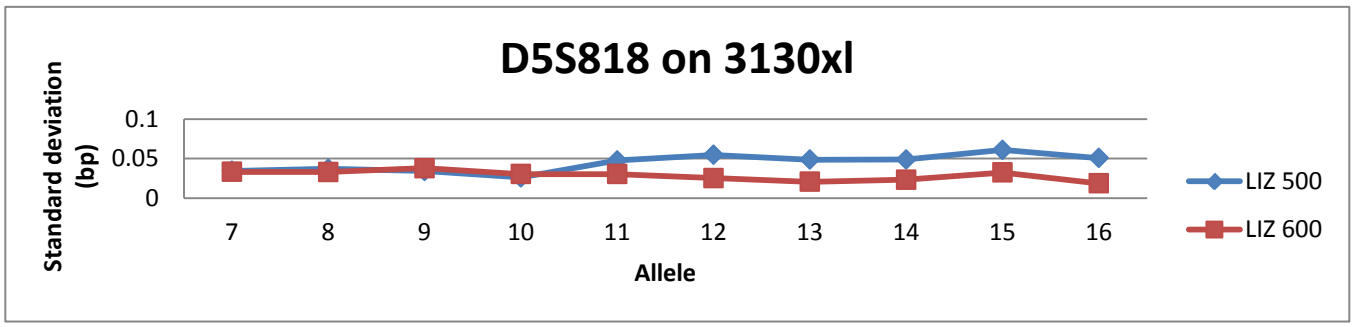


Figure 19: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at FGA on the 3130xl

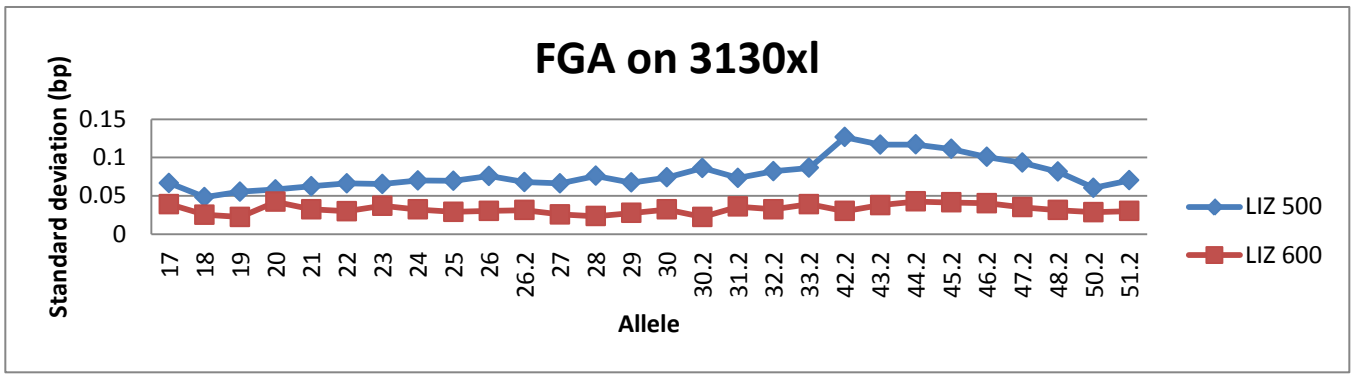


Figure 20: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at D8 on the 3500xl

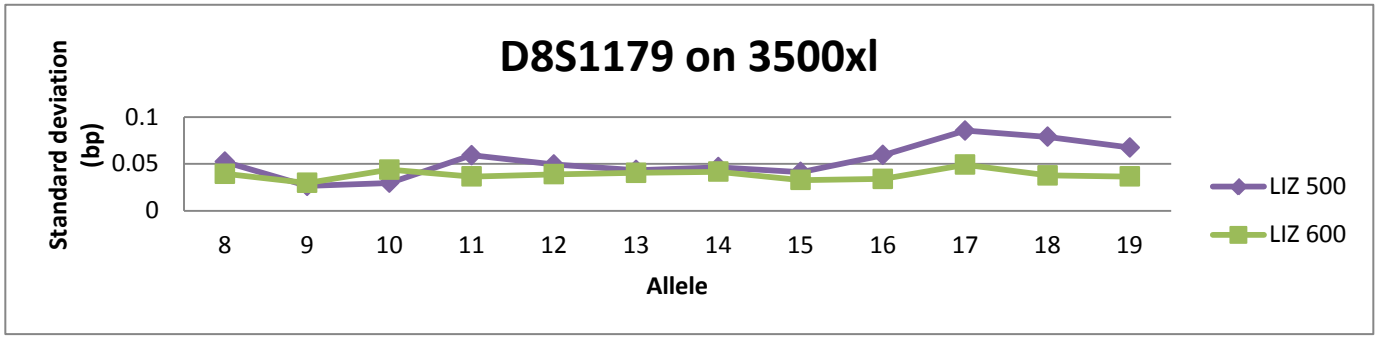


Figure 21: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at D21 on the 3500xl

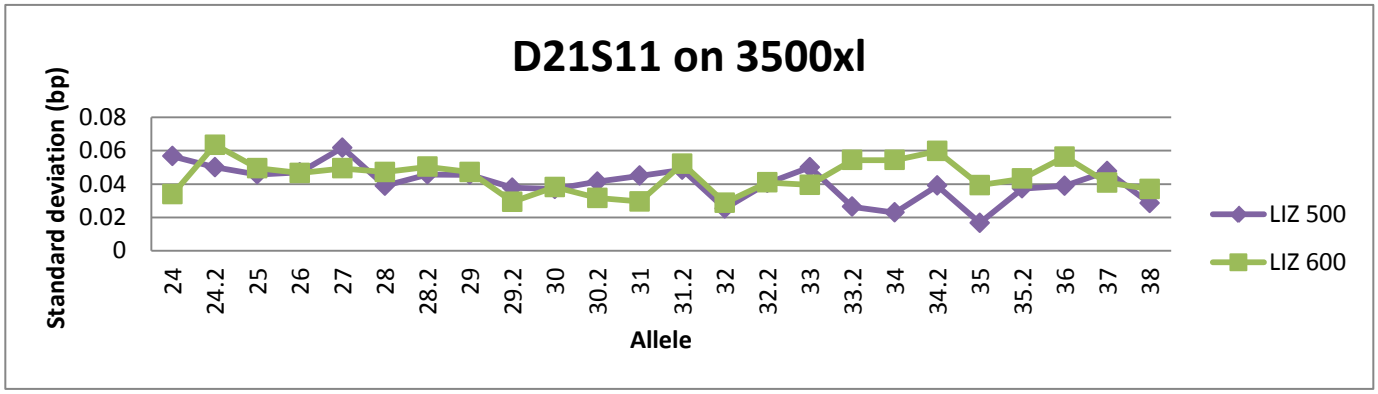


Figure 22: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at D7 on the 3500xl

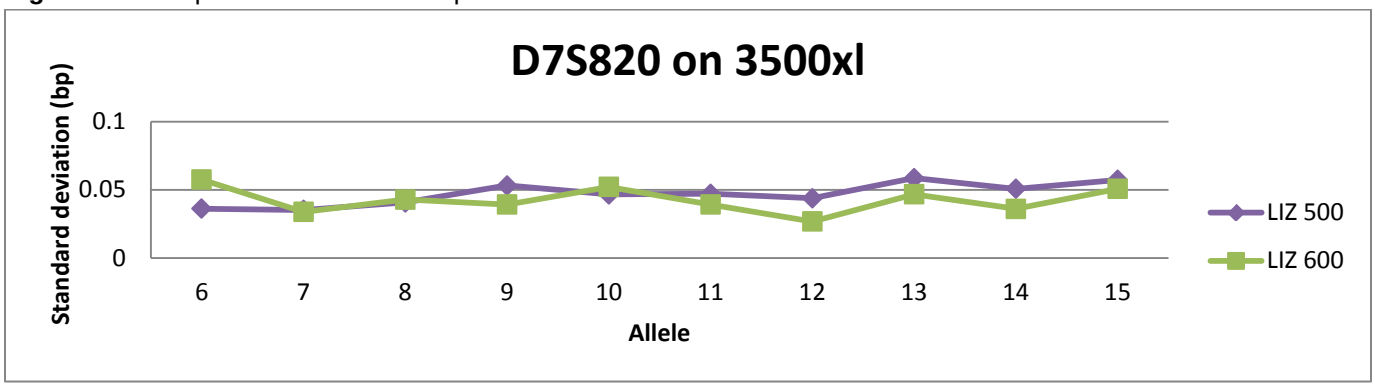


Figure 23: Comparison of allele base pair size between LIZ 500 & LIZ 600 at CSF1PO on the 3500xl

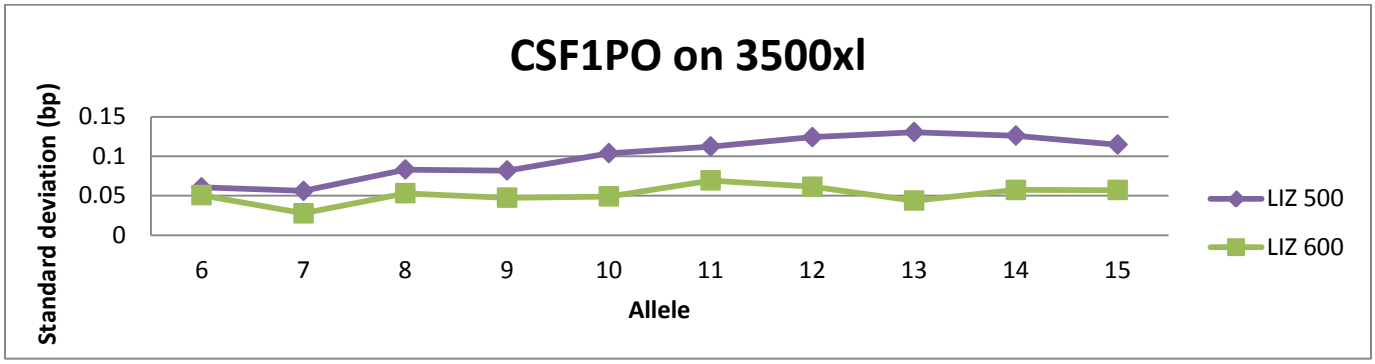


Figure 24: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at D3 on the 3500xl

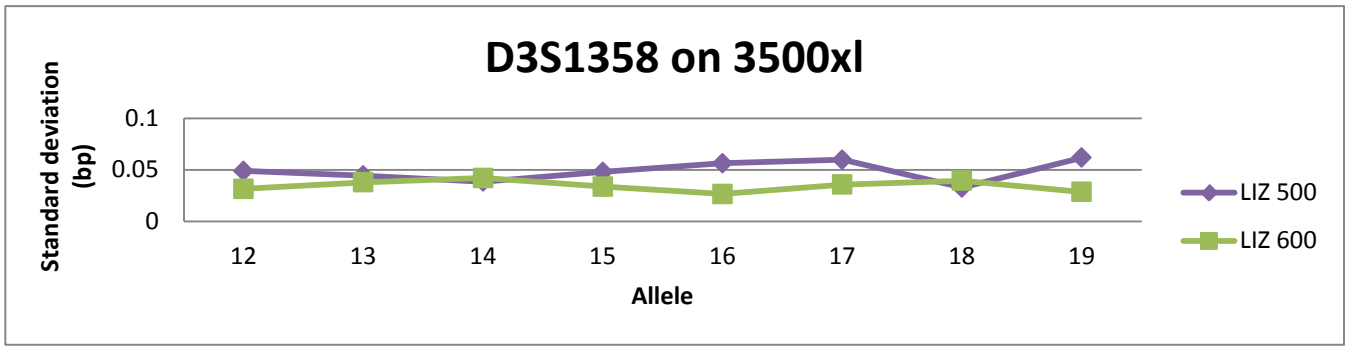


Figure 25: Comparison of allele base pair size between LIZ 500 & LIZ 600 at TH01 on the 3500xl

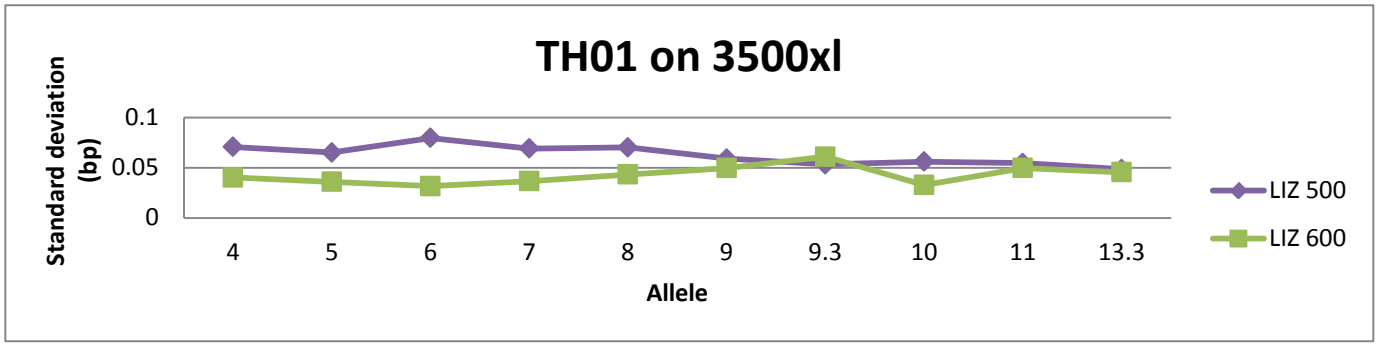


Figure 26: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at D13 on the 3500xl

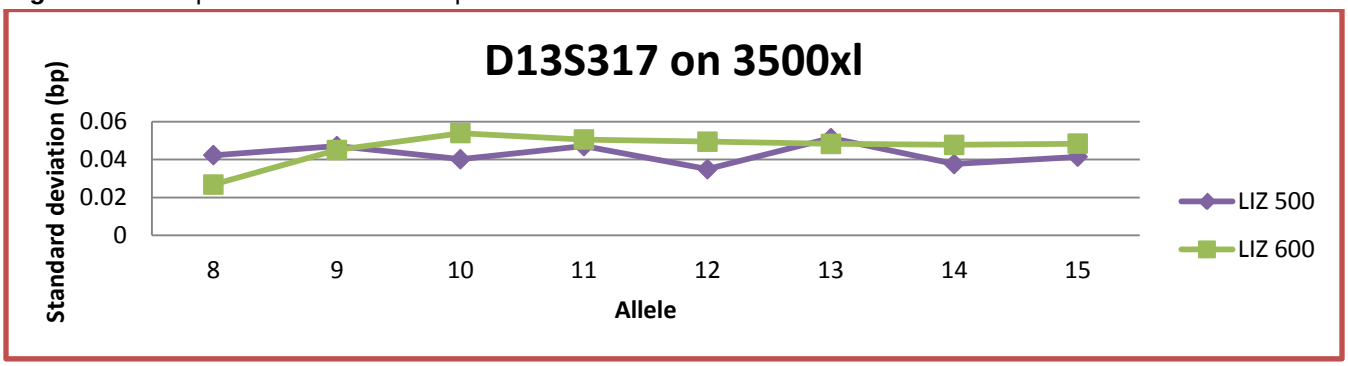


Figure 27: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at D16 on the 3500xl

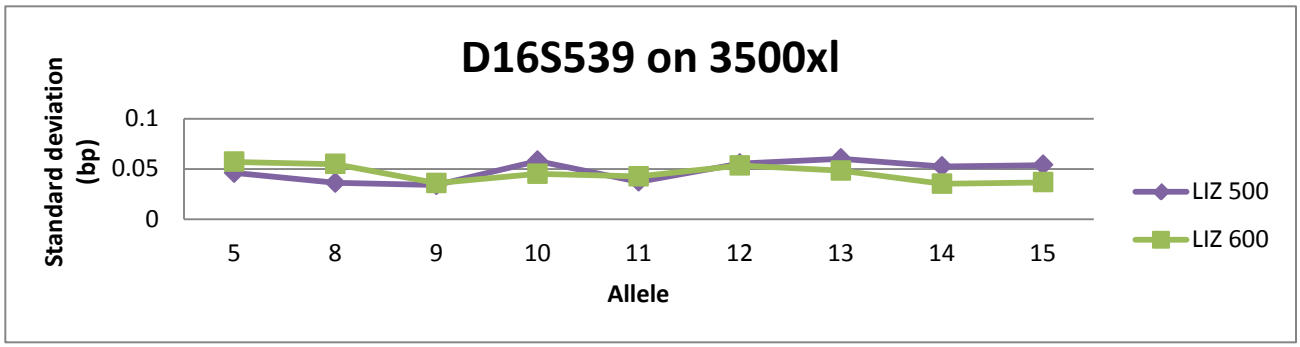


Figure 28: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at D2 on the 3500xl

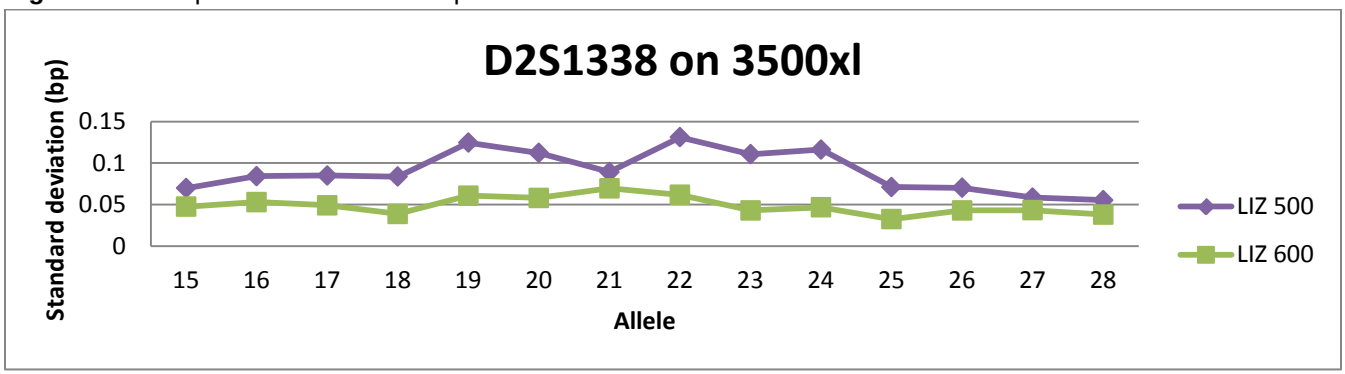


Figure 29: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at D19 on the 3500xl

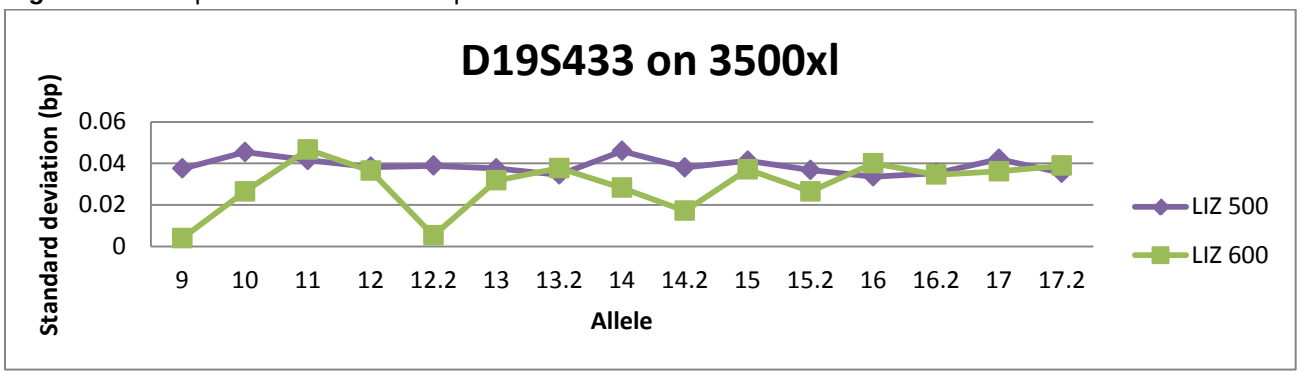


Figure 30: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at D3 on the 3500xl

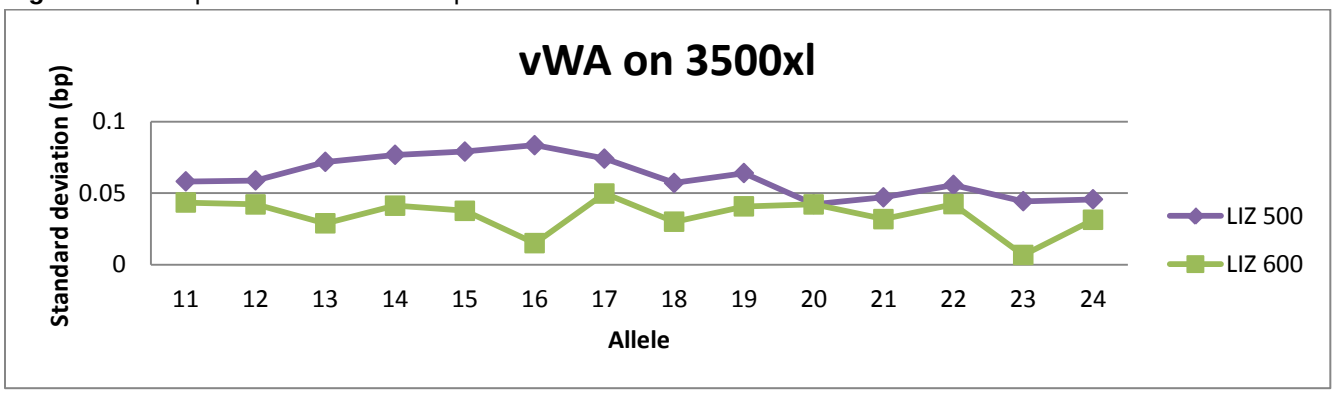


Figure 31: Comparison of allele base pair size between LIZ 500 & LIZ 600 at TPOX on the 3500xl

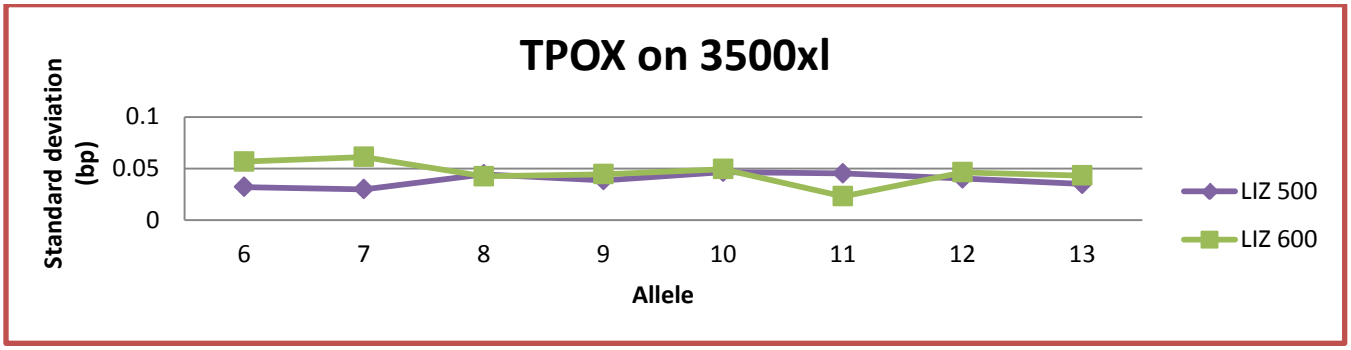


Figure 32: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at D18 on the 3500xl

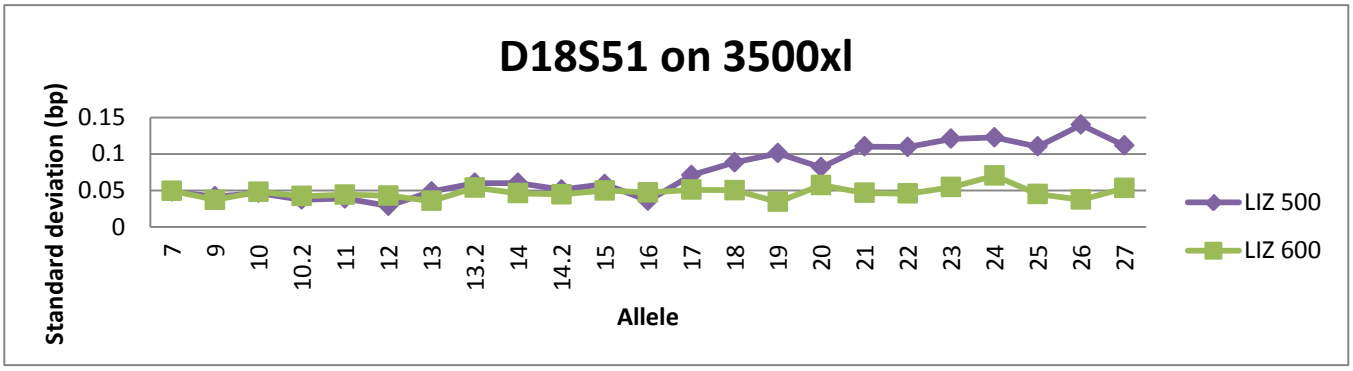


Figure 33: Comparison of allele base pair size between LIZ 500 & LIZ 600 at AMEL on the 3500xl

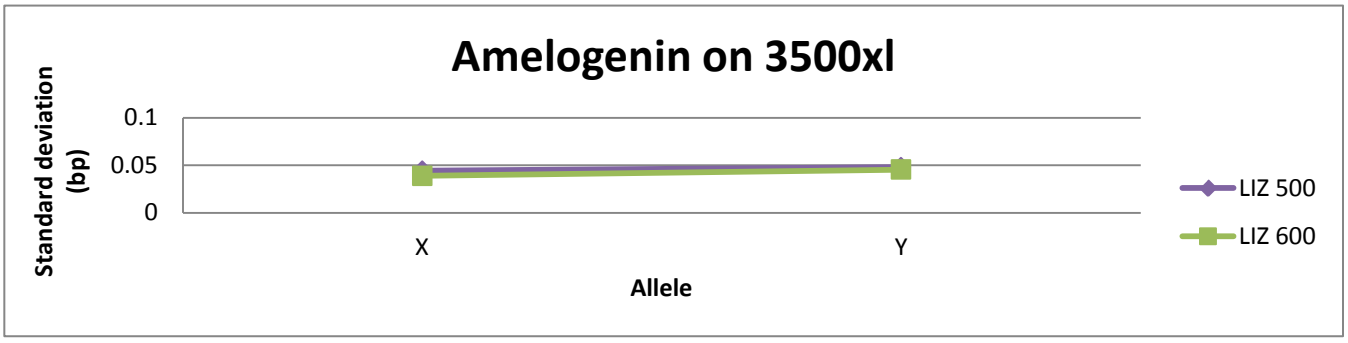


Figure 34: Comparison of allele base pair size variation between LIZ 500 & LIZ 600 at D5 on the 3500xl

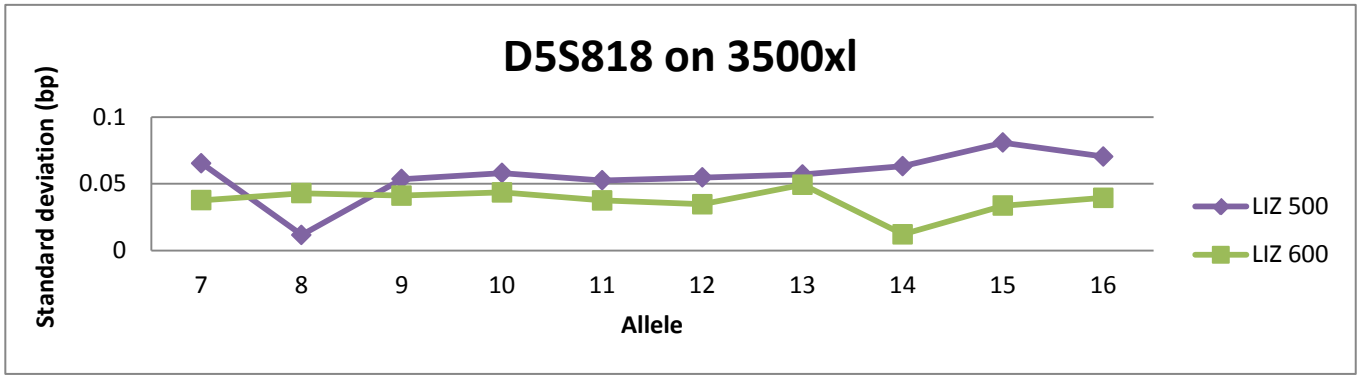
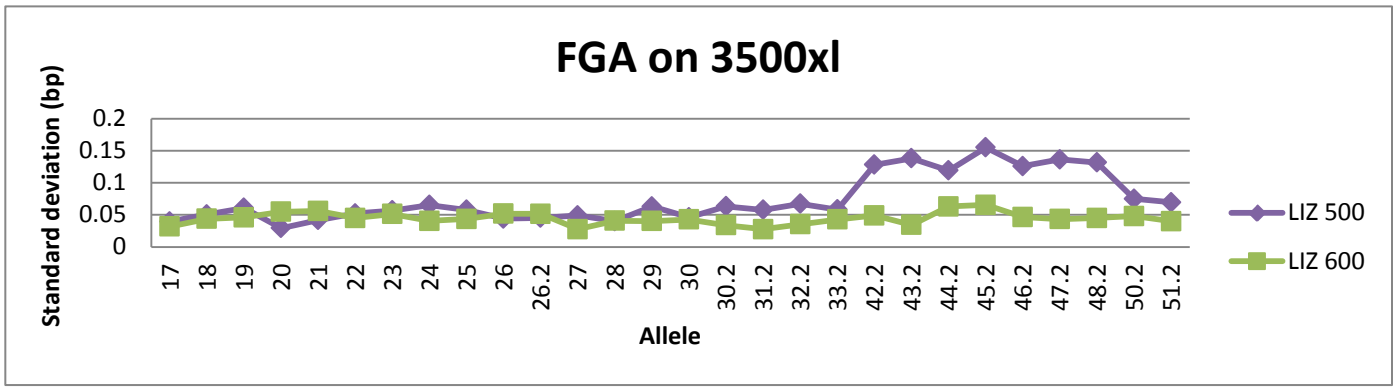
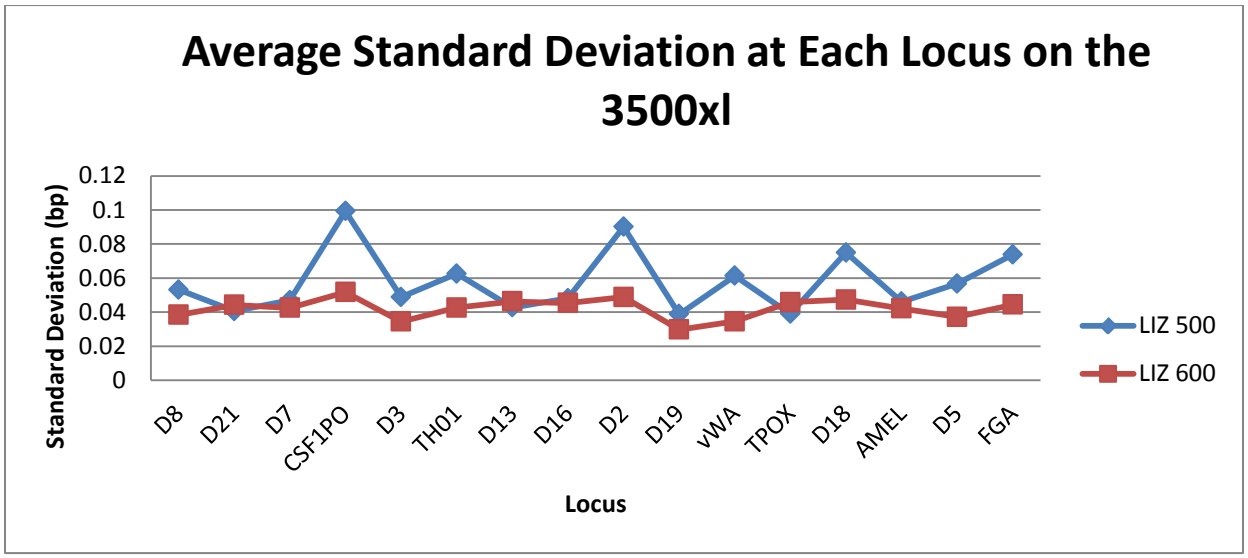


Figure 35: Comparison of allele base pair size between LIZ 500 & LIZ 600 at FGA on the 3500xl



The average standard deviation for each locus on the 3500xl using 0.3µL is displayed in Figure 36.

Figure 36: Average standard deviation for each locus on the 3500xl



LIZ Optimization

The peak heights of the size standard peaks consistently increased as the concentration of size standard was increased without an effect on the samples or ladder peak heights, which is to be expected. The average and minimum peak heights are shown in Table 2. Pull up was created in the 0.3 µL and 0.5 µL size standard concentration but not in the 0.1 µL. Samples analyzed for each concentration were two amp positives, two amp negatives, one extraction positive, two ladders, and one run negative.

	Average Peak Height in RFU			Minimum Peak Height
Size Standard concentration	**Samples**	**Ladders**	**All (sample and ladders)**	**All**
0.1µL Janus	373	676	449	113
Hand	916	652	850	155
0.3µL Janus	1729	2318	1876	694
Hand *	3350	1867	2954	457
0.5µL Janus	3755	3368	3658	689
Hand	4788	3436	4450	751

Table 2: Size standard calling peaks only

*One sample was eliminated from analysis due to bad injection and lowering of average peak heights

Injection Time

All injection times produced full profiles in concentrations of 5.0ng/µL – 0.31ng/µL, Dropout below the given threshold began to occur at 0.15ng at each injection time. Graphs of each concentration and injection time are shown in Figures 37 - 42. The average peak height, peak height standard deviation, max and min for each injection time can be seen in Tables 8 - 11 in the Sensitivity Study Section.

Figure 37: 5ng at 12, 18, 24, and 30 second injection times

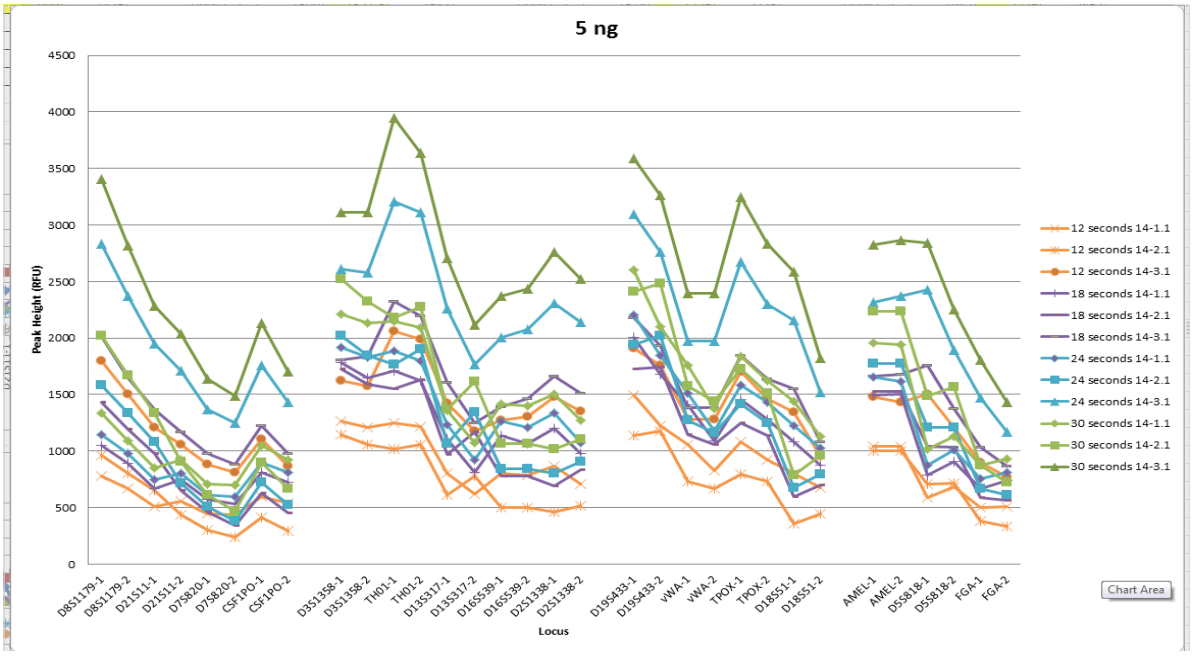


Figure 38: 2.5ng at 12, 18, 24, and 30 second injection times

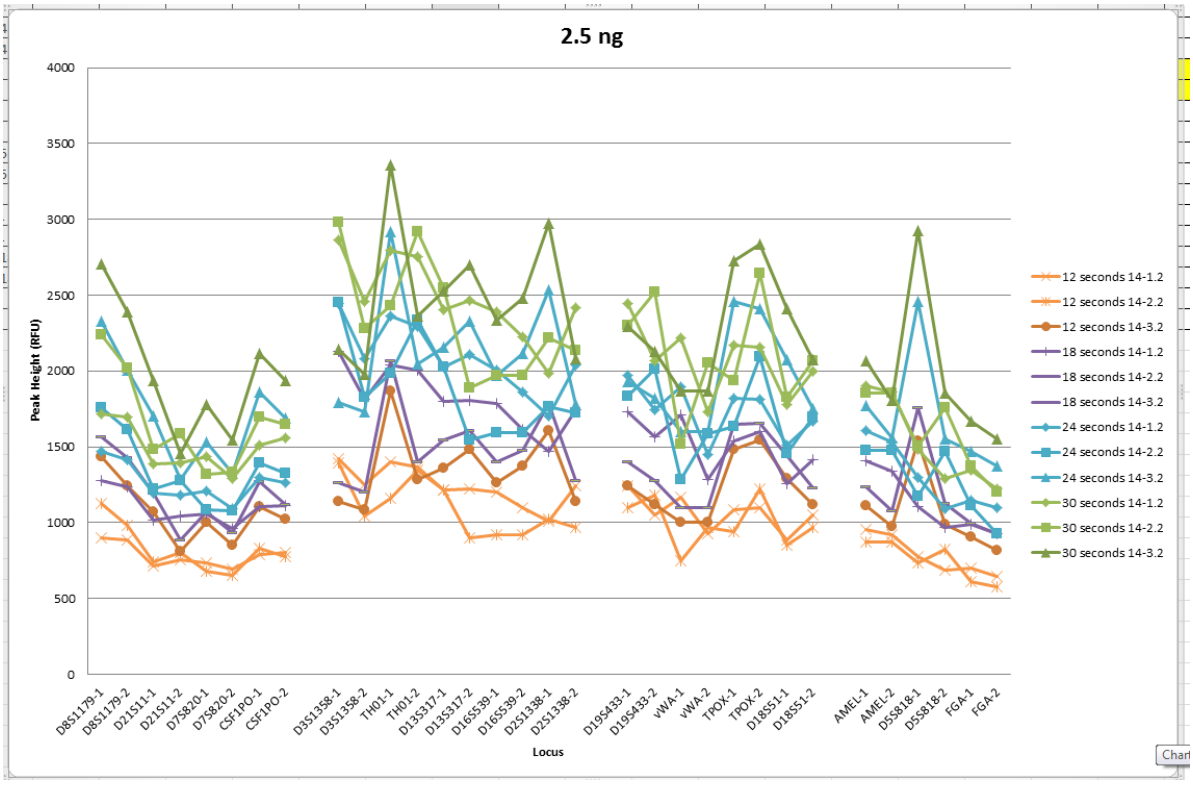


Figure 39: 2ng at 12, 18, 24, and 30 second injection times

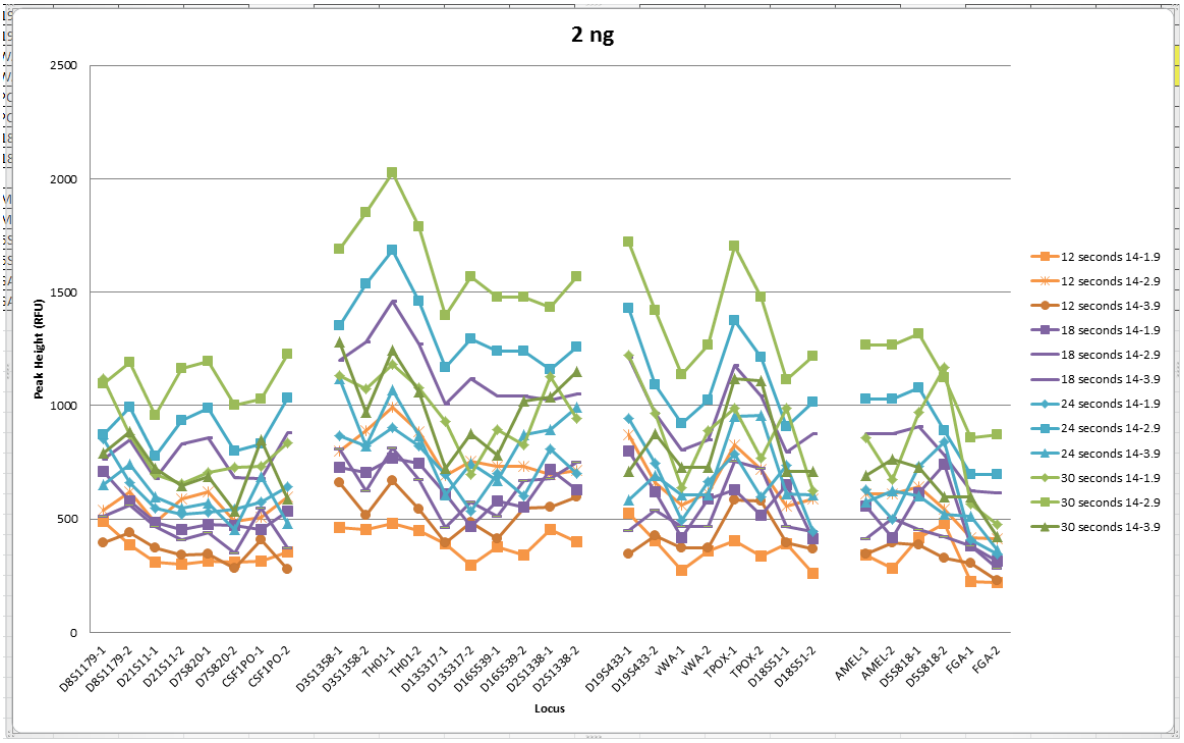


Figure 40: 1.25ng at 12, 18, 24, and 30 second injection times

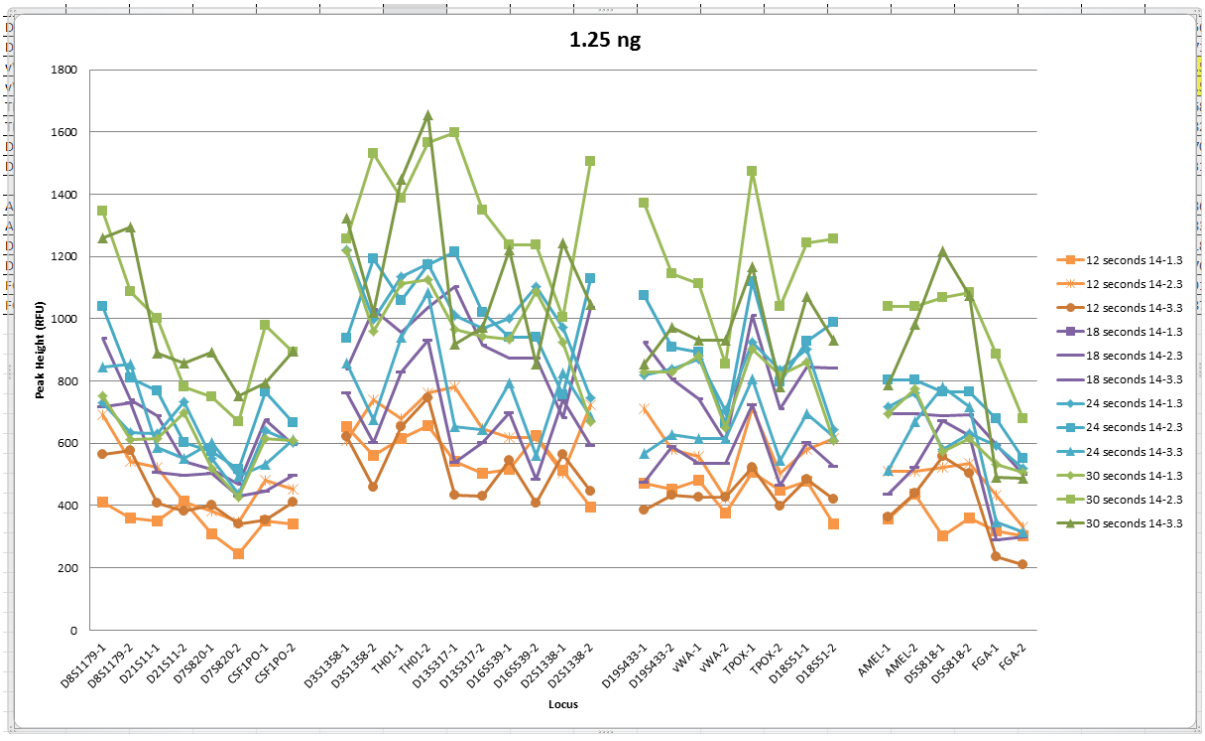


Figure 41: 0.62ng at 12, 18, 24, and 30 second injection times

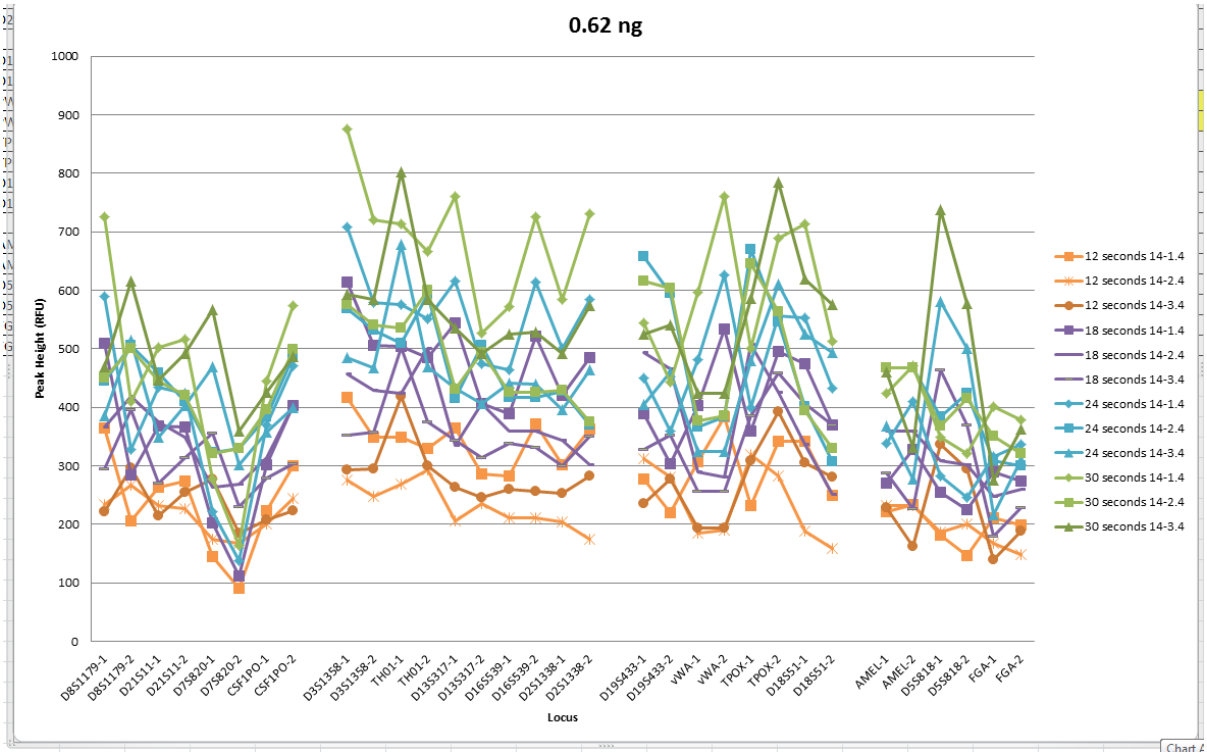
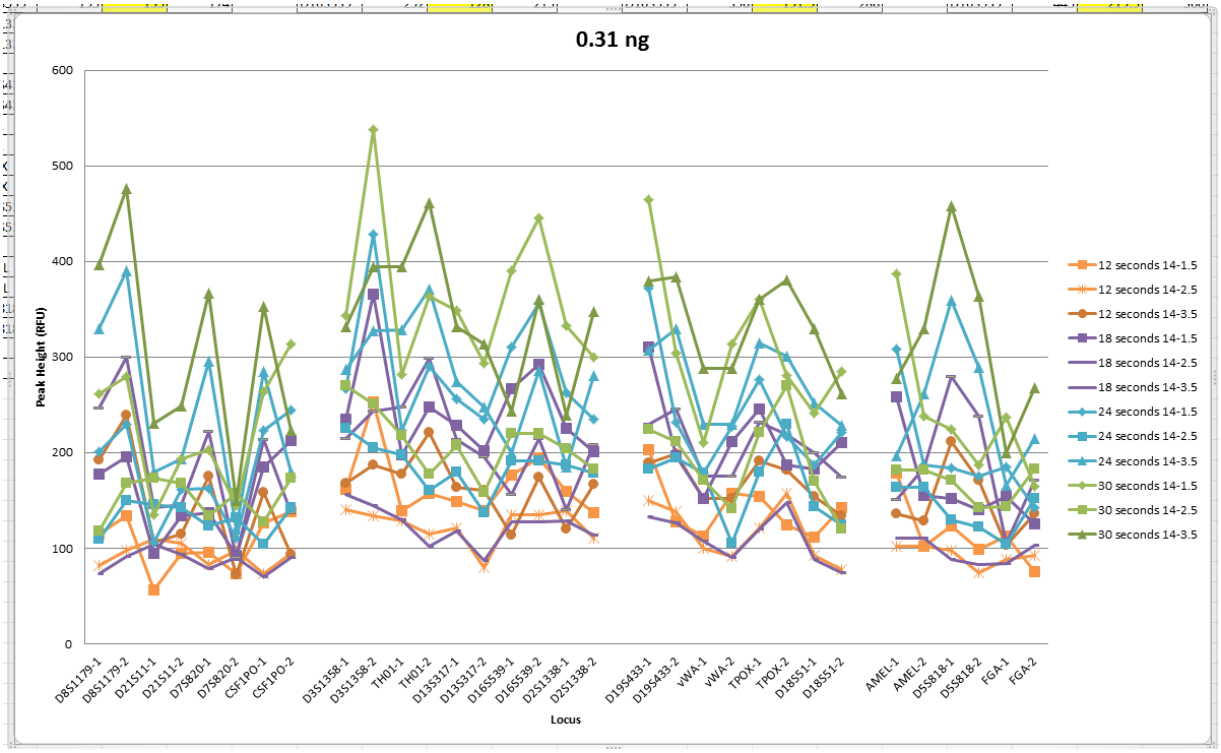


Figure 42: 0.31ng at 12, 18, 24, and 30 second injection times



Analytical Threshold

The analytical threshold was calculated by methods 1 and 2 for each of the injection times. Average, standard deviation, maximum, and minimum peak heights for each dye color (in relative fluorescence units), along with the calculated analytical threshold (RFU) can be seen in Tables 3 - 6.

Blue	All	12 sec	18 sec	24 sec	30 sec
Average	8.42	7.52	7.70	8.76	9.44
Standard Deviation	4.87	3.48	3.70	5.06	6.11
Maximum	46	29	38	42	46
Minimum	5	5	5	5	5
AT= $2(Y_{max}-Y_{min})$	92	58	76	84	92
AT= Avg+3(std)	23.02	17.97	18.81	23.94	27.77

Table 3: Blue dye channel results

Green	All	12 sec	18 sec	24 sec	30 sec
Average	14.46	14.15	14.01	14.35	15.37
Standard Deviation	6.23	5.13	5.36	6.07	7.99
Maximum	61	52	57	49	61
Minimum	5	5	5	5	5
AT= $2(Y_{max}-Y_{min})$	122	104	114	98	122
AT= Avg+3(std)	33.15	29.55	30.09	32.55	39.35

Table 4: Green dye channel results

Yellow	All	12 sec	18 sec	24 sec	30 sec
Average	25.87	25.21	25.59	25.74	26.88
Standard Deviation	9.61	7.58	9.13	9.81	11.35
Maximum	88	81	80	88	88
Minimum	8	9	11	9	8
AT= $2(Y_{max}-Y_{min})$	160	144	138	158	160
AT= Avg+3(std)	54.72	47.95	52.98	55.16	60.93

Table 5: Yellow dye channel results

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

Table 6: Red dye channel results

Sensitivity

In both sensitivity studies, full profiles were obtained at quantities of 5.0ng– 0.31ng, and dropout began to occur at 0.15ng below the given threshold at each injection time.

Sister allele peak height imbalance (<50%) is shown in Table 7 for the first sensitivity study and in Table 16 for the second study. The average peak height, peak height standard deviation, maximum, minimum, and combined peak height average for each injection time in the first study can be seen in Table 8-11 and in the second study Table 12-15. RFU levels were lower than expected for the sensitivity study so they were ran again to see if the RFU levels would be consistent with the first sensitivity studies. Also, when blood samples were ran they were extremely high compared to the first study so that was another reason the second study was conducted.

Locus	Sample	Concentration	Injection Time
D2S1338	14-1	0.15ng	18, 24, 30 sec
D7S820	14-3	0.31ng	All
D13S317	14-1	0.15ng	24 sec
D16S539	14-1	0.15ng	24 sec

Table 7: Sister Allele Peak Height Imbalance (<50%) for Sensitivity Study 1

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	12 seconds													
	14-1				14-2				14-3					
	Average	Std. dev	Max	Min	Average	Std. dev	Max	Min	Average	Std. dev	Max	Min	Combined Average	Combined Std. dev
5ng	996.69	227.06	1419	647	936.19	202.74	1393	577	1358.09	336.13	2059	775	1096.99	255.31
2.5ng	828.47	279.59	1498	437	681.75	283.61	1176	235	1196.41	247.38	1870	808	902.21	270.19
2ng	369.00	78.56	524	219	655.44	138.25	994	414	427.13	111.53	668	229	483.85	109.45
1.25ng	437.56	108.52	658	245	563.91	120.65	780	331	455.09	109.47	745	209	485.52	112.88
0.62ng	275.50	78.01	416	90	223.47	45.10	319	148	258.78	59.87	419	139	252.58	60.99
0.31ng	137.52	37.54	253	-	108.28	23.18	157	73	157.50	37.91	239	72	134.43	32.88
0.15ng	82.71	21.17	151	-	-	-	-	-	71.85	10.89	101	-	77.28	16.03
0.078ng	-	-	-	-	74.00	16.79	102	-	69.00	3.00	72	-	71.50	9.90
0.039ng	60.00	-	60	-	-	-	-	-	-	-	-	-	60.00	-

Table 8: 12-second injection time for Sensitivity Study 1

	18 seconds													
	14-1				14-2				14-3					
	Average	Std. dev	Max	Min	Average	Std. dev	Max	Min	Average	Std. dev	Max	Min	Combined Average	Combined Std. dev
5ng	1432.38	348.56	2126	925	*	*	*	*	1521.75	380.83	2326	863	1477.06	364.69
2.5ng	1125.47	391.80	2006	536	1046.94	419.82	1744	338	1326.66	273.01	2064	888	1166.35	361.54
2ng	572.50	124.26	798	316	941.31	204.88	1462	616	547.97	154.95	935	281	687.26	161.37
1.25ng	*	*	*	*	776.94	171.94	1104	467	575.34	139.89	930	290	676.14	155.92
0.62ng	387.09	114.26	613	112	361.09	74.36	507	247	330.47	71.03	503	180	359.55	86.55
0.31ng	199.06	59.63	366	92	106.00	22.86	156	70	200.31	50.59	300	90	168.46	44.36
0.15ng	97.23	32.62	225	-	76.33	11.95	109	-	81.55	15.96	117	-	85.04	20.18
0.078ng	64.67	1.70	67	-	76.86	17.44	114	-	66.00	6.23	78	-	69.17	8.46
0.039ng	109.00	-	109	-	-	-	-	-	-	-	-	-	109.00	-
* Failed injection, were taken out for calculations														

Table 9: 18-second injection time for Sensitivity Study 1

	24 seconds													
	14-1				14-2				14-3					
	Average	Std. dev	Max	Min	Average	Std. dev	Max	Min	Average	Std. dev	Max	Min	Combined Average	Combined Std. dev
5ng	1646.31	394.98	2444	1089	1588.13	359.55	2449	927	2150.81	534.81	3208	1168	1795.08	429.78
2.5ng	1244.00	425.28	2205	595	1186.50	490.51	2023	379	1903.91	387.27	2922	1284	1444.80	434.35
2ng	662.19	150.13	943	346	1094.31	241.44	1684	695	694.16	182.61	1117	365	816.89	191.39
1.25ng	800.84	202.23	1222	436	870.53	192.71	1214	518	663.75	157.86	1083	317	778.38	184.27
0.62ng	450.81	131.71	708	138	448.91	97.49	670	300	429.19	96.64	679	216	442.97	108.61
0.31ng	231.06	70.26	428	106	158.53	34.42	230	104	263.75	64.43	390	120	217.78	56.37
0.15ng	109.20	37.37	255	-	86.39	15.07	117	-	98.50	22.03	142	-	98.03	24.82
0.078ng	69.67	0.47	70	-	84.22	16.29	111	-	76.63	9.95	96	-	76.84	8.90
0.039ng	94.00	30.54	137	-	70.00	-	70	-	-	-	-	-	82.00	30.54

Table 10: 24-second injection time for Sensitivity Study 1

	30 seconds													
	14-1				14-2				14-3					
	Average	Std. dev	Max	Min	Average	Std. dev	Max	Min	Average	Std. dev	Max	Min	Combined Average	Combined Std. dev
5ng	1950.44	476.29	2862	1225	1970.91	449.69	2982	1202	2574.63	635.21	3944	1436	2165.32	520.40
2.5ng	1434.97	499.73	2606	699	1473.34	610.09	2524	465	2215.13	453.10	3359	1456	1707.81	520.97
2ng	875.38	196.39	1222	477	1340.16	287.58	2027	856	823.47	208.87	1281	421	1013.00	230.94
1.25ng	777.09	199.69	1218	428	1139.72	254.73	1599	671	1000.75	245.26	1656	487	972.52	233.23
0.62ng	549.75	162.93	875	163	452.84	91.49	646	320	524.81	116.34	802	276	509.14	123.58
0.31ng	291.53	90.91	538	135	183.06	39.43	270	118	325.94	76.67	476	150	266.84	69.00
0.15ng	132.17	48.35	319	-	98.71	17.81	125	-	114.90	30.71	185	-	115.26	32.29
0.078ng	87.40	9.69	101	-	87.25	23.39	166	-	85.85	13.79	114	-	86.83	15.62
0.039ng	97.25	42.12	170	-	71.00	-	71	-	-	-	-	-	84.13	42.12

Table 11: 30-second injection time for Sensitivity Study 1

	12 seconds									
	14-1 A				14-1 B					
	Average	Std. dev	Max	Min	Average	Std. dev	Max	Min	Combined Average	Combined Std. dev
5ng	1500.25	359.76	2189	761	1886.75	323.48	2444	1239	1693.50	341.62
2.5ng	1043.69	220.20	1473	697	1076.22	178.02	1454	642	1059.95	199.11
2ng	315.07	106.70	583	187	968.09	165.05	1267	610	641.58	135.88
1.25ng	346.38	87.24	586	210	412.66	83.66	564	236	379.52	85.45
0.62ng	*	*	*	*	252.41	53.40	360	172	252.41	53.40
0.31ng	*	*	*	*	121.68	33.92	188	-	121.68	33.92
0.15ng	76.80	17.08	107	-	79.42	8.78	94	-	78.11	12.93
0.078ng	-	-	-	-	67.00	-	67	-	67.00	-
0.039ng	-	-	-	-	*	*	*	*	-	-
* Failed injection, were taken out for calculations										

Table 12: 12-second injection time for Sensitivity Study 2

	18 seconds									
	14-1 A				14-1 B					
	Average	Std. dev	Max	Min	Average	Std. dev	Max	Min	Combined Average	Combined Std. dev
5ng	2401.56	580.20	3535	1206	3250.09	561.24	4298	2123	2825.83	570.72
2.5ng	1551.63	327.24	2188	1042	1556.41	260.24	2078	926	1554.02	293.74
2ng	468.63	152.04	862	274	1454.34	253.58	1917	910	961.49	202.81
1.25ng	549.69	139.01	940	333	688.25	138.44	940	403	618.97	138.72
0.62ng	*	*	*	*	398.59	81.75	557	261	398.59	81.75
0.31ng	*	*	*	*	189.50	58.58	301	71	189.50	58.58
0.15ng	*	*	*	*	100.94	21.55	141	-	100.94	21.55
0.078ng	86.17	14.51	107	-	75.00	-	75	-	80.58	-
0.039ng	-	-	-	-	-	-	-	-	-	-
* Failed injection, were taken out for calculations										

Table 13: 18-second injection time for Sensitivity Study 2

	24 seconds									
	14-1 A				14-1 B					
	Average	Std. dev	Max	Min	Average	Std. dev	Max	Min	Combined Average	Combined Std. dev
5ng	2956.81	712.36	4279	1489	4396.34	762.36	5742	2906	3676.58	737.36
2.5ng	1971.00	419.38	2762	1323	2052.66	343.41	2706	1221	2011.83	381.39
2ng	631.10	204.47	1153	364	2106.44	367.35	2738	1329	1368.77	285.91
1.25ng	722.03	182.17	1234	457	901.47	185.80	1237	537	811.75	183.98
0.62ng	*	*	*	*	526.00	110.31	747	352	526.00	110.31
0.31ng	*	*	*	*	236.97	73.99	373	89	236.97	73.99
0.15ng	122.75	30.47	189	-	120.52	35.52	186	-	121.64	33.00
0.078ng	91.29	21.22	139	-	92.00	25.46	110	-	91.64	-
0.039ng	69.00	-	69	-	-	-	-	-	-	-
* Failed injection, were taken out for calculations										

Table 14: 24-second injection time for Sensitivity Study 2

	30 seconds									
	14-1 A				14-1 B					
	Average	Std. dev	Max	Min	Average	Std. dev	Max	Min	Combined Average	Combined Std. dev
5ng	3750.53	918.33	5489	1876	5544.63	957.73	7295	3681	4647.58	938.03
2.5ng	2433.56	524.27	3450	1637	2633.63	450.34	3553	1554	2533.59	487.30
2ng	799.17	265.04	1480	447	2620.88	453.99	3444	1642	1710.02	359.52
1.25ng	907.88	231.89	1527	556	1190.41	242.57	1630	709	1049.14	237.23
0.62ng	*	*	*	*	673.22	140.08	960	440	673.22	140.08
0.31ng	*	*	*	*	317.66	100.04	498	115	317.66	100.04
0.15ng	144.38	34.58	205	-	144.15	47.50	231	-	144.27	41.04
0.078ng	108.25	27.55	169	-	101.40	24.64	137	-	104.83	-
0.039ng	-	-	-	-	70.00	-	70	-	-	-
* Failed injection, were taken out for calculations										

Table 15: 30-second injection time for Sensitivity Study 2

Locus	Sample	Concentration	Injection Time
CSF1PO	14-1 B	0.31ng	18, 24, 30 sec
TH01	14-1 B	0.15ng	24 and 30 sec
D19S433	14-1 B	0.15ng	24 and 30 sec

Table 16: Sister Allele Peak Height Imbalance (<50%) for Sensitivity Study 2

Precision

The migration of the 250 bp peak can be seen in Table 17 & 18. The average of both 0.3µL and 0.5µL LIZ 500 was 248.42 and the standard deviation was 0.11 bp. Precision for each locus and each dye channel can be seen in Table 19 & 20 (AMP + and ladder 1 study)~~,~~ and Table 21 & 22 (allelic ladder 2 study).

250bp Migration Study

	248.30
	248.31
	248.34
	248.24
	248.34
	248.40
	248.39
	248.47
	248.45
	248.54
	248.56
	248.61
Average	248.41
Standard Deviation	0.12
Minimum	248.24
Maximum	248.61

Table 17: LIZ 500- 0.3µL: 250 bp peak migration

	248.24
	248.28
	248.33
	248.37
	248.41
	248.47
	248.45
	248.40
	248.54
	248.48
	248.61
	248.50
Average	248.42
Standard deviation	0.11
Minimum	248.24
Maximum	248.61

Table 18: LIZ 500- 0.5µL: 250 bp peak migration

Allelic Ladder 1 and Amplification Positive Precision Study

Loci	STD
D8	0.04516
D21	0.049143
D7	0.052969
CSF1PO	0.063036
D3	0.036455
TH01	0.046782
D13	0.053622
D16	0.059618
D2	0.057762
D19	0.045037
vWA	0.044444
TPOX	0.058658
D18	0.048577
AMEL	0.041209
D5	0.040407
FGA	0.052009

Table 19: Standard deviation at each locus

	Average std
Blue channel	0.054385
Green channel	0.050539
Yellow channel	0.047825
Red channel	0.046409

Table 20: Average Standard deviation for each dye

Allelic Ladder 2 Precision Study

	Average standard deviation		
	1µL IDD	0.5µL IDD	1µL ID
D8	0.0352	0.0389	0.0383
D21	0.0406	0.0412	0.0404
D7	0.0406	0.0406	0.0413
CSF1PO	0.0508	0.0451	0.0444
D3	0.0366	0.0348	0.0358
TH01	0.0353	0.0412	0.0424
D13	0.0449	0.0434	0.0359
D16	0.0416	0.0449	0.0398
D2	0.0436	0.0494	0.0403
D19	0.0406	0.0410	0.0391
vWA	0.0377	0.0403	0.0406
TPOX	0.0478	0.0389	0.0415
D18	0.0436	0.0441	0.0382
AMEL	0.0353	0.0343	0.0454
D5	0.0360	0.0392	0.0394
FGA	0.0423	0.0404	0.0406

Table 21: Standard deviation at each locus

	Average standard deviation		
	1µL IDD	0.5µL IDD	1µL ID
Blue channel	0.0412	0.0413	0.0408
Green channel	0.0406	0.0435	0.0392
Yellow channel	0.0420	0.0418	0.0394
Red channel	0.0404	0.0398	0.0405

Table 22: Avg Standard dev for each dye channel

Contamination

There was no contamination seen between the samples and blanks when the plate was setup by hand. The first plate did not contain all samples when setup by the Janus™ so therefore that plate was not used for this study.

Concordance

Table 23 shows the previously analyzed profiles from the 3130xL that were compared to the samples ran on the 3500xL. The samples that could be visualized were concordant with these samples' profiles.

	Blue								Green										Yellow								Red					
Samples	D8		D21		D7		CSF1PO		D3		TH01		D13		D16		D2		D19		vWA		TPOX		D18		AMEL		D5		FGA	
Blood																																
1	8	13	30	31	8	9	11	12	15	17	6	9.3	10	14	12	14	19	19	13	15	15	16	8	11	15	16	X	X	12	12	21	24
2	11	14	28	30	8	8	11	12	17	17	7	9.3	10	12	12	13	20	20	14	16	18	19	8	11	11	11	X	Y	12	13	21	22
3	12	12	31	32	10	11	10	11	15	15	6	9.3	8	12	10	11	17	19	14	14	15	17	10	12	16	18	X	X	11	12	22	24
4	13	15	29	31	10	12	12	13	17	18	6	9.3	11	11	12	12	17	23	13	14	16	18	10	12	12	18	X	Y	12	12	19	26
5	12	13	28	30	10	11	11	12	15	17	6	9	11	12	9	11	19	20	14	14	16	18	9	10	13	15	X	X	11	13	20	22
6	13	14	29	30	11	12	12	13	15	18	7	9	9	11	13	13	20	22	12	12	17	18	11	12	16	20	X	Y	12	13	24	25
7	13	14	27	29	8	12	12	12	15	17	7	9	9	10	11	13	18	19	13	13	16	17	8	10	14	16	X	X	8	10	19	23
8	9	14	28	30	10	11	12	12	15	17	9.3	9.3	11	13	9	11	18	20	14	15	16	18	8	9	13	15	X	Y	12	14	20	21
9	13	14	30	31	10	10	11	12	17	17	6	8	9	12	12	13	21	22	12	13	16	16	8	8	10	15	X	Y	12	12	20	23
10	10	10	30	30	9	10	11	11	16	17	6	6	11	14	9	11	19	22	12	14	17	17	8	11	15	16	X	Y	13	13	20	22
11	15	15	29	31	10	10	10	12	16	17	8	9	13	14	9	12	14	17	13	14	16	17	8	8	12	14	X	X	11	12	22	25
12	10	13	29	30	9	12	10	10	15	18	7	8	11	12	12	12	20	25	14	14	16	18	8	11	14	14	X	Y	11	11	20	21
13	12	15	30	31	8	10	12	13	16	17	7	9.3	11	11	9	11	21	26	14	15	16	17	6	11	15	15	X	X	11	12	22	27
14	15	15	31	32	8	11	10	11	16	17	6	9.3	11	11	12	12	18	18	14	15	15	17	8	9	15	18	X	Y	11	13	21	24
15	10	14	30	30	10	11	10	11	17	17	6	7	10	11	13	14	19	24	14	15	18	19	8	9	13	15	X	Y	11	12	24	24
16	8	14	28	29	11	11	11	11	15	15	7	9.3	12	12	10	13	21	25	10	13	17	17	10	11	15	17	X	X	8	13	23	24
17	11	14	29	32	8	12	11	12	14	15	6	7	11	11	11	13	17	24	14	14	15	17	8	11	13	15	X	X	10	13	19	23
18	12	13	30	31	8	10	10	13	15	16	7	9.3	9	13	12	13	17	24	13	14	16	18	11	11	14	24	X	X	9	12	20	23
19	10	14	29	30	8	13	10	11	14	18	6	9.3	12	12	11	12	19	19	13	14	18	19	8	8	12	14	X	X	11	12	22	25
20	14	16	28	30	10	10	12	12	16	18	6	7	11	13	11	12	18	21	12	15	17	19	8	11	14	15	X	X	12	12	22	23
21	12	12	30	32	8	10	11	11	14	17	7	7	11	12	9	11	18	20	13	14	17	18	8	8	15	16	X	X	11	13	19	25
22	12	12	30	32	8	10	11	11	14	17	7	7	11	12	9	11	18	20	13	14	17	18	8	8	15	16	X	X	11	13	19	25
23	12	14	29	29	10	10	10	10	14	15	9.3	9.3	11	12	11	11	17	24	14	15	16	19	8	8	12	16	X	Y	9	11	22	23
24	12	14	29	29	10	10	10	10	14	15	9.3	9.3	11	12	11	11	17	24	14	15	16	19	8	8	12	16	X	Y	9	11	22	23
25	10	15	29	33	11	12	10	12	15	16	6	9.3	11	11	9	11	17	26	14	14	17	17	8	11	15	16	X	Y	10	12	18	22
26	10	15	29	33	11	12	10	12	15	16	6	9.3	11	11	9	11	17	26	14	14	17	17	8	11	15	16	X	Y	10	12	18	22
27	13	13	27	31	8	9	11	11	17	18	9	9.3	9	12	11	12	21	23	15	15	17	18	8	8	12	14	X	Y	10	12	23	25
28	13	13	27	31	8	9	11	11	17	18	9	9.3	9	12	11	12	21	23	15	15	17	18	8	8	12	14	X	Y	10	12	23	25
29	9	14	28	29	9	12	11	11	16	18	9	9.3	9	11	12	13	18	25	13	16	17	17	8	9	13	21	X	Y	12	13	19	22
30	9	14	28	29	9	12	11	11	16	18	9	9.3	9	11	12	13	18	25	13	16	17	17	8	9	13	21	X	Y	12	13	19	22
31	12	14	28	30	10	10	11	13	17	18	9.3	9.3	9	11	12	12	19	24	12	15	15	18	8	8	17	20	X	Y	11	13	22	23
32	12	14	28	30	10	10	11	13	17	18	9.3	9.3	9	11	12	12	19	24	12	15	15	18	8	8	17	20	X	Y	11	13	22	23
33	14	14	29	29	11	12	11	12	15	16	9	9.3	10	11	8	11	17	24	13	14	14	18	8	8	11	15	X	Y	10	12	20	21
34	14	14	29	29	11	12	11	12	15	16	9	9.3	10	11	8	11	17	24	13	14	14	18	8	8	11	15	X	Y	10	12	20	21
35	10	13	28	30	9	12	11	12	14	17	6	9.3	12	12	10	12	17	19	13	15	14	17	8	11	13	17	X	Y	11	12	23	24
36	10	13	28	30	9	12	11	12	14	17	6	9.3	12	12	10	12	17	19	13	15	14	17	8	11	13	17	X	Y	11	12	23	24
37	10	15	29	33	11	12	10	12	15	16	6	9.3	11	11	9	11	17	26	14	14	17	17	8	11	15	16	X	Y	10	12	18	22

	Blue								Green										Yellow								Red					
	D8		D21		D7		CSF1PO		D3		TH01		D13		D16		D2		D19		vWA		TPOX		D18		AMEL		D5		FGA	
Saliva																																
38	13	13	30	32	8	11	11	13	15	16	8	9	8	8	11	12	16	19	14	14	16	17	10	10	14	16	X	Y	10	13	20	24
39	9	16	30	32	10	11	12	13	16	17	8	9	9	11	12	12	17	25	13	16	19	19	10	11	17	17	X	Y	11	11	21	25
40	13	13	28	32	8	10	10	11	15	15	9	9.3	8	13	10	12	19	24	13	14	16	17	8	10	15	16	X	Y	10	13	23	24
41	13	15	28	29	9	11	12	12	14	15	8	9.3	11	11	11	13	17	18	14	15	15	19	8	11	14	15	X	X	11	11	22	23
42	13	16	29	30	7	9	10	11	14	14	6	9.3	11	11	9	13	16	20	13	15	16	18	8	8	14	15	X	X	13	15	19	24
43	13	15	29	29	11	11	11	12	15	18	6	9.3	11	12	11	14	20	24	16	16	16	17	9	9	15	17	X	Y	11	11	19	20
44	12	13	29	30	9	9	11	13	17	17	9	9.3	11	12	11	12	17	25	15	16	14	17	8	9	12	15	X	X	10	12	20	21
45	9	13	29	32	8	12	11	12	15	16	8	9.3	11	12	9	13	19	20	13	14	17	18	8	11	14	19	X	X	11	12	21	21

Table 23: 3130xl Sample Profiles

Reproducibility

The heights of each peak, as well as the average peak heights for each peak were recorded (Tables 24-63). The minimum and maximum peak heights were determined per injection and across all injections. Sample 27 had dropout occur at D7S820 and D13S317 for both alleles and it highlighted in yellow in Table 46.

9947A				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	13		7048		5095		4367		5503	
	D21S11	30		6430		4549		3758		4912	
	D7S820	10	11	3121	2836	2240	2027	1777	1629	2379	2164
	CSF1PO	10	12	3814	3263	2747	2360	2266	1949	2942	2524
	D3S1358	14	15	5100	4806	3678	3456	3285	3071	4021	3778
	TH01	8	9.3	5270	5159	3721	3645	3246	3163	4079	3989
	D13S317	11		9903		7309		5984		7732	
	D16S539	11	12	6037	5194	4386	3805	3606	3193	4676	4064
	D2S1338	19	23	4079	3922	2914	2859	2516	2424	3170	3068
	D19S433	14	15	3434	3505	2375	2478	2093	2145	2634	2709
	vWA	17	18	5093	4468	3641	3186	3116	2687	3950	3447
	TPOX	8		9271		6696		5693		7220	
	D18S51	15	19	4208	3860	3002	2738	2499	2341	3236	2980
	AMEL	X		6431		4665		4053		5050	
	D5S818	11		5147		3607		3033		3929	
	FGA	23	24	3146	2683	2269	1956	1866	1571	2427	2070
				2683	9903	1956	7309	1571	5984	1571	9903
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 24: Amp Positive (9947A)

1				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	8	13	19735	17797	18768	16813	14460	12837	17654	15816
	D21S11	30	31.2	15857	15140	14954	14027	11068	10510	13960	13226
	D7S820	8	9	15609	14898	14721	14000	10974	10502	13768	13133
	CSF1PO	11	12	15279	14026	13726	12717	11211	10292	13405	12345
	D3S1358	15	17	17109	16375	16489	15615	12891	12050	15496	14680
	TH01	6	9.3	17251	17593	16358	16118	12396	12499	15335	15403
	D13S317	10	14	14400	13037	13493	12320	10415	9555	12769	11637
	D16S539	12	14	22977	21338	21800	20333	17301	16010	20693	19227
	D2S1338	19		32522		32288		30061		31624	
	D19S433	13	15	20641	18895	19440	17516	14936	13683	18339	16698
	vWA	15	16	21746	19640	20273	18235	15817	14283	19279	17386
	TPOX	8	11	26024	21763	23921	20140	15794	15312	21913	19072
	D18S51	15	16	22996	20318	21468	19170	16943	15151	20469	18213
	AMEL	X		26145		27037		27965		27049	
	D5S818	12		28215		28372		22922		26503	
	FGA	21	24	11988	14755	11484	13943	11997	11106	11823	13268
				11988	32522	11484	32288	9555	30061	9555	32522
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 25: Sample 1 allele calls and peak heights

2				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	11	14	10997	9916	10034	8960	7926	7092	9652	8656
	D21S11	28	30	10235	10211	9054	9024	7116	6999	8802	8745
	D7S820	8		21066		19038		14882		18329	
	CSF1PO	11	12	10564	9727	9463	8880	7777	7178	9268	8595
	D3S1358	17		19718		17827		14625		17390	
	TH01	7	9.3	10182	10199	9110	9123	7225	7337	8839	8886
	D13S317	10	12	10220	10109	9113	9177	7376	7317	8903	8868
	D16S539	12	13	16037	14123	14305	12825	11626	10323	13989	12424
	D2S1338	20		26526		23882		19631		23346	
	D19S433	14	16.2	13531	12309	12234	11272	9848	8999	11871	10860
	vWA	18	19	12395	11513	11119	10386	8899	8275	10804	10058
	TPOX	8	11	13769	13188	12417	11913	10001	9568	12062	11556
	D18S51	11		30896		27307		22156		26786	
	AMEL	X	Y	10746	11134	9917	10064	8159	8088	9607	9762
	D5S818	12	13	12129	11042	10895	9931	8628	7856	10551	9610
	FGA	21	22	11422	11044	10227	9921	8111	7785	9920	9583
				9727	30896	8880	27307	6999	22156	6999	30896
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 26: Sample 2 allele calls and peak heights

3				Run Date: 7/12/13		Run Date: 7/15/13		Run Date: 7/16/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	12		32151		27809		27826		29262	
	D21S11	31	32.2	15846	15755	11823	11595	12042	11863	13237	13071
	D7S820	10	11	16723	15112	12238	11190	12341	11052	13767	12451
	CSF1PO	10	11	16078	14413	12221	11073	12376	10874	13558	12120
	D3S1358	15		32555		31873		33015		32481	
	TH01	6	9.3	25147	23723	19129	18058	20060	19158	21445	20313
	D13S317	8	12	17935	16174	13595	12484	14358	12906	15296	13855
	D16S539	10	11	27208	25077	20621	19013	21682	19596	23170	21229
	D2S1338	17	19	21620	20874	17063	16417	17707	16804	18797	18032
	D19S433	14		32443		31219		31440		31701	
	vWA	15	17	21780	20891	16571	15908	17593	16752	18648	17850
	TPOX	10	12	22755	21912	17263	16625	18588	17437	19535	18658
	D18S51	16	18	19931	19088	15481	14960	16179	15522	17197	16523
	AMEL	X		24378		27103		28149		26543	
	D5S818	11	12	24378	24378	14102	13281	14134	13277	17538	16979
	FGA	22	24	16438	15784	12450	11949	12646	12174	13845	13302
				14413	32555	11073	31873	10874	33015	10874	33015
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 27: Sample 3 allele calls and peak heights

4				Run Date: 7/12/13		Run Date: 7/15/13		Run Date: 7/16/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	13	15	24608	23669	17871	17252	21221	20665	21233	20529
	D21S11	29	31	20225	19539	14311	13775	17411	17008	16544	15941
	D7S820	10	12	17910	17040	12702	12240	15393	14872	15335	14717
	CSF1PO	12	13	17998	16479	13969	12853	16418	15000	16128	14777
	D3S1358	17	18	27392	24473	20501	18150	25967	22837	24620	21820
	TH01	6	9.3	30094	28154	22356	20753	28998	26797	27149	25235
	D13S317	11		32533		29527		32373		31478	
	D16S539	12		32525		32543		32643		32570	
	D2S1338	17	23	24410	20713	18443	16231	23728	20465	22194	19136
	D19S433	13	14	25156	21963	18904	16520	23981	21463	22680	19982
	vWA	16	18	26021	24649	19022	18249	25689	24082	23577	22327
	TPOX	10	12	25292	24518	18842	18004	24885	24258	23006	22260
	D18S51	12	18	24766	21595	18596	16335	24578	21256	22647	19729
	AMEL	X	Y	27766	27670	21078	20915	26357	26123	25067	24903
	D5S818	12		25130		28220		26436		26595	
	FGA	19	26	20250	17269	14604	12655	19132	16503	17995	15476
				16479	32533	12240	32543	14872	32643	12240	32643
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 28: Sample 4 allele calls and peak heights

6				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	13	14	22228	20059	23912	21595	17225	15560	21122	19071
	D21S11	29	30	17389	14692	18913	16012	13226	11300	16509	14001
	D7S820	11	12	12985	11618	14080	12631	9879	8989	12315	11079
	CSF1PO	12	13	11738	10747	12479	11632	9461	8754	11226	10378
	D3S1358	15	18	24782	22511	26996	24689	20329	18552	24036	21917
	TH01	7	9	22634	21383	24665	23347	18103	17190	21801	20640
	D13S317	9	11	18331	17438	19811	19212	14762	14122	17635	16924
	D16S539	13		32618		32571		32162		32450	
	D2S1338	20	22	14373	13181	15621	14469	11887	10887	13960	12846
	D19S433	12	12.2	19367	18527	20686	19862	15538	14854	18530	17748
	vWA	17	18	23854	21114	26167	22965	19012	17078	23011	20386
	TPOX	11	12	20206	18553	24045	20846	15978	15179	20076	18193
	D18S51	16	20	16958	14896	18477	16274	13720	12278	16385	14483
	AMEL	X	Y	26397	25226	28192	27338	21789	20467	25459	24344
	D5S818	12	13	17855	18512	19443	19902	14056	14557	17118	17657
	FGA	24	25	14433	13371	14391	14034	12116	10639	13647	12681
				10747	32618	11632	32571	8754	32162	8754	32618
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 29: Sample 6 allele calls and peak heights

7				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	13	14	15118	13591	15194	13542	11108	9945	13807	12359
	D21S11	27	29	11159	10728	11238	10839	8183	7730	10193	9766
	D7S820	8	12	9766	9239	10005	9395	7180	6740	8984	8458
	CSF1PO	12		15804		15870		12085		14586	
	D3S1358	15	17	17161	16919	17848	17086	13796	13273	16268	15759
	TH01	7	9	15606	15279	15683	15370	12056	11884	14448	14178
	D13S317	9	10	15591	14158	15933	14559	12047	10979	14524	13232
	D16S539	11	13	16834	15942	17567	16219	13303	12444	15901	14868
	D2S1338	18	19	12649	11605	13057	11797	9977	9128	11894	10843
	D19S433	13		27858		27702		21475		25678	
	vWA	16	17	16643	15471	16778	15588	12983	11886	15468	14315
	TPOX	8	10	13362	13041	13853	13545	10442	10184	12552	12257
	D18S51	14	16	12821	12409	13203	12818	9930	9697	11985	11641
	AMEL	X		28226		28043		22743		26337	
	D5S818	8	10	13591	12264	13505	12375	10328	9344	12475	11328
	FGA	19	23	11102	9831	11431	10065	8482	7552	10338	9149
				9239	28226	9395	28043	6740	22743	6740	28226
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 30: Sample 7 allele calls and peak heights

8				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	9	14	18344	15426	17566	14580	13228	10968	16379	13658
	D21S11	28	30.2	9027	8043	8612	7792	6288	5727	7976	7187
	D7S820	10	11	4619	3987	4471	3828	3257	2791	4116	3535
	CSF1PO	12		7711		7525		5906		7047	
	D3S1358	15	17	16741	15775	16324	15219	12566	11714	15210	14236
	TH01	9.3		23224		22648		16863		20912	
	D13S317	11	13	11363	9861	10914	9473	8323	7234	10200	8856
	D16S539	9	11	14268	12653	13898	12270	10506	9304	12891	11409
	D2S1338	18	20	7093	6517	6919	6451	5416	5031	6476	6000
	D19S433	14	15	10689	9182	10346	8843	7931	6805	9655	8277
	vWA	16	18	15956	14602	15274	14056	11634	10589	14288	13082
	TPOX	8	9	12988	11858	12581	11553	9445	8711	11671	10707
	D18S51	13	15	8808	8013	8582	7649	6574	5911	7988	7191
	AMEL	X	Y	20019	19783	19382	18945	14844	14488	18082	17739
	D5S818	12	14	10914	9367	10395	8759	7733	6641	9681	8256
	FGA	20	21	9825	8687	9342	8289	6995	6311	8721	7762
				3987	23224	3828	22648	2791	16863	2791	23224
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 31: Sample 8 allele calls and peak heights

9				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/17/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	13	14					23383	22703	23383	22703
	D21S11	30	31.2					18056	17437	18056	17437
	D7S820	10						30027		30027	
	CSF1PO	11	12					16122	14635	16122	14635
	D3S1358	17						32587		32587	
	TH01	6	8	Failed first 4 studies				28164	26551	28164	26551
	D13S317	9	12					20912	18629	20912	18629
	D16S539	12	13					29591	26075	29591	26075
	D2S1338	21	22					20117	18926	20117	18926
	D19S433	12	13					24931	22353	24931	22353
	vWA	16						32618		32618	
	TPOX	8						32719		32719	
	D18S51	10	15					25371	21775	25371	21775
	AMEL	X	Y					27975	27112	27975	27112
	D5S818	12						25068		25068	
	FGA	20	23					18918	17665	18918	17665
				0	0	0	0	14635	32719	14635	32719
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 32: Sample 9 allele calls and peak heights

10				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	10		31975		31618		25624		29739	
	D21S11	30		29878		28810		20931		26540	
	D7S820	9	10	14669	13545	13947	12997	10109	9193	12908	11912
	CSF1PO	11		26313		24993		18467		23258	
	D3S1358	16	17	19109	18936	18465	18298	14312	13745	17295	16993
	TH01	6		32122		31868		26006		29999	
	D13S317	11	14	15561	14513	14868	13774	11116	10138	13848	12808
	D16S539	9	11	22913	21563	21606	20767	16385	15428	20301	19253
	D2S1338	19	22	17369	16944	16479	16049	12519	12126	15456	15040
	D19S433	12	14	20578	18952	19624	17932	14814	13629	18339	16838
	vWA	17		32188		31938		25913		30013	
	TPOX	8	11	18917	17464	18328	17081	13369	12451	16871	15665
	D18S51	15	16	19805	17263	18755	16384	14160	12112	17573	15253
	AMEL	X	Y	19358	19033	18780	18349	14517	14107	17552	17163
	D5S818	13		27651		27908		22603		26054	
	FGA	20	22	15356	14609	14599	13867	10739	10079	13565	12852
				13545	32188	12997	31938	9193	26006	9193	32188
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 33: Sample 10 allele calls and peak heights

11				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	15		30512		29997		21975		27495	
	D21S11	29	31.2	15493	14834	15038	14652	10734	10420	13755	13302
	D7S820	10		28021		26921		19524		24822	
	CSF1PO	10	12	13926	12924	13634	12601	10119	9418	12560	11648
	D3S1358	16	17	20714	18593	19859	17996	14918	13367	18497	16652
	TH01	8	9	22660	20987	21791	20002	16177	14668	20209	18552
	D13S317	13	14	15714	14594	15026	13846	11055	10370	13932	12937
	D16S539	9	12	22126	20247	21487	19757	16014	14916	19876	18307
	D2S1338	14	17	19785	19173	19362	18714	14384	14023	17844	17303
	D19S433	13	14	21777	19441	21179	18815	15937	14238	19631	17498
	vWA	16	17	19282	17435	18672	16691	13886	12490	17280	15539
	TPOX	8		32381		32305		28484		31057	
	D18S51	12	14	19041	18192	18415	17850	13817	13292	17091	16445
	AMEL	X		25807		26512		26969		26429	
	D5S818	11	12	18255	15922	17472	15368	12801	11297	16176	14196
	FGA	22	25	15461	13726	14705	13181	10780	9750	13649	12219
				12924	32381	12601	32305	9418	28484	9418	32381
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 34: Sample 11 allele calls and peak heights

12				Run Date: 7/15/13		Run Date: 7/16/13		Run Date: 7/17/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	10	13	19405	18426	22916	21653	24238	23017	22186	21032
	D21S11	29	30.2	15345	15094	18903	18479	19900	19614	18049	17729
	D7S820	9	12	13669	13418	16743	15994	18140	17040	16184	15484
	CSF1PO	10		31557		32398		32499		32151	
	D3S1358	15	18	18290	17184	22341	21517	22740	21679	21124	20127
	TH01	7	8	24592	23276	30557	29353	30400	29620	28516	27416
	D13S317	11	12	13551	12609	17129	15500	17223	15775	15968	14628
	D16S539	12		32545		32617		32597		32586	
	D2S1338	20	25	20485	18153	24423	21502	25465	23275	23458	20977
	D19S433	14		32190		32463		32729		32461	
	vWA	16	18	19106	18201	24205	23345	24032	23351	22448	21632
	TPOX	8	11	27899	21268	32235	27003	32354	27462	30829	25244
	D18S51	14		32710		32653		32729		32697	
	AMEL	X	Y	21920	21353	26768	25944	25765	25762	24818	24353
	D5S818	11		27509		25382		25298		26063	
	FGA	20	21	16438	9867	20843	15850	20744	16237	19342	13985
				9867	32710	15500	32653	15775	32729	9867	32729
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 35: Sample 12 allele calls and peak heights

13				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	12	15	12176	11475	11861	11268	9093	8584	11043	10442
	D21S11	30	31.2	11480	11547	11188	11386	8375	8602	10348	10512
	D7S820	8	10	11057	11012	10744	10750	8146	8148	9982	9970
	CSF1PO	12	13	5477	5177	5537	5186	4289	4045	5101	4803
	D3S1358	16	17	12642	11446	12593	11351	10024	8975	11753	10591
	TH01	7	9.3	7333	7008	7050	6875	5597	5326	6660	6403
	D13S317	11		32203		32116		27733		30684	
	D16S539	9	11	11078	10785	10950	10680	8781	8397	10270	9954
	D2S1338	21	26	6447	6180	6261	6051	5041	4885	5916	5705
	D19S433	14	15.2	9738	9453	9646	9254	7755	7378	9046	8695
	vWA	16	17	15387	13979	15128	13727	12119	10709	14211	12805
	TPOX	6	11	6717	7041	6700	7141	5184	5545	6200	6576
	D18S51	15		25257		24754		19669		23227	
	AMEL	X		27276		26805		21416		25166	
	D5S818	11	12	14816	13487	14558	13028	11424	10125	13599	12213
	FGA	22	27	10944	10523	10688	10281	8322	7993	9985	9599
				5177	32203	5186	32116	4045	27733	4045	32203
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 36: Sample 13 allele calls and peak heights

14				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	15		28172		25764		21066		25001	
	D21S11	31.2	32.2	13528	12283	12373	11263	9839	8919	11913	10822
	D7S820	8	11	11620	10759	10761	9936	8474	7886	10285	9527
	CSF1PO	10	11	6848	6501	6288	5981	5038	4771	6058	5751
	D3S1358	16	17	15806	14301	14579	13013	12416	11037	14267	12784
	TH01	6	9.3	9166	8281	8358	7726	6883	6409	8136	7472
	D13S317	11		32404		32128		29409		31314	
	D16S539	12		26119		24230		20466		23605	
	D2S1338	18		18545		17192		14668		16802	
	D19S433	14	15	13154	11332	12046	10399	10037	8694	11746	10142
	vWA	15	17	17969	16726	16523	15190	13782	12879	16091	14932
	TPOX	8	9	8725	8476	8220	7788	6777	6386	7907	7550
	D18S51	15	18	14724	13924	13587	12665	11424	10786	13245	12458
	AMEL	X	Y	18381	18146	16938	16366	14116	13768	16478	16093
	D5S818	11	13	14681	14371	13245	12878	10967	10533	12964	12594
	FGA	21	24	12384	11758	11462	10899	9578	8919	11141	10525
				6501	32404	5981	32128	4771	29409	4771	32404
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 37: Sample 14 allele calls and peak heights

15				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	10	14	8793	7876	8391	7540	6127	5442	7770	6953
	D21S11	30		14176		13368		9613		12386	
	D7S820	10	11	6528	5998	6237	5749	4455	4082	5740	5276
	CSF1PO	10	11	3510	3528	3320	3311	2560	2495	3130	3111
	D3S1358	17		17452		16698		12746		15632	
	TH01	6	7	5060	4669	4878	4439	3585	3267	4508	4125
	D13S317	10	11	13909	12597	13335	12057	10059	9032	12434	11229
	D16S539	13	14	7789	7401	7558	7158	5700	5324	7016	6628
	D2S1338	19	24	4723	4265	4512	4120	3503	3167	4246	3851
	D19S433	14.2	15	6093	6256	5734	5934	4319	4424	5382	5538
	vWA	18	19	10557	9568	10062	8976	7492	6854	9370	8466
	TPOX	8	9	4743	4813	4584	4725	3450	3486	4259	4341
	D18S51	13	15	9058	8534	8348	7984	6366	6055	7924	7524
	AMEL	X	Y	10806	10585	10383	10269	7879	7623	9689	9492
	D5S818	11	12	9035	8205	8416	7670	6127	5708	7859	7194
	FGA	24		13257		12729		9493		11826	
				3510	17452	3311	16698	2495	12746	2495	17452
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 38: Sample 15 allele calls and peak heights

16				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	8	14	17469	15382	12508	11130	9640	8415	13206	11642
	D21S11	28	29	11319	10677	8156	7707	5843	5582	8439	7989
	D7S820	11		16064		11531		8332		11976	
	CSF1PO	11		12371		8923		6842		9379	
	D3S1358	15		30623		22631		17618		23624	
	TH01	7	9.3	8553	7996	6298	5904	4721	4352	6524	6084
	D13S317	12		29809		21548		16481		22613	
	D16S539	10	13	15031	13126	10760	9605	8005	6974	11265	9902
	D2S1338	21	25	7687	6915	5642	4993	4326	3846	5885	5251
	D19S433	10	13	14140	11975	10486	8876	7840	6536	10822	9129
	vWA	17		31605		24420		18230		24752	
	TPOX	10	11	9781	9407	7016	6866	5152	4945	7316	7073
	D18S51	15	17	13157	12145	9389	8638	7272	6639	9939	9141
	AMEL	X		27637		24229		18731		23532	
	D5S818	8	13	14861	13251	10969	9623	8141	7040	11324	9971
	FGA	23	24	11529	10206	8317	7405	6394	5635	8747	7749
				6915	31605	4993	24420	3846	18731	3846	31605
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 39: Sample 16 allele calls and peak heights

18				Run Date: 7/11/13		Run Date: 7/15/13		Run Date: 7/17/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	12	13			20779	18749	21256	19256	21018	19003
	D21S11	30	31.2			15155	14376	15483	14636	15319	14506
	D7S820	8	10			14332	13204	14675	13418	14504	13311
	CSF1PO	10	13			12786	11959	12585	11729	12686	11844
	D3S1358	15	16			17153	15437	18573	16850	17863	16144
	TH01	7	9.3			13890	13210	14748	14233	14319	13722
	D13S317	9	13			13945	12899	15165	13716	14555	13308
	D16S539	12	13	Failed 3 out of 5		22123	20128	23285	20942	22704	20535
	D2S1338	17	24			20339	16922	20713	17306	20526	17114
	D19S433	13	14			20524	18537	21616	19418	21070	18978
	vWA	16	18			22233	21223	23586	22561	22910	21892
	TPOX	11				32328		32434		32381	
	D18S51	14	24			24044	17586	24057	17702	24051	17644
	AMEL	X				23772		23562		23667	
	D5S818	9	12			20139	17767	20947	18714	20543	18241
	FGA	20	23			18266	17215	19175	17782	18721	17499
				0	0	11959	32328	11729	32434	11729	32434
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 40: Sample 18 allele calls and peak heights

21				Run Date: 7/15/13		Run Date: 7/16/13		Run Date: 7/17/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	12		31210		31441		32004		31552	
	D21S11	30	30.2	12808	12263	13220	12776	14871	14199	13633	13079
	D7S820	8	10	12530	11923	12951	12284	14563	13849	13348	12685
	CSF1PO	11		27460		28473		30997		28977	
	D3S1358	14	17	16506	15123	16741	15805	19594	18255	17614	16394
	TH01	7		32310		32340		32591		32414	
	D13S317	11	12	12025	11466	12718	11810	14509	13688	13084	12321
	D16S539	9	11	23235	22005	24570	22874	28105	26198	25303	23692
	D2S1338	18	20	20167	19013	21039	19092	24100	22561	21769	20222
	D19S433	13	14.2	18238	17054	18890	17857	21786	20309	19638	18407
	vWA	17	18	18385	16470	19510	17674	22237	19854	20044	17999
	TPOX	8		32842		32859		33154		32952	
	D18S51	15	16	20659	18842	21549	19517	24417	22223	22208	20194
	AMEL	X		26013		25853		25141		25669	
	D5S818	11	13	15924	15196	17035	16112	18320	17470	17093	16259
	FGA	19	25	14159	13079	15122	13941	16184	15023	15155	14014
				11466	32842	11810	32859	13688	33154	11466	33154
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 41: Sample 21 allele calls and peak heights

23				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	12	14	10656	10602	10321	10198	7193	7029	9390	9276
	D21S11	29		15394		14747		9979		13373	
	D7S820	10		10581		10023		6906		9170	
	CSF1PO	10		11177		10734		7702		9871	
	D3S1358	14	15	13188	12337	12800	11966	9092	8602	11693	10968
	TH01	9.3		15776		14987		10485		13749	
	D13S317	11	12	10865	9891	10416	9588	7407	6708	9563	8729
	D16S539	11		24525		23846		16865		21745	
	D2S1338	17	24	7707	6261	7392	6009	5399	4409	6833	5560
	D19S433	14	15	9086	7485	8660	7258	6101	5081	7949	6608
	vWA	16	19	11812	10931	11428	10693	8083	7510	10441	9711
	TPOX	8		19861		19061		13383		17435	
	D18S51	12	16	9441	8551	9007	8152	6470	5846	8306	7516
	AMEL	X	Y	11735	11579	11383	11156	7972	7874	10363	10203
	D5S818	9	11	8720	8296	8365	7870	5766	5427	7617	7198
	FGA	22	23	7411	6790	7014	6448	5015	4557	6480	5932
				6261	24525	6009	23846	4409	16865	4409	24525
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 42: Sample 23 allele calls and peak heights

24				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	12	14	11952	12035	11616	11838	7737	7797	10435	10557
	D21S11	29		20884		20612		13404		18300	
	D7S820	10		19160		18755		12190		16702	
	CSF1PO	10		18545		18216		12610		16457	
	D3S1358	14	15	15543	14854	15456	14894	10362	9909	13787	13219
	TH01	9.3		20086		19887		12906		17626	
	D13S317	11	12	15268	14090	15216	14009	9935	9170	13473	12423
	D16S539	11		32579		31785		20953		28439	
	D2S1338	17	24	11749	10323	11563	10111	7718	6813	10343	9082
	D19S433	14	15	12032	10610	11905	10396	7865	6844	10601	9283
	vWA	16	19	14032	13303	13843	13196	8991	8688	12289	11729
	TPOX	8		26544		26424		17405		23458	
	D18S51	12	16	14035	12645	13777	12364	9139	8298	12317	11102
	AMEL	X	Y	13231	13134	13200	13045	8696	8626	11709	11602
	D5S818	9	11	11102	10660	10790	10317	6984	6763	9625	9247
	FGA	22	23	10815	9712	10533	9538	6936	6305	9428	8518
				9712	32579	9538	31785	6305	20953	6305	32579
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 43: Sample 24 allele calls and peak heights

25				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	10	15	6524	5718	6328	5579	4692	4063	5848	5120
	D21S11	29	33.2	6101	5932	5874	5666	4333	4188	5436	5262
	D7S820	11	12	6410	6222	6307	6011	4617	4460	5778	5564
	CSF1PO	10	12	6792	6322	6572	6104	5027	4719	6130	5715
	D3S1358	15	16	6869	6643	6787	6450	5069	4783	6242	5959
	TH01	6	9.3	6426	6193	6213	5989	4613	4494	5751	5559
	D13S317	11		13662		13374		9837		12291	
	D16S539	9	11	9253	8773	9075	8654	6767	6483	8365	7970
	D2S1338	17	26	8713	7470	8567	7300	6575	5626	7952	6799
	D19S433	14		16384		16089		12185		14886	
	vWA	17		13504		13209		9814		12176	
	TPOX	8	11	7884	7688	7742	7578	5841	5707	7156	6991
	D18S51	15	16	9320	8771	9054	8576	6909	6534	8428	7960
	AMEL	X	Y	6816	6799	6689	6723	4926	5006	6144	6176
	D5S818	10	12	7254	7546	6951	7274	5024	5333	6410	6718
	FGA	18	22	6707	6443	6465	6278	4778	4681	5983	5801
				5718	16384	5579	16089	4063	12185	4063	16384
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 44: Sample 25 allele calls and peak heights

26				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	10	15	9077	8284	8191	7211	6338	5650	7869	7048
	D21S11	29	33.2	8561	8204	7534	7192	5783	5562	7293	6986
	D7S820	11	12	9069	8812	7994	7708	6267	6071	7777	7530
	CSF1PO	10	12	9730	9515	8500	8361	7005	6763	8412	8213
	D3S1358	15	16	10187	9246	9093	8320	7158	6482	8813	8016
	TH01	6	9.3	11115	10841	9651	9517	7666	7345	9477	9234
	D13S317	11		18352		16135		12811		15766	
	D16S539	9	11	14185	13243	12386	11679	9853	9370	12141	11431
	D2S1338	17	26	13439	11800	11735	10262	9615	8508	11596	10190
	D19S433	14		23983		21126		16717		20609	
	vWA	17		20196		17670		14005		17290	
	TPOX	8	11	12596	12382	11098	11004	8905	8584	10866	10657
	D18S51	15	16	13181	12196	11471	10696	9331	8647	11328	10513
	AMEL	X	Y	10340	10008	9236	8968	7237	6964	8938	8647
	D5S818	10	12	9890	11068	8823	9781	6756	7576	8490	9475
	FGA	18	22	10044	9486	8810	8284	6873	6495	8576	8088
				8204	23983	7192	21126	5562	16717	5562	23983
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 45: Sample 26 allele calls and peak heights

27				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	13		5143		4742		3813		4566	
	D21S11	27	31.2	721	403	639	358	514	289	625	350
	D7S820	8	9	96	71	93		66		85	71
	CSF1PO	11		434		378		339		384	
	D3S1358	17	18	172	394	171	364	130	291	158	350
	TH01	9	9.3	1528	1550	1403	1394	1126	1126	1352	1357
	D13S317	9	12								
	D16S539	11	12	1271	1064	1169	955	965	827	1135	949
	D2S1338	21	23	2146	1790	1913	1645	1680	1384	1913	1606
	D19S433	15	15.2	3089	3135	2821	2920	2363	2461	2758	2839
	vWA	17	18	2690	2324	2460	2128	1989	1763	2380	2072
	TPOX	8		12183		11041		9269		10831	
	D18S51	12	14	604	457	580	421	498	366	561	415
	AMEL	X	Y	4591	4280	4342	4044	3460	3336	4131	3887
	D5S818	10	12	882	787	822	702	674	562	793	684
	FGA	23	15	584	443	552	384	460	346	532	391
				71	12183	93	11041	66	9269	66	12183
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 46: Sample 27 allele calls and peak heights
*Yellow indicates dropout

28				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	13		15375		13487		11114		13325	
	D21S11	27	31.2	8239	7609	7244	6762	5834	5445	7106	6605
	D7S820	8	9	8705	8249	7605	7147	6099	5838	7470	7078
	CSF1PO	11		18538		16135		13702		16125	
	D3S1358	17	18	5490	10412	4866	9348	4030	7825	4795	9195
	TH01	9	9.3	10030	10325	8734	9080	7302	7613	8689	9006
	D13S317	9	12	8571	8110	7677	7155	6330	5914	7526	7060
	D16S539	11	12	11445	10813	10113	9401	8432	7919	9997	9378
	D2S1338	21	23	10516	10207	9223	8939	7796	7713	9178	8953
	D19S433	15	15.2	9834	9434	8669	8407	7323	6982	8609	8274
	vWA	17	18	8836	8033	7705	7036	6391	5786	7644	6952
	TPOX	8		21354		18714		15502		18523	
	D18S51	12	14	10805	10369	9439	9067	7823	7561	9356	8999
	AMEL	X	Y	8467	8143	7599	7275	6204	6017	7423	7145
	D5S818	10	12	8324	9263	7293	8087	6005	6542	7207	7964
	FGA	23	15	7731	7449	6797	6521	5599	5346	6709	6439
				5490	21354	4866	18714	4030	15502	4030	21354
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 47: Sample 28 allele calls and peak heights

29				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	9	14	8524	7083	7797	6336	6206	5099	7509	6173
	D21S11	28	29	7548	7014	6732	6312	5312	4951	6531	6092
	D7S820	9	12	7044	6811	6332	5973	5031	4831	6136	5872
	CSF1PO	11		14206		12586		10394		12395	
	D3S1358	16	18	6465	6409	5862	5790	4757	4722	5695	5640
	TH01	9	9.3	6775	6534	6038	5811	4923	4754	5912	5700
	D13S317	9	11	7902	7421	7065	6600	5775	5438	6914	6486
	D16S539	12	13	9600	8823	8557	7940	7109	6472	8422	7745
	D2S1338	18	25	9036	8324	8141	7483	6728	6286	7968	7364
	D19S433	13	16	8650	8205	7830	7490	6333	6124	7604	7273
	vWA	17		14417		12989		10605		12670	
	TPOX	8	9	7924	8063	7182	7154	5758	5915	6955	7044
	D18S51	13	21	8967	7985	7978	7037	6649	5949	7865	6990
	AMEL	X	Y	7772	7403	7059	6663	5726	5500	6852	6522
	D5S818	12	13	10260	7866	9203	6881	7338	5630	8934	6792
	FGA	19	22	6909	6974	6168	6251	5043	5026	6040	6084
				6409	14417	5790	12989	4722	10605	4722	14417
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 48: Sample 29 allele calls and peak heights

30				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	9	14	7210	5884	7447	6062	5575	4517	6744	5488
	D21S11	28	29	6096	5625	6337	5770	4641	4200	5691	5198
	D7S820	9	12	5549	5198	5717	5339	4154	3905	5140	4814
	CSF1PO	11		12240		12563		9518		11440	
	D3S1358	16	18	5938	5475	6137	5675	4691	4291	5589	5147
	TH01	9	9.3	5059	5600	5209	5813	3966	4397	4745	5270
	D13S317	9	11	5816	5719	6174	5940	4681	4491	5557	5383
	D16S539	12	13	9230	8156	9608	8533	7379	6439	8739	7709
	D2S1338	18	25	8180	7226	8452	7499	6480	5708	7704	6811
	D19S433	13	16	8125	7681	8524	7981	6493	6045	7714	7236
	vWA	17		13591		13890		10652		12711	
	TPOX	8	9	7849	7460	8248	7709	6102	5830	7400	7000
	D18S51	13	21	8197	7326	8552	7575	6524	5776	7758	6892
	AMEL	X	Y	6947	6663	7301	7044	5470	5190	6573	6299
	D5S818	12	13	9014	6888	9325	6994	6974	5189	8438	6357
	FGA	19	22	6819	6267	7085	6513	5232	4849	6379	5876
				5059	13591	5209	13890	3905	10652	3905	13890
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 49: Sample 30 allele calls and peak heights

31				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	12	14	13361	13396	13791	13834	10303	10541	12485	12590
	D21S11	28	30	10508	9911	10890	10361	8121	7594	9840	9289
	D7S820	10		17089		18180		13194		16154	
	CSF1PO	11	13	8238	7937	8569	8410	6491	6252	7766	7533
	D3S1358	17	18	16479	14569	17142	15321	13361	11937	15661	13942
	TH01	9.3		22456		23845		17908		21403	
	D13S317	9	11	14454	13476	15339	14256	11640	10887	13811	12873
	D16S539	12		31223		32043		25803		29690	
	D2S1338	19	24	10429	9318	11175	10053	8490	7565	10031	8979
	D19S433	12	15	12407	10761	12781	11359	9825	8688	11671	10269
	vWA	15	18	15204	14324	16088	14846	12188	11401	14493	13524
	TPOX	8		26660		28131		21292		25361	
	D18S51	17	20	11509	10473	12134	10987	9182	8336	10942	9932
	AMEL	X	Y	15122	14596	15704	15176	11999	11535	14275	13769
	D5S818	11	13	11514	10733	11814	11040	8763	8198	10697	9990
	FGA	22	23	10371	9290	11035	9842	8182	7373	9863	8835
				7937	31223	8410	32043	6252	25803	6252	32043
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 50: Sample 31 allele calls and peak heights

32				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	12	14	12933	12832	11900	11697	9141	9155	11325	11228
	D21S11	28	30	12424	11978	11180	10897	8741	8429	10782	10435
	D7S820	10		22004		20148		15660		19271	
	CSF1PO	11	13	10752	10637	9809	9692	8052	7767	9538	9365
	D3S1358	17	18	17862	16163	16556	15044	12830	11697	15749	14301
	TH01	9.3		27490		25335		19822		24216	
	D13S317	9	11	17079	16345	15705	15187	12353	11979	15046	14504
	D16S539	12		32448		32115		27732		30765	
	D2S1338	19	24	14578	13431	13339	12522	10712	10062	12876	12005
	D19S433	12	15	15202	13974	13939	12968	10868	9980	13336	12307
	vWA	15	18	16298	15293	14691	14099	11427	10828	14139	13407
	TPOX	8		31599		30756		24148		28834	
	D18S51	17	20	14811	13782	13613	12721	10972	10167	13132	12223
	AMEL	X	Y	15149	15151	14117	13891	10871	10838	13379	13293
	D5S818	11	13	13232	12509	11931	11335	9261	8689	11475	10844
	FGA	22	23	13177	11577	12004	10681	9303	8326	11495	10195
				10637	32448	9692	32115	7767	27732	7767	32448
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 51: Sample 32 allele calls and peak heights

33				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	14		20413		18828		14120		17787	
	D21S11	29		21902		19784		14614		18767	
	D7S820	11	12	11331	10331	10294	9194	7673	7031	9766	8852
	CSF1PO	11	12	12947	11667	11508	10645	9007	8381	11154	10231
	D3S1358	15	16	12648	11500	11719	10556	8777	7949	11048	10002
	TH01	9	9.3	12221	12814	11121	11676	8363	8935	10568	11142
	D13S317	10	11	12153	11279	10965	10114	8323	7661	10480	9685
	D16S539	8	11	17038	16489	15558	15030	12024	11568	14873	14362
	D2S1338	17	24	16454	14646	14983	13294	11683	10568	14373	12836
	D19S433	13	14	15813	14103	14340	13055	10900	9955	13684	12371
	vWA	14	18	12823	12005	11490	10888	8817	8372	11043	10422
	TPOX	8		31830		30446		20886		27721	
	D18S51	11	15	16398	14951	14470	13300	11495	10392	14121	12881
	AMEL	X	Y	12526	12022	11521	11016	8722	8319	10923	10452
	D5S818	10	12	13217	12446	11845	11249	8900	8331	11321	10675
	FGA	20	21	12392	6426	11175	6491	8369	7559	10645	6825
				6426	31830	6491	30446	7031	20886	6426	31830
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 52: Sample 33 allele calls and peak heights

34				Run Date: 7/15/13		Run Date: 7/16/13		Run Date: 7/17/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	14		17389		19203		17893		18162	
	D21S11	29		15750		17366		16038		16385	
	D7S820	11	12	7975	7525	8698	8123	8070	7623	8248	7757
	CSF1PO	11	12	10057	9314	10684	9955	9987	9336	10243	9535
	D3S1358	15	16	9511	8273	10714	9287	9665	8444	9963	8668
	TH01	9	9.3	10075	10375	11508	11830	10359	10619	10647	10941
	D13S317	10	11	7971	7431	9091	8278	8108	7555	8390	7755
	D16S539	8	11	14099	13321	15544	14744	14258	13345	14634	13803
	D2S1338	17	24	13608	11625	15142	12852	13714	11830	14155	12102
	D19S433	13	14	13075	11713	14511	13025	13131	11995	13572	12244
	vWA	14	18	11449	10238	12979	11597	11661	10365	12030	10733
	TPOX	8		31111		31929		30948		31329	
	D18S51	11	15	12897	11759	14271	12917	12891	11695	13353	12124
	AMEL	X	Y	10985	10716	12134	11789	10989	10642	11369	11049
	D5S818	10	12	10214	9311	11352	10466	10265	9413	10610	9730
	FGA	20	21	10015	5723	11127	5665	10182	5202	10441	5530
				5723	31111	5665	31929	5202	30948	5202	31929
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 53: Sample 34 allele calls and peak heights

35				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	10	13	20027	17369	18221	15914	14005	12440	17418	15241
	D21S11	28	30	16526	16021	14852	14218	11354	11177	14244	13805
	D7S820	9	12	16473	14945	14584	12995	11424	10467	14160	12802
	CSF1PO	11	12	18332	16581	14553	13341	13309	12035	15398	13986
	D3S1358	14	17	17414	16597	15934	15173	12506	12029	15285	14600
	TH01	6	9.3	19116	18366	16892	16098	13504	12953	16504	15806
	D13S317	12		28657		25599		20314		24857	
	D16S539	10	12	24063	23590	21518	20747	17498	16866	21026	20401
	D2S1338	17	19	23342	22077	20322	19200	16884	16173	20183	19150
	D19S433	13	15	21338	19437	19084	17584	15252	13860	18558	16960
	vWA	14	17	19916	18935	17600	16991	14011	13436	17176	16454
	TPOX	8	11	23282	25704	20260	20531	16768	15987	20103	20741
	D18S51	13	17	22995	21251	20163	18455	16564	15277	19907	18328
	AMEL	X	Y	20661	19929	18885	18276	14877	14359	18141	17521
	D5S818	11	12	19083	18947	17108	17223	13437	13326	16543	16499
	FGA	23	24	17859	12856	15976	13087	12508	11266	15448	12403
				12856	28657	12995	25599	10467	20314	10467	28657
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 54: Sample 35 allele calls and peak heights

36				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	10	13	14658	13008	14160	12421	10838	9471	13219	11633
	D21S11	28	30	12898	12450	12010	11880	8988	8810	11299	11047
	D7S820	9	12	12480	12199	12021	11524	9039	8779	11180	10834
	CSF1PO	11	12	13879	12739	13029	12210	10291	9668	12400	11539
	D3S1358	14	17	12906	12237	12568	12147	9640	9152	11705	11179
	TH01	6	9.3	13276	13042	12649	12574	9680	9545	11868	11720
	D13S317	12		24156		22913		17825		21631	
	D16S539	10	12	18327	17177	17557	16429	13424	12938	16436	15515
	D2S1338	17	19	17190	16522	16586	15732	12902	12478	15559	14911
	D19S433	13	15	16066	15349	15626	14692	11957	11348	14550	13796
	vWA	14	17	14326	13921	13698	13280	10553	10200	12859	12467
	TPOX	8	11	15728	15806	15006	15082	11550	11550	14095	14146
	D18S51	13	17	16394	15738	15410	14942	11997	11830	14600	14170
	AMEL	X	Y	14532	14014	13982	13747	10616	10493	13043	12751
	D5S818	11	12	14653	14878	13914	14049	10606	10700	13058	13209
	FGA	23	24	13167	11634	12558	11121	9628	8409	11784	10388
				11634	24156	11121	22913	8409	17825	8409	24156
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 55: Sample 36 allele calls and peak heights

37				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	10	15	10503	8527	9816	7839	7694	6069	9338	7478
	D21S11	29	33.2	8872	8111	8221	7504	6465	5849	7853	7155
	D7S820	11	12	9306	8958	8503	8316	6718	6497	8176	7924
	CSF1PO	10	12	9882	9501	9056	8736	7335	7202	8758	8480
	D3S1358	15	16	9370	8652	8823	7990	7003	6479	8399	7707
	TH01	6	9.3	10370	9749	9620	9070	7732	7294	9241	8704
	D13S317	11		16848		15642		12523		15004	
	D16S539	9	11	12675	11947	11676	11259	9499	8958	11283	10721
	D2S1338	17	26	12690	11312	11748	10557	9574	8666	11337	10178
	D19S433	14		22432		20887		16966		20095	
	vWA	17		18419		16957		13752		16376	
	TPOX	8	11	11545	10841	10657	10020	8632	8014	10278	9625
	D18S51	15	16	12300	11272	11353	10452	9272	8447	10975	10057
	AMEL	X	Y	9696	9557	9170	8865	7335	7192	8734	8538
	D5S818	10	12	10398	11089	9551	10308	7562	8096	9170	9831
	FGA	18	22	9347	8858	8769	8071	6893	6467	8336	7799
				8111	22432	7504	20887	5849	16966	5849	22432
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 56: Sample 37 allele calls and peak heights

1S				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	13		2281		2140		1618		2013	
	D21S11	30	32.2	1355	1169	1251	1101	928	799	1178	1023
	D7S820	8	11	1331	1115	1229	1047	927	766	1162	976
	CSF1PO	11	13	1126	1117	1030	1031	805	802	987	983
	D3S1358	15	16	1673	1693	1572	1612	1226	1257	1490	1521
	TH01	8	9	1176	1191	1088	1123	843	878	1036	1064
	D13S317	8		3351		3178		2430		2986	
	D16S539	11	12	1483	1364	1396	1311	1062	1018	1314	1231
	D2S1338	16	19	1171	1005	1100	975	854	738	1042	906
	D19S433	4	14.2	1345	1459	1249	1351	977	1037	1190	1282
	vWA	16	17	1569	1495	1480	1385	1139	1090	1396	1323
	TPOX	10		2530		2392		1813		2245	
	D18S51	14	16	1534	1425	1431	1340	1126	1047	#REF!	1271
	AMEL	X	Y	1514	1750	1399	1632	1067	1265	1327	1549
	D5S818	10	13	1531	1256	1404	1134	1089	884	1341	1091
	FGA	20	24	1099	1013	1033	932	795	719	976	888
				1005	3351	932	3178	719	2430	719	3351
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 57: Sample 1S (38) allele calls and peak heights

2S				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	9	16	1050	796	1064	806	778	595	964	732
	D21S11	30.2	32.2	843	895	868	909	612	644	774	816
	D7S820	10	11	791	721	806	745	572	532	723	666
	CSF1PO	12	13	477	408	485	416	362	311	441	378
	D3S1358	16	17	1454	1417	1475	1425	1105	1061	1345	1301
	TH01	8	9	506	518	516	529	385	398	469	482
	D13S317	9	11	1725	1658	1811	1695	1360	1283	1632	1545
	D16S539	12		1677		1727		1303		1569	
	D2S1338	17	25	639	537	661	565	499	420	600	507
	D19S433	13	16	1181	1026	1179	1009	908	762	1089	932
	vWA	19		2562		2544		1930		2345	
	TPOX	10	11	538	478	539	487	414	354	497	440
	D18S51	17		1812		1830		1399		1680	
	AMEL	X	Y	1414	1342	1411	1326	1052	1000	1292	1223
	D5S818	11		1953		1929		1461		1781	
	FGA	21	25	902	692	952	708	674	512	#REF!	637
				408	2562	416	2544	311	1930	311	2562
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 58: Sample 2S (39) allele calls and peak heights

3S				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	13		2988		3025		1837		2617	
	D21S11	28	32.2	1623	1449	1684	1473	1009	878	1439	1267
	D7S820	8	10	1259	1220	1300	1286	785	758	1115	1088
	CSF1PO	10	11	1335	1334	1334	1369	854	877	1174	1193
	D3S1358	15		4329		4465		2733		3842	
	TH01	9	9.3	1495	1491	1518	1543	893	939	1302	1324
	D13S317	8	13	2675	2424	2754	2524	1682	1534	2370	2161
	D16S539	10	12	1958	1806	2020	1894	1241	1192	1740	1631
	D2S1338	19	24	1487	1484	1579	1516	983	951	1350	1317
	D19S433	13	14	2190	1865	2246	1901	1399	1171	1945	1646
	vWA	16	17	2071	1946	2142	1980	1317	1204	1843	1710
	TPOX	8	10	1407	1518	1448	1563	894	982	1250	1354
	D18S51	15	16	1887	1785	1923	1837	1196	1143	1669	1588
	AMEL	X	Y	1987	1885	2061	1963	1259	1171	1769	1673
	D5S818	10	13	1798	1569	1820	1590	1089	971	1569	1377
	FGA	23	24	1523	1445	1538	1483	959	909	1340	1279
				1220	4329	1286	4465	758	2733	758	4465
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 59: Sample 3S (40) allele calls and peak heights

4S				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	13	15	1163	1022	1079	945	795	688	1012	885
	D21S11	28	29	1211	1198	1087	1071	795	798	1031	1022
	D7S820	9	11	1034	927	966	846	705	638	902	804
	CSF1PO	12		1984		1845		1387		1739	
	D3S1358	14	15	1689	1507	1616	1404	1191	1057	1499	1323
	TH01	8	9.3	1133	1215	1070	1104	778	850	994	1056
	D13S317	11		3218		2997		2252		2822	
	D16S539	11	13	1151	1044	1075	981	807	738	1011	921
	D2S1338	17	18	1134	1111	1053	1010	829	805	1005	975
	D19S433	14	15	1861	1748	1738	1635	1313	1237	1637	1540
	vWA	15	19	1400	1419	1305	1294	978	981	1228	1231
	TPOX	8	11	1106	1194	986	1100	767	822	953	1039
	D18S51	14	15	1296	1395	1225	1279	913	977	1145	1217
	AMEL	X		3379		3159		2334		2957	
	D5S818	11		2807		2502		1818		2376	
	FGA	22	23	1323	1244	1240	1153	942	855	1168	1084
				927	3379	846	3159	638	2334	638	3379
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 60: Sample 4S (41) allele calls and peak heights

6S				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	13	15	13634	13100	12827	12372	9103	8716	11855	11396
	D21S11	29		29378		27486		19870		25578	
	D7S820	11		29803		27953		19765		25840	
	CSF1PO	11	12	15149	13352	14055	12394	10446	9427	13217	11724
	D3S1358	15	18	14145	13935	13444	13257	9674	9466	#REF!	12289
	TH01	6	9.3	17377	16876	16455	15886	11819	11684	15217	14815
	D13S317	11	12	16945	16216	16118	15513	11545	11290	14869	14340
	D16S539	11	14	18481	18130	17568	16893	12825	12578	16291	15867
	D2S1338	20	24	13490	12876	12513	11949	9185	8746	11729	11190
	D19S433	16		31338		30474		22785		28199	
	vWA	16	17	16600	15497	15619	14500	11486	10672	14568	13556
	TPOX	9		31509		30393		21782		27895	
	D18S51	15	17	17811	17237	16783	16075	12360	12116	15651	15143
	AMEL	X	Y	18400	18259	17403	17103	12804	12620	16202	15994
	D5S818	11		28250		27756		19786		25264	
	FGA	19	20	15300	13874	14081	13155	10246	9332	13209	12120
				12876	31509	11949	30474	8716	22785	8716	31509
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 61: Sample 6S (43) allele calls and peak heights

7S				Run Date: 7/11/13		Run Date: 7/12/13		Run Date: 7/15/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	12	13	8997	7442	8710	7189	6551	5380	8086	6670
	D21S11	29	30	8151	7221	7796	6958	5961	5217	7303	6465
	D7S820	9		13601		13166		9925		12231	
	CSF1PO	11	13	5821	5102	5623	4912	4507	3858	5317	4624
	D3S1358	17		14805		14279		10950		13345	
	TH01	9	9.3	6333	6394	6124	6166	4653	4800	5703	5787
	D13S317	11	12	9764	8708	9477	8378	7258	6540	8833	7875
	D16S539	11	12	9066	8358	8876	8114	6895	6377	8279	7616
	D2S1338	17	25	5912	4795	5746	4648	4450	3614	5369	4352
	D19S433	15	16	10901	9664	10673	9449	8317	7350	9964	8821
	vWA	14	17	9492	8542	9184	8172	7119	6372	8598	7695
	TPOX	8	9	6815	6285	6635	6089	5149	4755	6200	5710
	D18S51	12	15	9237	8406	8919	8099	7069	6436	8408	7647
	AMEL	X		21547		20636		16222		19468	
	D5S818	10	12	8351	9568	7961	9201	5978	6825	7430	8531
	FGA	20	21	7950	7601	7604	7252	5807	5576	7120	6810
				4795	21547	4648	20636	3614	16222	3614	21547
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 62: Sample 7S (44) allele calls and peak heights

8S				Run Date: 7/11/13		Run Date: 7/15/13		Run Date: 7/17/13			
		ALLELE 1	ALLELE 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	HEIGHT 1	HEIGHT 2	AVERAGE 1	AVERAGE 2
	D8S1179	9	13			14728	12313	20082	16747	17405	14530
	D21S11	29	32.2			12739	11882	17392	15984	15066	13933
	D7S820	8	12			12977	12231	17704	16637	15341	14434
	CSF1PO	11	12			13927	12969	18635	17368	16281	15169
	D3S1358	15	16			16962	15000	22290	19800	19626	17400
	TH01	8	9.3			16923	17036	21903	22509	19413	19773
	D13S317	11	12			15171	14206	19529	18497	17350	16352
	D16S539	9	13	Failed 3 out of 5		19293	17157	24833	22083	22063	19620
	D2S1338	19	20			16667	15231	21305	19943	18986	17587
	D19S433	13	14			17944	15826	22861	20483	20403	18155
	vWA	17	18			15669	14131	20632	18830	18151	16481
	TPOX	8	11			26381	16669	32414	22235	29398	19452
	D18S51	14	19			17322	15544	22240	20463	19781	18004
	AMEL	X				27700		25137		26419	
	D5S818	11	12			14951	11980	19654	15869	17303	13925
	FGA	21				13229		12727		12978	
				0	0	11882	27700	12727	32414	11882	32414
				MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX

Table 63: Sample 8S (45) allele calls and peak heights

Stutter

From the samples that were analyzed in this study, the calculated n-4 stutter ratios for each locus and Applied Biosystems® marker specific stutter ratios are displayed in Table 64. Comparison between the manufacture and calculated stutter percentages plus three standard deviations can be seen in Figure 43. Fifteen percent filter line is bolded.

	Data Points	Average	Min	Max	S.D.	(+3) S.D.	ABI Stutter ratios
D8S1179	253	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%

Table 64: MSP calculated n-4 stutter ratios compared to Applied Biosystems® stutter ratios

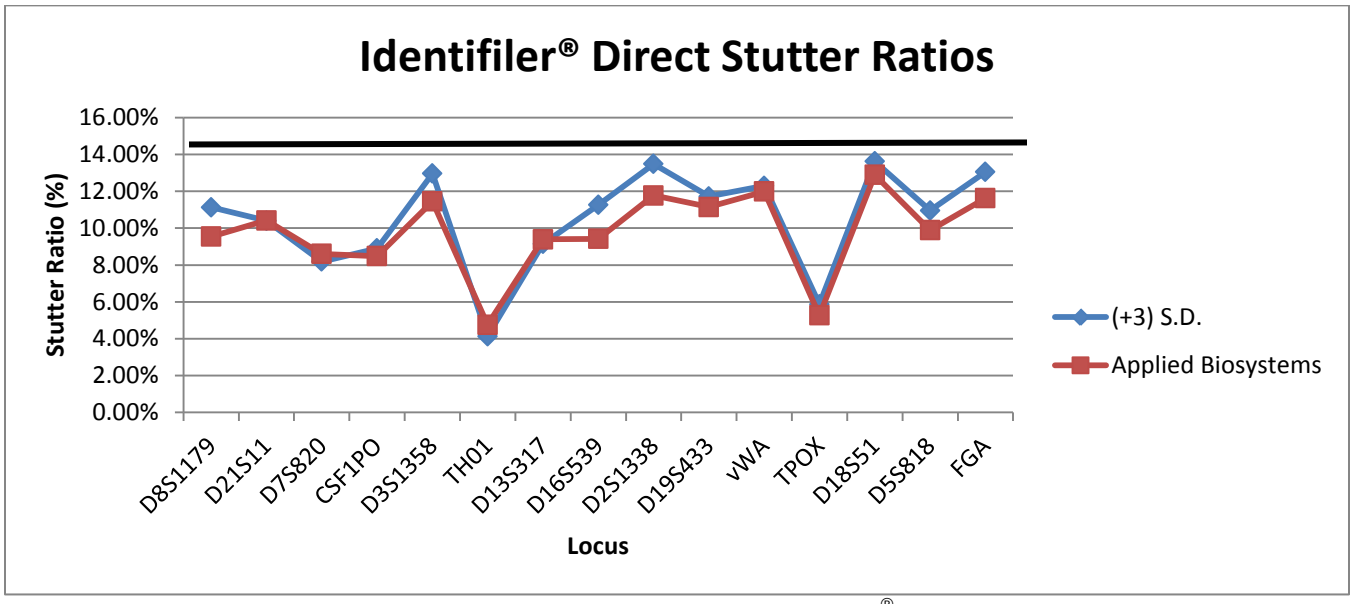

Figure 43: MSP calculated stutter ratios compared to Applied Biosystems®

Heterozygote Balance

All sister allele peak height ratios were calculated for each sample in the reproducibility studies then averaged together. The average peak height ratios are shown in Table 65. Sample 27 showed dropout and irregular peak height imbalance, therefore highlighted in yellow in Table 65. MSPFTC's peak height ratio threshold is 50% for reference samples. Based on this study MSPFTC will continue to use this threshold.

Sample	Average PHR
9947A	91.4%
1	91.8%
2	94.6%
3	94.2%
4	92.4%
5	-
6	92.0%
7	93.6%
8	89.5%
9	92.3%
10	93.8%
11	92.2%
12	91.7%
13	94.8%
14	93.1%
15	93.6%
16	90.2%
17	-
18	90.2%
19	-
20	-
21	93.4%
22	-

Sample	Average PHR
23	91.6%
24	93.4%
25	94.7%
26	94.0%
27	79.8%
28	91.4%
29	92.7%
30	90.2%
31	92.8%
32	94.8%
33	90.7%
34	88.9%
35	93.4%
36	95.6%
37	92.7%
1S	91.2%
2S	88.8%
3S	93.3%
4S	93.1%
5S	-
6S	95.5%
7S	89.4%
8S	88.8%

Table 65: Average Peak Height Ratios for Reproducibility Study

Discussion

LIZ Comparison

Allele migration for most of the loci in this study, with the exception of D7S820, D16S539, D19S433, and TH01, produced concordant results between the 3130xL and the 3500xL. D7S820 and D16S539 showed lower standard deviation on the 3130xL for LIZ 600 v2.0 but were consistent on the 3500xL whereas TH01 and D19S433 had lower standard deviation on the 3500xL for LIZ 600 v2.0 but were consistent the 3130xL. As is illustrated in Figures 4- 35, allele sizing variation across alleles and across loci is reduced when using GeneScan™ LIZ 600 v2.0 Size Standard.

When comparing the data obtained from just the 3500xL, all of the loci, with the exception of D13S317 and TPOX, showed that LIZ 600 v2.0 gave equal or more consistent base pair sizing than samples with LIZ 500. Exceptions are outlined in red in Figures 26 and 31.

Overall, LIZ 600 v2.0 gave equal or more consistent base pair sizing at each allele in each locus than LIZ 500 on the 3500xL. Concordance was obtained between the two different genetic analyzers.

The average standard deviation for each locus on the 3500xl is displayed in Figure 36. Both size standards showed high precision (less than 0.15 bp standard deviation) on both instruments but LIZ 600 v2.0 had improved precision overall.

LIZ Optimization

When determining the amount of LIZ that would be used in the master mix, two things must be considered: pull up in the negative controls and peak heights of the size standard. The 0.1µL did not show any pull up from the size standard but the two other concentrations created pull up in the blue dye.

The size standard peak heights consistently increased as the concentration of size standard was increased without an effect on the samples or ladder peak heights, which is to be expected. The average and minimum peak heights are shown in Table 2. On average, the plate processed by hand showed similar but slightly higher RFU values.

Based on this information the optimal amount of LIZ 600 v2.0 size standard was concluded to be 0.1µL. This concentration gave consistent base pair sizing at each allele in each locus and no extraneous peaks or artifacts, such as pull up, were called in any of the other dyes with a threshold of 50RFU. After completing the reproducibility study with actual blood and saliva card samples, the LIZ 600 v2.0 concentration was increased to 0.2µL to overcome pull up peaks, which were created by the intense allele peaks, in the size standard that was causing improper sizing of the size standard peaks.

Injection Time

All injection times produced full profiles in tested quantities of 5.0ng – 0.31ng dropout began to occur at 0.15ng below the given threshold at each injection time. The average peak height, peak height standard deviation, maximum and minimum for each injection time can be seen in Tables

8 - 11 in the Sensitivity Study Section. All the injection times showed acceptable peak height values therefore, the injection time was determined to stay as the manufacturers recommended injection of 1.2 kilovolts for 24 seconds. No artifacts were called in any of the injection times; all were under the 15% filter. Graphs of each concentration and injection time are shown in Figures 37 - 42.

Analytical Threshold

The analytical threshold was calculated by methods 1 and 2 for each of the injection times. Average, standard deviation, maximum, and minimum peak heights for each dye color in relative fluorescence units (RFU), along with the analytical threshold (RFU), calculated can be seen in Tables 3 - 6.

Method 2, which was recommended by IUPAC, was used to determine the appropriate analytical threshold for each injection time because MSPFTC has used this for all of their other validations and wanted to continue to use this method. The highest values from this method came from the red dye channel because it had the most baseline noise. All dye channel thresholds were chosen by rounding up (in increments of 5) from the red dye channel. Analytical thresholds were set to 60 RFU for 12 and 18 seconds, 65 RFU for 24 seconds, and 70 RFU for 30 seconds.

Sensitivity

In both sensitivity studies, full profiles were obtained in tested quantities of 5.0ng – 0.31ng and dropout began to occur at 0.15ng below the given threshold at each injection time (see analytical threshold results for threshold determined at each injection time). Two exceptions occurred, one

in each sensitivity study, but for the same sample and injection time. One allele dropped out in 0.31ng at a 12 seconds injection time; in the first sensitivity study it was in 14-1 and in the second sensitivity study it was in 14-1 B. Samples had relatively good peak heights and no off scale peaks.

Sister allele peak height imbalance (<50%) is shown in Table 7 for the first sensitivity study and in Table 16 for the second study. The average peak height, peak height standard deviation, maximum, minimum, and combined peak height average for each injection time in the first study can be seen in Table 8-11 and in the second study Table 12-15.

Overall, full profiles could be obtained within the range of 5ng - 0.31ng without dropout occurring or off scale peaks with the exception of one allele dropping out in the 12-second injection time. Heterozygote peak imbalance occurred at 0.31ng and 0.15ng. Dropout occurred consistently at 0.15ng and below.

The second sensitivity study showed evidence that the 5ng and 2.5ng concentrations were switched in samples 14-1 and 14-2, so the data was placed into the table correctly. Sample 14-1 and 14-2 at concentration 2ng was switched and this was determined based on the genotypes so these were also placed in the results table correctly.

Precision

Precision for each locus and each dye channel can be seen in Table 19 & 20 (AMP + and ladder 1 study), and Table 21 & 22 (allelic ladder 2 study). For the allelic ladder 1 study, 26 ladder and

3 amplification positive samples failed because the Janus™ failed to place the sample into the well on the CE plate therefore allelic ladder 2 precision study was conducted. The ladder plate 2 was set up by hand so the sample was insured to be in the well. All ladders passed on this study. All the precision studies at all loci, alleles, and dye channels had a standard deviation lower than the recommended 0.15bp.

Contamination

The contamination study plate was set up by the Janus™ and 15 samples out of 24 (7 amp negatives and 8 extraction positives) were not pipetted into the CE tray from the amplification tray. No contamination was observed in the samples that were injected but this plate was re-setup by hand to insure each sample was placed in the intended well. The amplification negative samples that were in the checkerboard pattern with the extraction positive samples did not show any contamination for the plate set up by hand.

Concordance and Reproducibility

Samples 5, 17, 19, 20, 22, and 5S failed each injection and no profile was shown in Genemapper® ID-X v 1.3. Select samples (3, 4, 9, 12, 18, 21, 34, and 8S) were re-setup and ran with a higher concentration of LIZ to counteract the oversaturation pull up peaks from the samples that were causing the LIZ to size incorrectly. The master mix for plates ran on July 15, 16, and 17 contained 8.8µL Hi-Di formamide with 0.2µL ILS 600, per sample.

For the reproducibility study, each sample that passed, a table was made showing the concordant profiles from each injection that matched the known profile on file. The heights of each peak as

well as the average peak heights for each peak were recorded (Tables 24-63). The minimum and maximum peak heights were determined per injection and across all injections.

For the concordance study, sample 11 had dropout at FGA for one allele. All other samples were concordant with the expected genotypes as previously determined on the 3130xL. See Table 23 for previously analyzed profiles from the 3130xL. A plate was run on the 3130xL with the same samples and oversaturation was also seen on this plate causing the LIZ to fail stating no sizing data.

For both the concordance and reproducibility studies, sample 27 had dropout occur at D7S820 and D13S317 for both alleles and is highlighted in yellow in Table 46. Sample 27 also showed irregular peak heights between loci and imbalance between alleles; this could be due to being a fatal blood sample.

Identical and concordant genotypic results were obtained when comparing the 3130xL to the 3500xL genetic analyzer in 36 out of 45 cases. Due to oversaturation of the CCD camera, the LIZ 600 v2.0 was unable to size correctly each time therefore causing the LIZ to fail stating “no sizing data”. Not all profiles could be compared due to this issue. Although profiles were not generated for these samples, the raw data showed that DNA was amplified and detected by the 3500xL. Samples that were reproducibly seen were identical to the expected profiles.

All other peak heights were fairly consistent with minimal variability with the exception of the injection on July 15 (Reproducibility 3) had consistently lower peak heights. The variances were minimal and did not cause concern that dropout was occurring.

Stutter

From the samples that were analyzed in this study, the calculated n-4 stutter ratios for each locus and Applied Biosystems® marker specific stutter ratios are displayed in Table 64. The stutter percentages provided from Applied Biosystems® were based on treated paper (FTA® cards) for the Identifiler® Direct Amplification kit. These can be found in the AmpFlSTR® Identifiler® Direct PCR Amplification Kit User Guide on page 80. Comparison between the manufacturer and calculated stutter percentages plus three standard deviations can be seen in Figure 43. Fifteen percent filter line is bolded.

The calculated negative stutter values were consistent with the provided Applied Biosystems® stutter percentages. Previously MSPFTC set a 15% stutter filter (for reference samples) when using Identifiler® Direct Kit on the 3130xL. This study has shown that a 15% stutter filter for reference samples is still appropriate when using the 3500xL.

Heterozygous Balance

As stated in the reproducibility discussion section, samples 5, 17, 19, 20, 22, and 5S failed each injection and no profile was shown in Genemapper® ID-X v 1.3. All other peak height ratios were calculated for each sample in the reproducibility studies then averaged together. The average peak height ratios are shown in Table 65.

Samples 27, 34, 2S, 7S, and 8S were all lower than 90% balance but above the sister allele peak height imbalance (<50%). Sample 27 showed dropout and irregular peak height imbalance, therefore highlighted in yellow in Table 65. The results obtained showed that all samples consistently produced balanced peak height ratios within the expected range for heterozygote peaks. The lowest peak imbalance, excluding sample 27, was 88.8%.

Conclusions

Based on all results obtained from this internal validation, the following settings and parameters will be used in the future in Massachusetts State Police Forensic and Technology Center's DNA unit. LIZ 600 v2.0 size standard will be used in the capillary electrophoresis master mix at an amount of 0.2µL per sample. Samples will be injected at the manufacturers recommended injection of 1.2 kilovolts for 24 seconds. When analyzing the data, an analytical threshold will be set to 60 RFU for 12 and 18 second injections, 65 RFU for 24 second injections, and 70 RFU for 30 second injections.

It was observed, in the sensitivity study, that full profiles could be obtained with 0.31ng of DNA and higher with dropout occurring consistently at 0.15ng and below. Heterozygote peak imbalance occurred at 0.31ng and 0.15ng. Also, the heterozygote balance study showed that all samples consistently produced balanced peak height ratios within the expected range. The lowest peak imbalance, excluding sample 27, was 88.8%.

All loci, alleles, and dye channels tested in the precision study had less variation than the recommended 0.15bp for each study. Contamination did not occur in wells, across sample wells, or in wells in a sequential injection using Identifiler® Direct that were run on the 3500xL.

Identical and concordant genotypic results were obtained when comparing the 3130xL to the 3500xL genetic analyzer in 36 out of 45 cases but due to oversaturation of the CCD camera not all profiles could be compared. Although profiles were not generated for these samples, the raw data showed that DNA was amplified and detected by the 3500xL. Samples that were reproducibly seen were identical to the expected profiles.

The calculated negative stutter values (i.e. n-4 stutter) were consistent with the provided Applied Biosystems® stutter percentages. Previously MSPFTC set a 15% stutter filter when using Identifiler® Direct Kit on reference samples on the 3130xL. From the evidence provided from this validation study and after evaluating more samples, MSPFTC will decide whether to use the calculated stutter percentages as a stutter guideline or to continuing to use a 15% filter when using the Identifiler® Direct kit for the 3500xL Genetic Analyzer.

Future Needs

Massachusetts State Police Forensic and Technology Center needs to complete a few more studies to further add supporting evidence for this validation. Another sensitivity study should be conducted with samples on FTA® cards. 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. The signal intensities that we were seeing with our sensitivity studies were

drastically lower than the signal intensities observed when blood samples were used in the concordance and reproducibility studies. Also, a cycle number study should be conducted because of the oversaturation of the CCD camera we were getting with the concordance and reproducibility studies. The cycle number for blood card samples may need to decease so oversaturation doesn't affect the LIZ sizing. Another LIZ optimization may need to be conducted for the Janus™ if the cycle number changes for blood card samples. More non- probative samples should be run to increase the amount of observed alleles for stutter at all loci. This study would help MSPFTC to decide if they will use a 15% filter or if they will use the recommended stutter percentages that were determined from this validation.

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References

1. Applied Biosystems® by Life Technologies™. AmpFℓSTR® Identifiler® Direct PCR Amplification Kit User Guide. Carlsbad, CA: Life Technologies™ Corporation 2012.
2. Applied Biosystems®. Applied Biosystems® 3500/3500xL Genetic Analyzer User Guide. Foster City, CA: Life Technologies™ Corporation. 2010.
3. Applied Biosystems®. Applied Biosystems® 3500/3500xL Genetic Analyzer User Bulletin. Foster City, CA: Life Technologies™ Corporation 2010.
4. "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>.
5. 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].
6. 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.
7. "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>.
8. 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.

9. 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>.
10. 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>.
11. 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.
12. 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.

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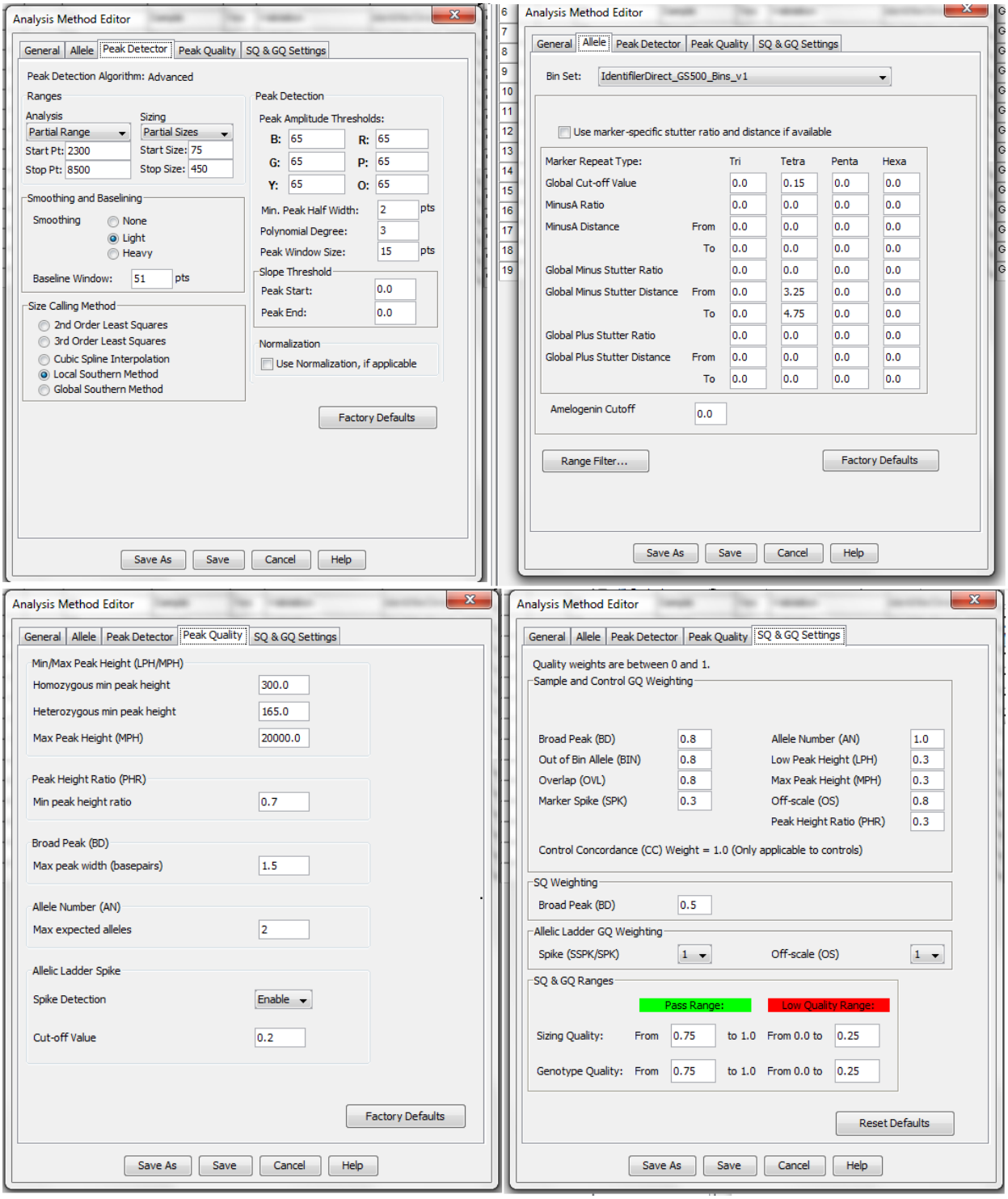
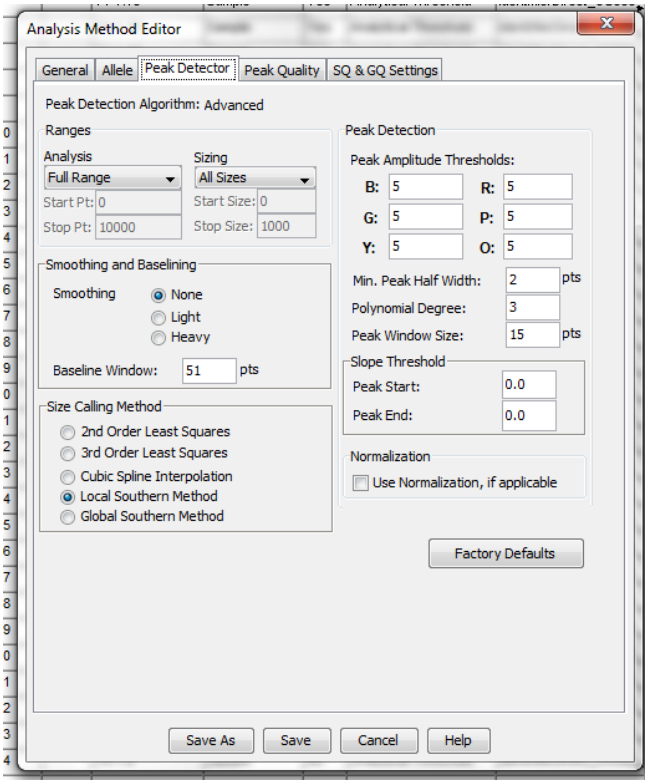
Appendices

Appendix I: Analysis Methods

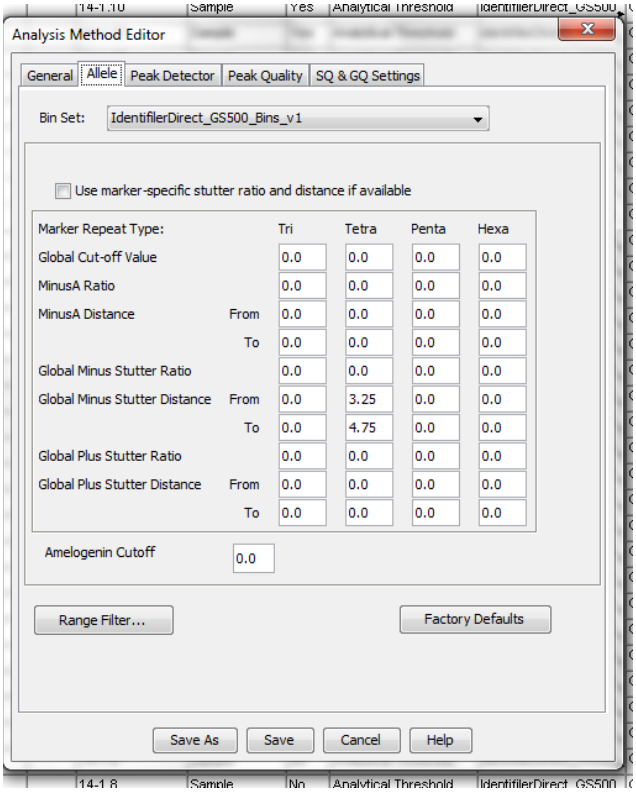
Validation:



Analytical Threshold: (only showing differences from Validation analysis method)



Stutter: (only showing differences from Validation analysis method)

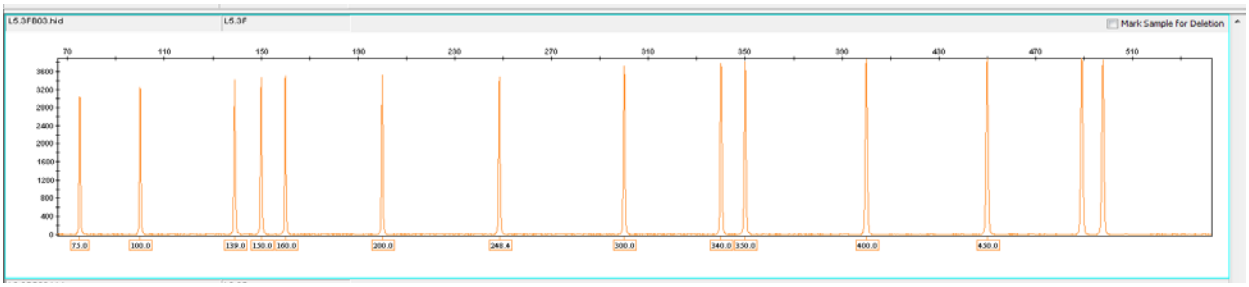


Appendix II: Amplification Parameters

Initial Incubation	95°C	11 minutes
Denature	94°C	20 seconds
Anneal	59°C	2 minutes
Extension	72°C	1 minute
26 cycles of Denature, Anneal, and Extension		
Final Elongation	60°C	25 minutes
Hold	4°C	Forever

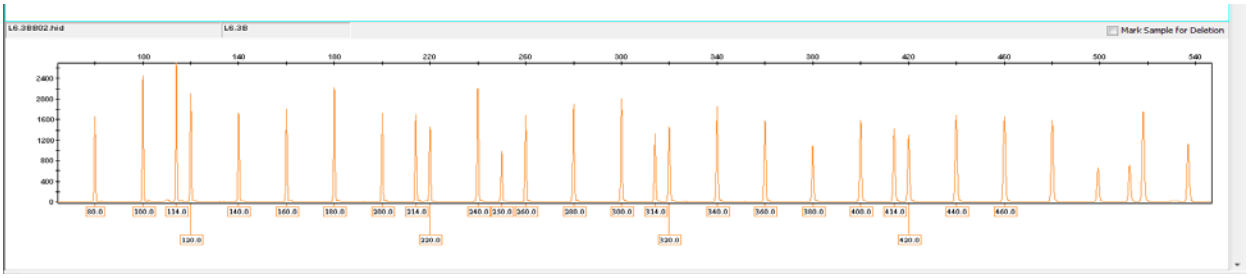
Amplification plate setup master mix recipe: 12.5µl of both Identifiler® Direct master mix and primer set.

Appendix III: LIZ size standard comparison



LIZ 500 size standard

Fragments 35, 50, 75, 100*, 139, 150, 160*, 200*, 250*, 300*, 340*, 350, 400*, 450, 490 and 500*



LIZ 600 v2.0 size standard

Fragments 20, 40, 60, 80, 100*, 114, 120, 140, 160*, 180, 200*, 214, 220, 240, 250*, 260, 280, 300*, 314, 320, 340*, 360, 380, 400*, 414, 420, 440, 460, 480, 500*, 514, 520, 540, 560, 580, and 600

* = in both size standards

Appendix IV: Cost of Supplies and Reagents for 3500xL

Product	Catalog Number	Unit Size	Price
3500xl Genetic Analyzer for Human Identification	4406016	1 system	$183,400.00
3500xl HID Install Kit	4405777	1 kit	$6,804.00
AB Assurance, 3500xl 1 PM HID	ZG11SC3500XLHID	1	$14,637.92
Genemapper® ID-X Software v1.3 (full upgrade)	4473495	1 CD	$2,575.00
Genemapper® ID-X Software v1.3 Client Install Licenses	4473494	10 licenses	$73,600.00
3500xl Capillary Array - 36 cm	4404687	1 array	$1,750.00
DS-33 GeneScan™ Installation Standard	4376911	1 kit	$411.00
LIZ 600 v2.0 Size Standard	4408399	800 reactions	$405.00
POP-4	4393710	384 samples	$198.00
POP-4	4393710	960 samples	$500.00
Cathode Buffer Containers	4408256	4 pack	$154.00
Anode Buffer Containers	4393927	4 pack	$112.00
Cathode Buffer Septa	4410715	10 each	$357.00
Conditioning Reagent	4393718	1 unit	$27.42
Identifiler® Direct Kit	4467831	200 tests	$4,040.00
Identifiler® Direct Kit	4408580	1000 tests	$20,410.00