A VALIDATION STUDY OF THE APPLIED BIOSYSTEMS™ GLOBALFILER™ PCR AMPLIFICATION KIT ON THE APPLIED BIOSYSTEMS™ 3500XL GENETIC ANALYZER

INTERNSHIP PRACTICUM

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Table of Contents

Table of Figures

Table of Tables

Introduction

The main focus of this internship was the validation of the Applied Biosystems™ GlobalFiler™ PCR Amplification Kit on the Applied Biosystems™ 3500xL Genetic Analyzer. This validation study was undertaken at The Harris County Institute of Forensic Sciences (HCIFS). The HCIFS is located in downtown Houston and provides services to the Forensic Pathology Service, the Harris County Sheriff's Office, the Houston Police Department, and the surrounding counties, such as Montgomery County and Fort Bend County. [1]. The HCIFS Genetics Laboratory receives approximately 250 to 300 cases per month, and employs 53 staff members. The lab currently has the Applied Biosystems™ GlobalFiler™ PCR Amplification Kit validated for the Applied Biosystems™ 3130xL Genetic Analyzer but now have purchased the Applied Biosystems™ 3500xL Genetic Analyzer. The change in the detection platform required the laboratory to perform an internal validation as outlined by Scientific Working Group on DNA Analysis Methods Validation Guidelines [2]. The validation study included several key components; known and non-probative evidence samples or mock evidence samples, sensitivity and stochastic studies, precision and accuracy studies, mixture studies, a degradation study and a contamination assessment. These were assessed for the laboratory following the suggestions provided in the SWGDAM 2016 Validation Guidelines for DNA Analysis Methods. These guidelines address the considerations the FBI Quality Assurance Standards for Forensic DNA Testing Laboratories has described for internal validation.

Significance

The Applied Biosystems™ 3130xL Genetic Analyzer currently in use with HCIFS has already been validated, but since Applied Biosystems™ is no longer supporting this genetic

analyzer and will not be performing preventative maintenance for the instrument, HCIFS is now replacing it with the Applied Biosystems™ 3500xL Genetic Analyzer. The project is significant because without a validating this new instrument, the HCIFS laboratory will be unable to employ the Applied Biosystems™ 3500xL Genetic Analyzer for forensic case work. This validation is in accordance with the FBI Quality Assurance Standards, section 8.3.3, which states "A complete change of detection platform or test kit (or laboratory assembled equivalent) shall require internal validation studies" [3].

Background

The Harris County Institute of Forensic Sciences' Crime Laboratory has a long-standing history since 1986 of performing analyses for the Forensic Pathology Service and for local law enforcement agencies. The HCIFS utilizes five forensic disciplines; Drug Chemistry, Firearms Identification, Forensic Toxicology, Trace Evidence, and Forensic Genetics. The Forensic Genetics Laboratory analyzes biological evidence sample such as blood, semen, muscle and bone. The DNA in these samples are extracted, purified and tested to obtain a DNA profile, which then may go on to link evidence found at a crime scene with an individual [1]. The Forensics Genetics laboratory, in alignment with the HCIFS overall goal "strives for continuous improvement using state-of-the-art technology and analytical methods." [1].

The fact that Applied Biosystems is phasing out the Applied Biosystems™ 3130xL Genetic Analyzer, gives HCIFS the opportunity to invest in the Applied Biosystems™ 3500xL Genetic Analyzer, the next generation of genetic analyzer, to help them accomplish this aim. While the Applied Biosystems™ 3130xL Genetic Analyzer is used in labs that need basic sequencing with an expanded capacity, the Applied Biosystems™ 3500xL Genetic Analyzer is easier to use while maintaining the versatility of different laboratory applications within a

process-controlled environment. This includes analysis software that provides real time assessment of the quality of data. [4]. The Applied Biosystems™ 3130xL Genetic Analyzer is more simplistic in its application, has low maintenance and, but is less flexible forensic analysis owing to a limited number of dye channels and capillaries. The Applied Biosystems™ 3500xL Genetic Analyzer can analyze up to six dyes, compared to the Applied Biosystems™ 3130xL Genetic Analyzer five dyes, which is significant because fewer dye channels can lead to lower resolution between peaks and is not able to assay as many loci, whereas more channels mitigate this issue. Unlike the Applied Biosystems™ 3130xL Genetic Analyzer, the Applied Biosystems™ 3500xL Genetic Analyzer has radio frequency identification (RFID) technology. This technology tracks volume of consumables, sample information and records data and administrative information automatically; which eliminates manual data input errors, ensures a more verifiable result and improves instrument troubleshooting. The Applied Biosystems™ 3500xL Genetic Analyzer also uses a solid-state long-life laser, versus the Argon-ion multi-line, single mode laser in the Applied Biosystems™ 3130xL Genetic Analyzer. This solid-state laser reduces the instruments energy consumption thereby extending the lifetime of the instrument. The solid-state laser improves temperature control that results in reduced signal variation between capillaries leading to more consistent data and shorter run times. [4].

Research Design and Methodology

For each component required in an internal validation, peak height and peak size were obtained and used to carry out the studies. Peak height is based on Relative Fluorescence Unit (RFU) distributions. This RFU data comes from the intensity of the fluorescently-labeled amplicons, which corresponds to the amount of DNA in a sample. Peak size is the base pair range, or how long the amplicon fragment is. All alleles are matched to an allelic

ladder that has a specific nucleotide number corresponding with it. This allelic ladder is shown in Figure 1.

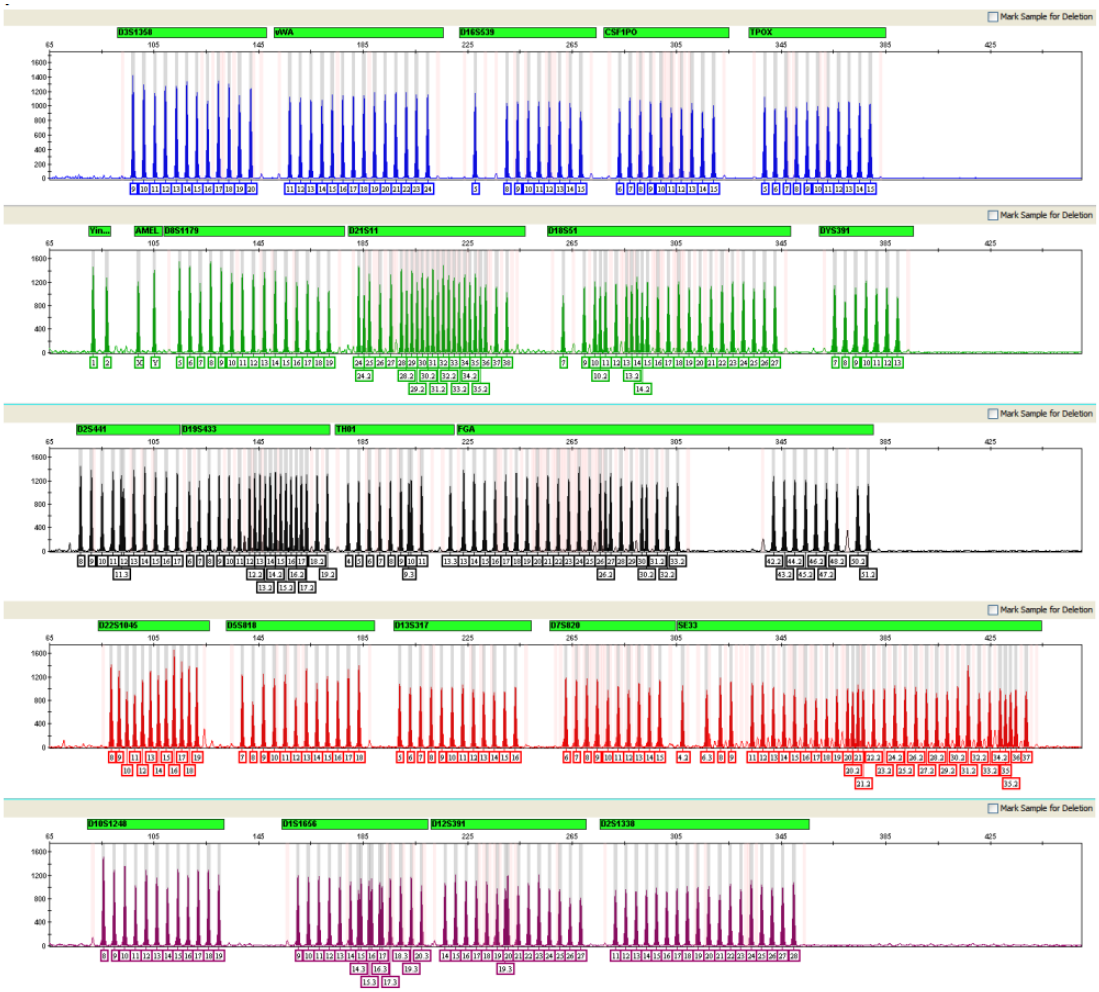

Figure 1: Electropherogram of the GlobalFiler™ Allelic Ladder from the GlobalFiler™ PCR Amplification Kit User Guide. [6]

The full profiles obtained from the mock-evidence samples will also be used to compare to the profiles of the reference samples. The mean and the standard deviation of the peak height, and peak size was then established and these statistics are depicted as a graphical or tabular evaluation. These evaluations will then be incorporated into the Genetics Laboratory's Standard Operating Procedures (SOPs) as methods of interpretation or protocol when using the Applied Biosystems™ 3500xL Genetic Analyzer. The internal validation included studies of sensitivity and stochastic studies, precision and accuracy studies known and non-probative

evidence, or mock evidence samples, mixture studies, a degradation study and a contamination assessment [2]. Sensitivity and stochastic studies are used to determine sensitivity levels of the instrument. From these studies, such details as detection limit, stochastic limit, peak height ratio and signal to noise ratio were determined for the Applied Biosystems™ 3500xL Genetic Analyzer. In the precision and accuracy studies, the aim is to prove conformity to the developmental validation. For the studies to conform, "the sizing precision [should] not exceed a standard deviation of 0.15 base pairs (bp) within an injection" and "the size range [should] not exceed 0.5 bp per injection." which is determined with three times the standard deviation. [10]. These studies will also be able to determine the repeatability, and the reproducibility of the protocols and the instrument. In this sense, repeatability is the determination that the same instrument can be precise and accurate, and reproducibility is the determination that different instruments can be precise and accurate. According to the FBI Standards the definition of precise is that it "characterizes the degree of mutual agreement among a series of individual measurements, values and/or results", and the definition of accurate "is the degree of conformity of a measured quantity to its actual (true) value" [3]. The mixture studies included mixed DNA samples and will assist in establishing mixture interpretation guidelines for this instrument, such as peak height ratios between major and minor contributors. The known and non-probative evidence or mock evidence samples are used to evaluate the currently established methods of sample analysis intended for profiles that will be inputted into a database specifically the HCIFS internal DNA database and the FBI Expanded Population Database from 2015. [2]. Lastly, a contamination assessment will evaluate if the instrument detects any exogenous DNA that may originate from any samples, reagents, the analyst, or the laboratory environment. [2].

Protocols

The DNA from all samples, excepting the organic mock case samples, was extracted with the QIAgen DNA Investigator kit and purified with QIAsymphony® SP instrument. Three DNA samples were extracted organically, and two other samples were extracted differentially. Some of the DNA was then run through a 7500 Real-Time PCR System with the use of the Applied Biosystems™ Quantifiler™ Trio DNA Quantification Kit to determine the concentration of DNA in each sample. Initially, the standard dilution series was made, which are 5 standards at the concentration of 50 ng/µL, 5 ng/µL, 0.5 ng/µL, 0.05 ng/µL, and 0.005 ng/µL. These were created from the stock and dilution buffer that is included in the kit. For the next step, 8 µL of Quantifiler™ Trio Primer Mix was mixed with 10 µL of Quantifiler™ THP PCR Reaction Mix per sample and 18 µL of this master mix was aliquoted into each well. 2 µL of each sample was also pipetted into corresponding wells. [5]. The DNA then underwent Polymerase Chain Reaction (PCR) utilizing the Applied Biosystems™ GlobalFiler™ PCR Amplification Kit. After vortexing, 7.5 µL of the GlobalFiler™ Master Mix was combined with 2.5 µL of the GlobalFiler™ Primer Set in each well. Then 13 µL of TE^{-4} buffer and 2 µL of each sample was pipetted into the corresponding wells, and then placed into the Applied Biosystems Veriti™ 96- Well Thermal Cycler. The instrument set-up for PCR conditions include the initial incubation step is 95°C for 1 minute, the denature and annealing/extending step is 94°C 10 seconds then 59°C for 90 seconds for 29 or 30 cycles. The final extension is at 60°C for 10 minutes, and then the final hold is at 4°C. [6]. This protocol can be seen in Table 1. Taking into consideration that this validation will be using the Applied Biosystems Veriti™ Thermal Cycler and the Applied Biosystems™ 3500xL Genetic Analyzer. The validation team decided to also try 28 cycles due to concerns of blowout in the 29 and 30 cycles. This in light of a technical note from ThermoFisher Scientific recommending 28 cycles on the Applied Biosystems™ 3130xL Genetic Analyzer.

While there is no technical note regarding the Applied Biosystems™ 3500xL Genetic Analyzer, the note does state that "the results obtained and conclusions generated are highly likely to be applicable to other HID STR chemistries and capillary electrophoresis instruments". [7] This conclusion is further backed by other internal validations performed by other laboratories. One lab also used a Veriti® 96-Well Thermal Cycler using 28 cycles for GlobalFiler™ PCR Amplification Kit on the 3500xL Genetic Analyzer. [8], and referenced a UNT Health Science Center internal validation where "blood and buccal samples were 27 and 28 cycles, respectively" using a GeneAmp™ PCR System 9700 were analyzed on the 3500xL Genetic Analyzer. [9].

Table 1: PCR parameters and conditions from GlobalFiler™ PCR amplification kit user guide, for which 28 cycles was used [6].

Initial incubation step	Cycle (29 or 30 cycles)		Final extension	Final hold
	Denature	Anneal/Extend		
HOLD	CYCLE		HOLD	HOLD
95°C, 1 minute	94°C, 10 seconds	59°C, 90 seconds	60°C, 10 minutes	4°C, Up to 24 hours[1]

The amplicons were then run through capillary electrophoresis using the Applied Biosystems™ 3500xL Genetic Analyzer. Genotype analysis was completed using the GeneMapper™ ID-X Client Version 1.4. The specifics regarding specimens examined and experimental designs of the various validation components is outlined in detail below.

Specimens Examined

All the samples used were pre-existing as per the IRB exemption. Saliva and bloodstain samples were collected from staff members, liquid semen samples were pre-purchased, and swab samples were collected from cups, keyboards or phones. The samples that were chosen was due to the fact that these samples were used in the previous 310xL Genetic Analyzer

validation. Also included in the studies are the Applied Biosystems™ Quantifiler™ THP DNA Standard, which is "used to generate the DNA quantification standards dilution series". This "consists of pooled human male genomic DNA". [5]. TE^{-4} Buffer was used as negative controls, and all the positive controls were the Applied Biosystems™ DNA Control 007, which is "A positive control for evaluating the efficiency of the amplification step and STR genotyping using the GlobalFiler™ kit Allelic Ladder." [6]. The profile for the positive control is shown in Figure 2 below.

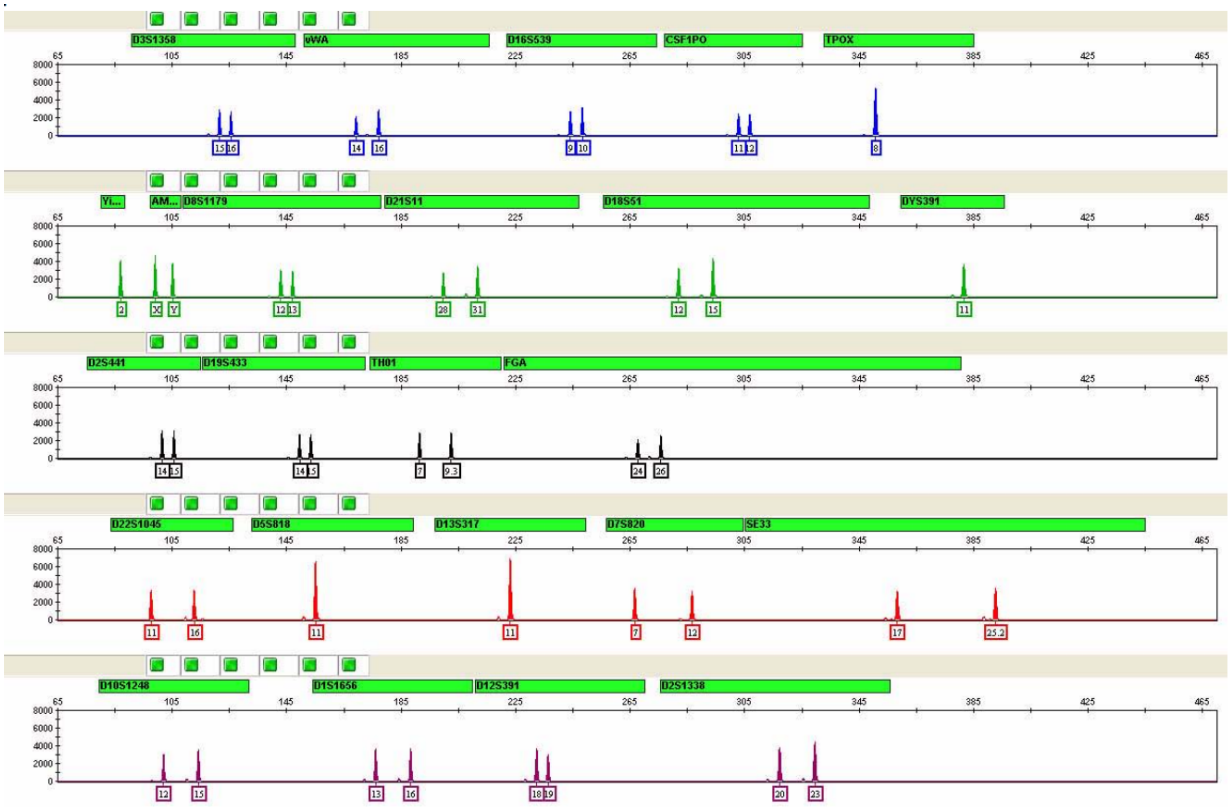

Figure 2: Positive control 007 (1 ng) profile from GlobalFiler™ PCR Amplification Kit User Guide [6].

Analytical Threshold

An analysis method was created on GeneMapper™ ID-X to detect all peaks above 1 RFU, which included the background noise. The analytical threshold was found using this RFU data from all the negatives and reagent blanks and applying them to the Limit Of Detection (LOD) or the Limit Of Quantitation (LOQ). Limit of Detection has been defined by the

International Union of Pure and Applied Chemists (IUPAC) as "the smallest measure that can be detected with reasonable certainty" [11]. IUPAC does not have a definition for Limit of Quantitation, however many internal validation studies define it as "the estimated limit in which the signal is not only reliably detected but also the peak height is reliably measured" [12].

These equations are as such:

$$\text{LOD} = \text{Mean RFU value} + 3 \text{ X SD}$$

$$\text{LOQ} = \text{Mean RFU value} + 10 \text{ X SD}$$

The standard deviation is found with this equation:

$$SD = \sqrt{\frac{\Sigma(x-\mu)^2}{N}}$$

Where x represents each RFU value, μ is the mean of all the RFU value, Σ is the sum, and N is the number of values.

Sensitivity, Stochastic and Stutter Study

Two samples (VD280 and VD285) were used for the sensitivity study. These samples were chosen because these samples were also used for the internal validation study for the Applied Biosystems™ 3130xL Genetic Analyzer, and therefore would be easy to compare stochastic threshold and stutter ratios [13]. They were quantified and each was diluted to 2 ng, 1.5 ng, 1ng, 750pg, 500pg, 250pg, 125pg, 62.5pg, 31.25pg, 15.625pg, and 7.81pg [6]. Each dilution series was pipetted into two well plates in duplicate. They were then re-quantified to confirm their concentrations and then each plate was amplified separately, one at 28 cycles and one at 29 cycles. Amplified products were combined with GeneScan 600 LIZ and deionized formamide then were injected for 22, 23 and 24 seconds on the Applied Biosystems™ 3500xL Genetic Analyzer.

After analyzing, the stochastic threshold was first determined. The PHR data was used from heterozygous peaks that do not have dropout or are blown out. The stochastic threshold was determined using a multinomial logistical regression of two variables, peak height and percent dropout. The equation for logistic regression is as such:

$$y = \frac{L}{1 + Ae^{-Bx}}$$

Where L equals 1, A is $e^{y-intercept}$ and B is equal to the equation below.

$$B = inverse\ (\ln\left(\frac{Y}{1-Y}\right))$$

Where Y is the percent dropout of the inputted data.

The percent profile was also calculated to determine when dropout occurred. While this is not a typical method of calculating stochastic threshold, HCIFS uses this method because in case work samples and low-yield DNA, evaluating percent dropout is important when comparing it to peak height. [14].

The stutter ratio from 1ng to 7.81pg was calculated with the equation:

Stutter Ratio = Stutter PH/ True PH

Then the average stutter ratio that was obtained was added to three times the standard deviation and compared to the manufactures ratio and adjusted in GeneMapper™ ID-X stutter software accordingly. The maximum ratio, number of forward stutter and number of minus 8 stutter was also determined.

Accuracy and Precision

There were 10 samples (VD457, 477, 320, 344, 170, 474, 286, 363, 186, and 396) that were amplified separately by two different analysts two times for the reproducibility and repeatability study, and 10 samples injected 5 times each for the precision and accuracy study. GeneMapper™ ID-X was used to find the average base pair size, maximum and minimum base pair size, the standard deviation and 3 times the standard deviation for each

precision sample and locus, and the alleles for each locus were compared between each amplification for reproducibility and repeatability. It is also important to note that while most laboratories use the GlobalFiler™ Allelic Ladder to calculate accuracy and precision, the FBI standards do not specify what kind of samples to use for the study. Therefore, as these samples were also used for the 3130xL genetic analyzer validation, it was prudent to use these samples again, so that concordance between the two different instruments can be determined.

Mixture Study

The mixture study included 4 biological samples (VD160, VD286, VD173, and VD300) in 3 different mixtures at 5 ratios. These ratios were replicated twice. These samples were chosen because the previous internal validation for the Applied Biosystems™ 3130xL used these samples, and therefore it would be simpler to compare ratios between the two studies [15]. Table 2 below outlines how each of these references were mixed. The mixtures were analyzed in GeneMapper™ ID-X and then the ratios between major and minor contributor were calculated.

Table 2: Mixture study with number of contributors, samples used and mixture ratio.

Contributors	**Sample**	**Mixture Ratio**				
2	VD160	10	5	3	2	1
	VD286	1	1	1	1	1
3	VD160	10	5	3	2	1
	VD286	1	1	1	1	1
	VD173	1	1	1	1	1
4	VD160	10	5	3	2	1
	VD286	1	1	1	1	1
	VD173	1	1	1	1	1
	VD300	1	1	1	1	1

Degradation Study

There were 3 single source samples that were degraded in an autoclave for 30, 60 and 90 minutes for the degradation samples [16]. This can be seen in Table 3. Each sample was quantified before degradation to determine the concentration of DNA before and after degradation, and how much they were degraded by.

Table 3: Degradation sample names and how many minutes each were degraded in an autoclave.

Sample name	Minutes in Autoclave
VD345	30
VD205	60
VD441	90

First the samples were quantified and diluted to an equal 140 ng/µl. After autoclaving, they were quantified, amplified in triplicate at 28 cycles and injected at 24 seconds. They were then analyzed on GeneMapper™ ID-X to obtain the profiles of each sample and compare them to the reference samples.

Non Probative-Mock Evidence Sample Evaluation

The mock evidence samples included a total of 11 samples, two that were organically extracted samples and two differential samples, then three pre-collected touch DNA swabs, two blood samples and two saliva samples that were extracted via the QIAgen DNA Investigator kit and purified with the QIAsymphony® SP instrument. Table 4 shows the names of the samples, the type of sample and how the DNA was extracted.

Table 4: Case type samples used with sample name, sample type and extraction type

Sample name	Sample Type	Extraction Type
VD472	Saliva	Organic
VD83	Blood	Organic
1A1-NS	Non-Sperm	Differential
1A1-S	Sperm	Differential
11A1-NS	Non-Sperm	Differential
11A1-S	Sperm	Differential
VD474	Saliva	QIAgen
Q-479	Saliva	QIAgen
K9	Blood	QIAgen
K10	Blood	QIAgen
DY-Keyboard	Touch DNA	QIAgen
SK-Cup	Touch DNA	QIAgen
SK-Phone	Touch DNA	QIAgen

The mock evidence samples were then analyzed and compared to known profiles to ensure concordance.

Contamination Assessment

Contamination was assessed based on the number of reagent blanks and negatives that were created throughout the study. 24 reagent blanks and negatives were analyzed, which included 10 reagent blanks made specifically for the contamination study. After the first analysis, the 10 reagent blanks were re-made and then all the samples were ran again under different

conditions. The contamination assessment utilized GeneMapper™ ID-X software to determine if any peaks fell above the analytical threshold in the negative controls and reagent blanks.

Results

While I was responsible for most of the studies; those being analytical threshold, 29 cycles sensitivity study, 29 cycle stutter study, precision and accuracy, mixture, case type, contamination and degradation; A second analyst and the validation manager were responsible for the 28 cycles sensitivity study, 28 cycles stutter study, the concordance between the 3130xL and the 3500xL, and stochastic threshold.

Analytical threshold

The first 24 reagent blanks and negatives data was exported to Microsoft® Excel where the means and standard deviations of the noise peak heights were calculated and sorted by dye channel. From the mean and standard deviation, the LOD and LOQ were found. The results of the negative LOD and LOQ are shown in Table 5 below.

Table 5: The mean, standard deviation, LOD and LOQ of all the peaks above 1 RFU in the reagent blanks and negative samples. (N=3025)

Color	Mean	Standard Deviation	LOD	LOQ	Final
Blue	10.0449587	2.9008007	18.74736	39.05297	40
Green	17.1370492	4.87820547	31.77167	65.9191	65
Yellow	8.87525355	2.60374646	16.68649	34.91272	35
Red	13.6218075	3.84476825	25.15611	52.06949	55
Purple	15.3442838	4.3385785	28.36002	58.73007	60

The LOD and LOQ were evaluated using reference samples and the negative controls, and it was determined that when applying the LOD several false peaks remained in the

GeneMapper™ ID-X generated profiles, and LOQ was able to filter out the false peaks while keeping true peaks that had low RFU values. Therefore, a new analysis method was created in GeneMapper™ ID-X using the LOQ. Each LOQ value was rounded to the nearest 5 when inputted into GeneMapper™ ID-X. [17].

Sensitivity study

Once the average peak heights of all alleles for each sample were calculated, cycle it was determined that either the 28 cycles for 24 seconds or 29 cycles for 22 seconds showed the ideal data, due to drop-out, lack of resolution or blowout in the other samples. Comparing the average peak heights for both samples, it was found that 28 cycles for 24 seconds created more stable data across all concentrations of DNA. This can be seen in Figure 3 where 28 cycles for 24 seconds has an R^2 value of 0.94 compared with 0.91. The closer the R^2 value comes to one, the better the data fits the regression line. The graphs for the comparison of injection times within each cycle can be found in Appendix A in figures S13 and S14.

Comparing the peak height of all the alleles on both samples between both cycle numbers, the 28 cycle and 24 second injection time has the highest R^2 value with 0.58. This is shown in Figure 4. In Appendix A, Figures S1 to S12 are the average peak height values for each sample, cycle and injection time showing the trend line and R^2 value. Comparing the average peak cycles vs the amount of DNA between the samples, the highest R^2 value for sample VD280 was 0.765 from 29 cycles for 22 seconds and the highest R^2 value for sample VD 285 was 0.41 from 28 cycles for 24 seconds.

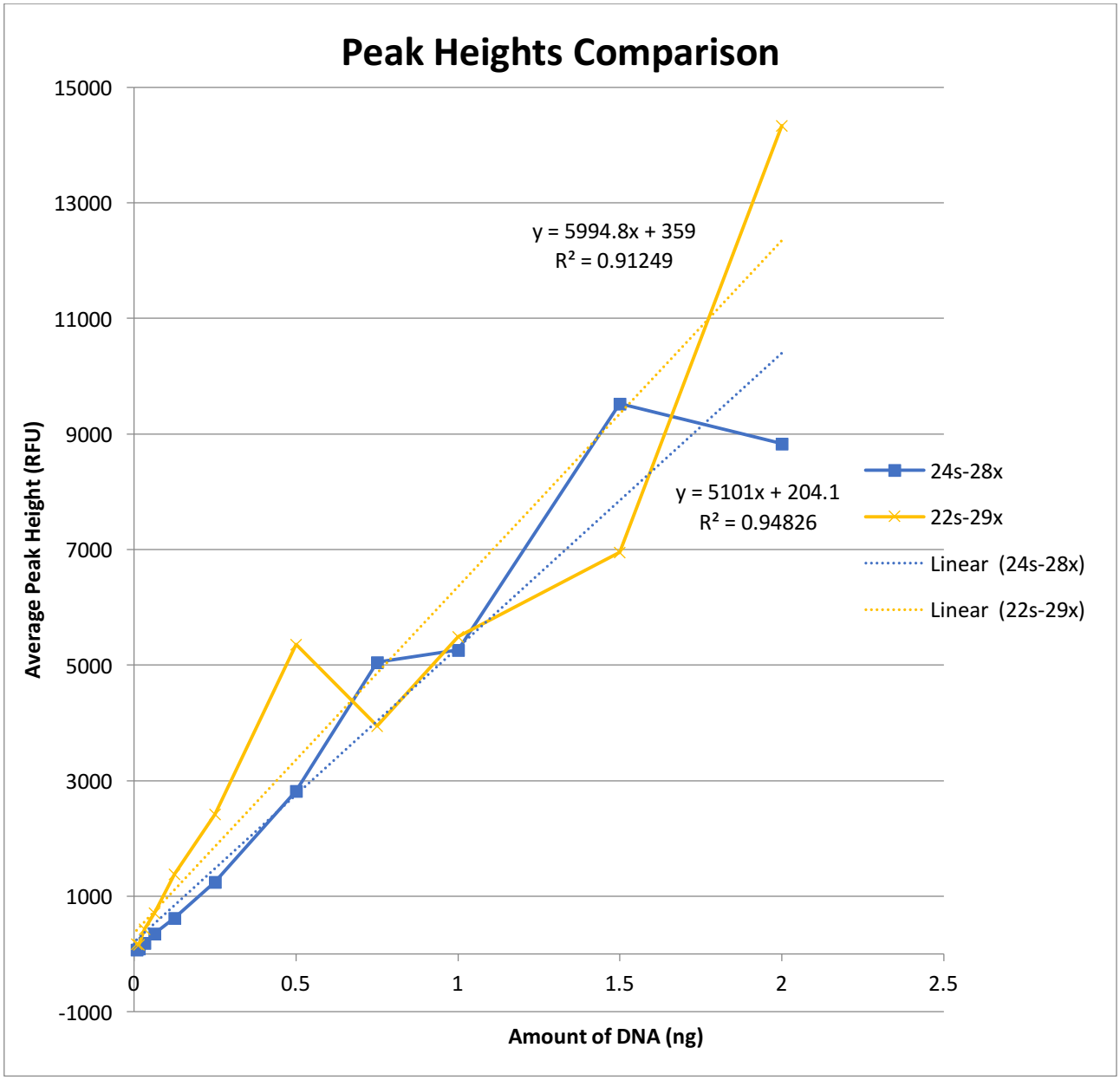

Figure 3: Peak heights for DNA samples injected at 28 cycles for 24 seconds and 29 cycles for 22 seconds. Trend-lines show the equation and R^2 values for the overall average peak heights between both protocols.

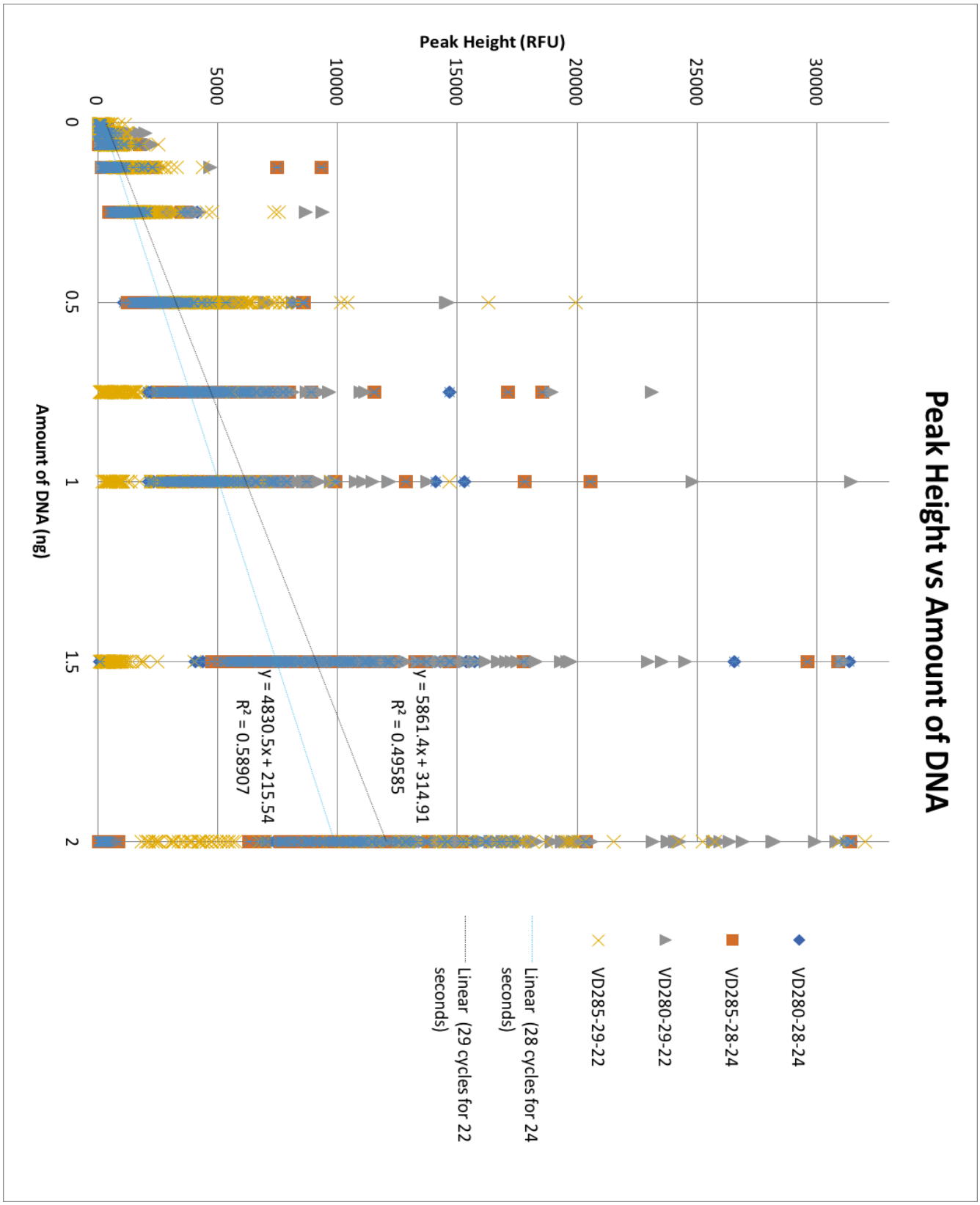

Figure 4: Peak height vs amount of DNA for VD280 and VD285 at 28 cycles for 24 seconds and at 29 cycles for 22 seconds. Trend lines show the equation and R^2 value for each protocol combining the two samples.

After looking at the percent profile detected for each concentration, it was determined that dropout started to be observed at 62.5pg, with the exceptions of 2ng on VD285 at 28 cycles for 24 seconds and 1.5ng and 750pg on VD280 at 29 cycles for 22 seconds. Figure 5 depicts the number of alleles detected for 28 cycles injected for 24 seconds and for 29 cycles injected at 22 seconds. The expected number of alleles in VD280 is 43 and the expected number of alleles in VD285 is 42. The tables showing percent profile at each cycle for each injection time Appendix B, tables S1 to S12.

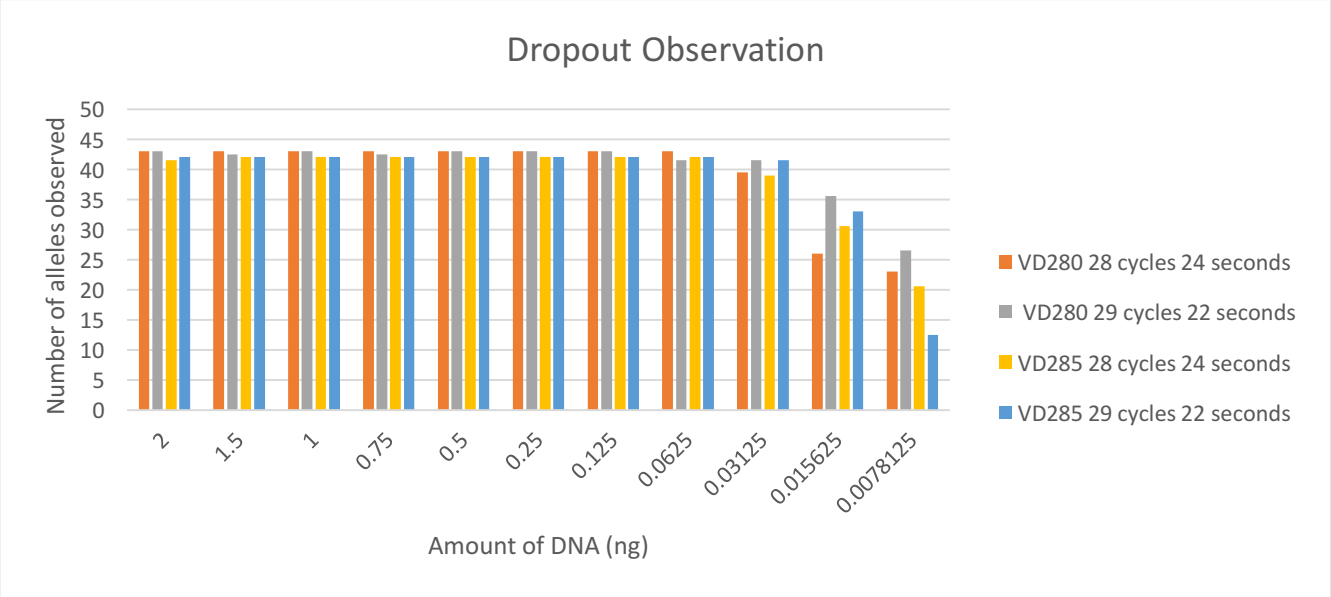

Figure 5: Graph observing the dropout of alleles for VD280 and VD285 at 28 cycles for 24 seconds and at 29 cycles for 22 seconds.

It was determined that the optimal cycle number is 28 cycles and the best injection time is 24 seconds. Each R^2 value for 28 cycles and 24 seconds is higher showing that the data is more consistent in peak height and average peak height. Over the dilution series, the 28 cycles 24 seconds protocol is more consistent in the allele dropout. The optimal concentration of DNA is 500pg or 0.5ng of DNA based on the distributions seen in the data. The outliers seen at 0.5ng were from the 29 cycles and 22 seconds protocol and therefore aren't included in the final analysis.

Stochastic Threshold

The highest RFU that showed when dropout occurred in the sensitivity data was 349 RFUs. Several other data points were found from the sensitivity samples that also had dropout at different RFUs. The Logistic Regression formula was applied to this data and the data points are graphed in Figure 6 along with the Logistical Regression line.

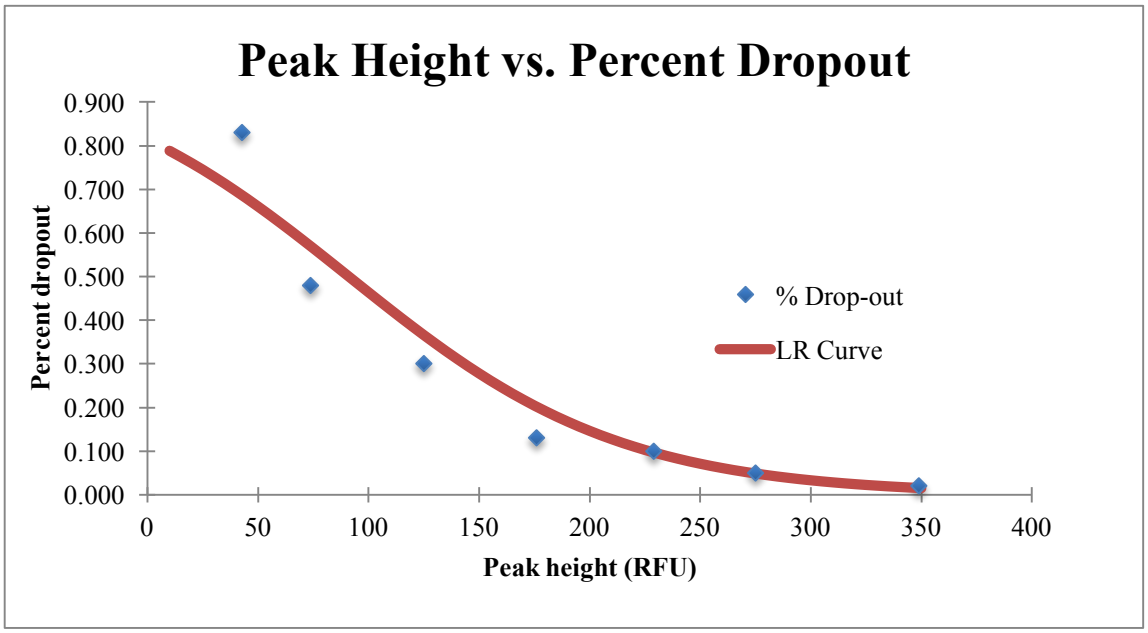

Figure 6: Data points for % dropout are the observed dropout in the data. The logistic regression curve is the model of percent dropout based on peak height.

The optimal stochastic threshold is when the logistic regression model determines when dropout will be below 1 percent. Due to rounding, it was determined that 400 RFUs was the optimal stochastic threshold, which is shown in Table 6. In this table, it is shown that while the dropout percentage falls below 1 percent at 380 RFU; though to be more conservative, the Genetics Lab has chosen 400 RFU to mitigate the proximity to the 1 percent dropout that 380 RFUs has. The whole table is shown in appendix B, table S9, to show the regression in numerical form.

Table 6: Logistical regression table showing the RFUs below 2% dropout.

RFU	DO %
340	1.736
350	1.48
360	1.262
370	1.075
380	0.915
390	0.78
400	0.664
410	0.565
420	0.481
430	0.409
440	0.348
450	0.296
460	0.252
470	0.214
480	0.182
490	0.155
500	0.132

Precision and Accuracy Study

After analyzing the samples in GeneMapper™ ID-X, it was determined that VD170 will be eliminated from the data due to degradation and VD363 will also be eliminated due to contamination. The degradation was due to improper storage of the sample and contamination was due to human error.

As per the manufacturer's developmental validation guidelines, the standard deviation of the allele peak sizes cannot exceed 0.15 bp for precision and three times the standard deviation of the cannot exceed ± 0.5 base pairs for accuracy. The internal validation indicates that the standard deviation did not exceed 0.12 bp and three times the standard deviation did not

exceed 0.35 base pairs. Therefore, each value that was detected was concordant to the manufactures developmental guidelines. The data for samples VD186 and VD286 is shown in Tables 7 and 8, with the other samples provided in tables S14 to S19 in appendix B.

Table 7: Precision and accuracy study of VD186 with average, 3 times standard deviation, minimum and maximum peak size.

Sample	Marker	Allele 1	Allele 2	Avg 1	SD1	3*SD 1	Min1	Max1	Avg 2	SD2	3*SD2	Min 2	Max 2
VD186	AMEL	X		98.77	0.05	0.15	98.73	98.83					
	CSF1PO	12		307.08	0.06	0.17	307.02	307.14					
	D10S1248	13	14	106.11	0.03	0.08	106.07	106.14	110.17	0.07	0.2	110.08	110.27
	D12S391	17	18	228.61	0.07	0.21	228.52	228.67	232.51	0.06	0.18	232.48	232.62
	D13S317	10	12	218.93	0.06	0.18	218.88	219	227.13	0.05	0.15	227.1	227.22
	D16S539	10	12	248.26	0.05	0.16	248.2	248.3	256.28	0.02	0.07	256.27	256.32
	D18S51	17	19	301.8	0.02	0.05	301.77	145.68	309.76	0.07	0.2	309.68	155.69
	D19S433	13	15.2	145.67	0.02	0.05	145.64	145.68	155.63	0.06	0.17	155.58	155.69
	D1S1656	11	14	168.03	0.06	0.17	167.98	168.13	180.09	0.05	0.15	180	180.11
	D21S11	31.2	32.2	97.45	0.05	0.15	213.57	213.68	115.44	0.05	0.15	217.66	217.77
	D22S1045	11	17	190.4	0.04	0.12	97.38	97.48	197.27	0.01	0.03	115.43	115.45
	D2S1338	19	21	313.27	0.12	0.33	313.18	313.41	321.65	0.11	0.16	321.57	321.68
	D2S441	12.3	14	96.37	0.05	0.16	96.3	96.42	101.28	0.01	0.03	101.27	101.29
	D3S1358	15	16	121.34	0.04	0.13	121.26	121.36	125.22	0.06	0.17	125.16	125.27
	D5S818	8	12	142.76	0.04	0.13	142.68	142.79	159.14	0.05	0.15	159.05	159.16
	D7S820	10	12	278.7	0.06	0.19	278.63	278.75	286.68	0.02	0.07	286.65	286.7
	D8S1179	12	13	142.82	0.06	0.17	142.78	142.89	146.95	0.05	0.15	146.92	147.03
	DYS391												
	FGA	24	25	267.56	0.07	0.2	267.5	267.64	271.59	0.01	0.03	271.57	271.59
	SE33	17	28.2	358.3	0.01	0.03	358.29	358.31	404.61	0.06	0.19	404.53	404.68
	TH01	9.3	10	303.88	0.07	0.2	202.44	202.63	324.79	0.03	0.09	203.4	203.47
	TPOX	8	11	351.07	0.05	0.15	350.99	351.12	363.24	0.06	0.19	363.13	363.28
	vWA	13	15	164.8	0.03	0.1	164.78	164.86	173.01	0.05	0.15	172.94	173.05
	Yindel												

Table 8: Precision and accuracy study of VD286 with average, 3 times standard deviation, minimum and maximum peak size.

Sample	Marker	Allele 1	Allele 2	Avg 1	SD1	3*SD 1	Min1	Max1	Avg 2	SD2	3*SD2	Min 2	Max 2
VD286	AMEL	X		98.79	0.05	0.15	98.74	98.83					
	CSF1PO	11		303.10	0.06	0.17	303.04	303.17					
	D10S1248	15	16	114.21	0.04	0.13	114.19	114.29	118.11	0.03	0.09	118.09	118.16
	D12S391	18	20	232.55	0.06	0.17	232.48	232.62	240.48	0.05	0.14	240.45	240.56
	D13S317	11		223.08	0.06	0.19	223.02	223.19					
	D16S539	9		244.16	0.04	0.12	244.10	244.21					
	D18S51	16	18	297.84	0.08	0.23	297.73	137.78	305.76	0.04	0.11	305.71	149.68
	D19S433	11	14	137.77	0.01	0.03	137.75	137.78	149.64	0.03	0.10	149.62	149.68
	D1S1656	12	15	172.05	0.04	0.12	172.00	172.11	184.23	0.06	0.17	184.17	184.29
	D21S11	28	33.2	112.51	0.05	0.14	199.67	199.78	221.86	0.02	0.05	221.83	221.87
	D22S1045	16	17	182.27	0.04	0.12	112.44	112.54	115.45	0.04	0.12	115.43	115.52
	D2S1338	17	21	305.28	0.04	0.13	305.22	305.33	321.58	0.06	0.17	321.55	321.68
	D2S441	11	12	89.04	0.03	0.08	89.01	89.07	93.24	0.04	0.12	93.20	93.30
	D3S1358	15	17	121.37	0.05	0.14	121.35	121.46	129.50	0.06	0.17	129.47	129.60
	D5S818	12	13	159.18	0.04	0.13	159.16	159.26	163.19	0.04	0.12	163.12	163.22
	D7S820	9	12	274.71	0.04	0.13	274.67	274.77	286.68	0.05	0.14	286.64	286.73
	D8S1179	12	14	142.78	0.01	0.03	142.77	142.80	151.10	0.03	0.09	151.08	151.14
	DYS391												
	FGA	23	25	263.54	0.09	0.26	263.39	263.61	271.58	0.06	0.18	271.51	271.66
	SE33	19	29.2	366.41	0.05	0.16	366.35	366.47	408.59	0.08	0.23	408.51	408.70
	TH01	6	7	187.35	0.05	0.16	187.28	187.40	191.42	0.05	0.15	191.37	191.49
	TPOX	8	11	351.10	0.05	0.15	351.06	351.18	363.26	0.09	0.27	363.17	363.41
	vWA	15	16	173.01	0.04	0.12	172.98	173.05	177.05	0.01	0.03	177.04	177.06
	Yindel												

Reproducibility and Repeatability

After analyzing the samples in GeneMapper™ ID-X, it was determined that VD170 will be eliminated from the data due to degradation and VD363 will also be eliminated due to contamination. The degradation was due to improper storage of the sample and contamination was due to human error. For each of the other samples, all the alleles were concordant between the separate amplification plates. The comparison between profiles is

shown in Tables 9 and 10 for samples VD186 and VD286 below, and the other samples provided in tables S20 to S25 in appendix B. All the samples were compared to their reference samples from the 3130xL genetic analyzer and the profiles were concordant

Table 9: Reproducibility of VD186 with each marker of each allele compared with each analyst.

Sample		**Analyst 1**		**Analyst 2**	
	Marker	**Allele 1**	**Allele 2**	**Allele 1**	**Allele 2**
VD186	AMEL	X	-	X	-
	CSF1PO	12	-	12	-
	D10S1248	13	14	13	14
	D12S391	17	18	17	18
	D13S317	10	12	10	12
	D16S539	10	12	10	12
	D18S51	17	19	17	19
	D19S433	13	15.2	13	15.2
	D1S1656	11	14	11	14
	D21S11	31.2	32.2	31.2	32.2
	D22S1045	11	17	11	17
	D2S1338	19	21	19	21
	D2S441	12.3	14	12.3	14
	D3S1358	15	16	15	16
	D5S818	8	12	8	12
	D7S820	10	12	10	12
	D8S1179	12	13	12	13
	DYS391	-	-	-	-
	FGA	24	25	24	25
	SE33	17	28.2	17	28.2
	TH01	9.3	10	9.3	10
	TPOX	8	11	8	11
	vWA	13	15	13	15
	Yindel	-	-	-	-

Table 10: Reproducibility of VD286 with each marker of each allele compared with each analyst.

Sample		Analyst 1		Analyst 2	
	Marker	**Allele 1**	**Allele 2**	**Allele 1**	**Allele 2**
VD286	AMEL	X	-	X	-
	CSF1PO	11	-	11	-
	D10S1248	15	16	15	16
	D12S391	18	20	18	20
	D13S317	11	-	11	-
	D16S539	9	-	9	-
	D18S51	16	18	16	18
	D19S433	11	14	11	14
	D1S1656	12	15	12	15
	D21S11	28	33.2	28	33.2
	D22S1045	16	17	16	17
	D2S1338	17	21	17	21
	D2S441	11	12	11	12
	D3S1358	15	17	15	17
	D5S818	12	13	12	13
	D7S820	9	12	9	12
	D8S1179	12	14	12	14
	DYS391	-	-	-	-
	FGA	23	25	23	25
	SE33	19	29.2	19	29.2
	TH01	6	7	6	7
	TPOX	8	11	8	11
	vWA	15	16	15	16
	Yindel	-	-	-	-

Stutter Study

Stutter ratios were calculated between minus stutter and the corresponding true peaks for each marker. Plus and minus 8 stutter were also counted. Three times the standard deviation

of the average stutter ratio was added to the average for each marker, which was then compared to the manufactures stutter ratio. Ratios higher than the manufacturers recommended settings are bolded in Tables 11 and 12. It has been recommended that the ratios that were higher than the manufactures should be adjusted to the higher ratio on the GeneMapper™ ID-X on the Panel Manager. Figure 7 shows an example of stutter.

Table 11: Stutter ratio and the count of forward and minus 8 stutter for 28 cycles. The bolded numbers are larger than the manufactures recommended ratios.

Locus	Manufacturer Stutter Ratio (%)	Max Stutter Ratio (%)	Average + 3SD (%)	Number of Forward Stutter	Number of (-8) Stutter
D3S1358	10.98	7.0	7.8	15	3
VWA	10.73	10.1	9.1	0	2
D16S539	9.48	6.5	6.3	3	0
CSF1PO	8.77	7.0	7.1	7	0
TPOX	5.55	2.2	2.3	0	0
D8S1179	9.6	11.4	**13.1**	45	11
D21S11	10.45	13.7	**10.6**	15	1
D18S51	12.42	12.8	10.5	16	8
D2S441	8.1	7.2	**9.2**	13	0
D19S433	9.97	7.1	8.0	0	0
THO1	4.45	4.3	4.4	0	0
FGA	11.55	7.8	7.5	0	0
D22S1045	-	16.4	**14.1**	45	17
D5S818	9.16	8.9	8.6	6	0
D13S317	9.19	13.0	**14.2**	29	2
D7S820	8.32	4.9	5.6	6	0
SE33	14.49	12.7	12.2	22	4
D10S1248	11.46	11.1	**11.9**	4	23
D1S1656	12.21	11.5	10.1	29	13
D12S391	13.66	13.7	13.3	6	10
D2S1338	11.73	14.5	11.6	7	11
			Total	268	105

Table 12: Stutter ratio and the count of forward and minus 8 stutter for 29 cycles. The highlighted numbers are larger than the manufactures recommended ratios.

Locus	Manufacturer Stutter Ratio (%)	Max Stutter Ratio (%)	Average + 3SD (%)	Number of Forward Stutter	Number of (-8) Stutter
D3S1358	10.98	8.5556	9.1	1	0
VWA	10.73	9.2437	9.254	0	3
D16S539	9.48	-	-	-	-
CSF1PO	8.77	11.18	**10.17**	2	0
TPOX	5.55	2.1666	2.512	0	0
D8S1179	9.6	13.498	**14.19**	2	0
D21S11	10.45	15.438	**12.28**	8	0
D18S51	12.42	15.084	9.062	6	5
D2S441	8.1	6.5133	7.108	0	0
D19S433	9.97	8.6227	6.771	0	0
THO1	4.45	1.6037	1.604	0	0
FGA	11.55	6.4622	6.495	0	0
D22S1045	-	10.064	**6.854**	14	0
D5S818	9.16	9.7035	**10.05**	7	0
D13S317	9.19	11.312	5.49	6	1
D7S820	8.32	4.6843	4.207	4	1
SE33	14.49	53.674	**24.47**	7	3
D10S1248	11.46	18.116	**15.91**	5	0
D1S1656	12.21	12.706	**13.28**	8	7
D12S391	13.66	14.286	**24.2**	0	1
D2S1338	11.73	9.8696	**12.67**	0	6
			Total	20	20

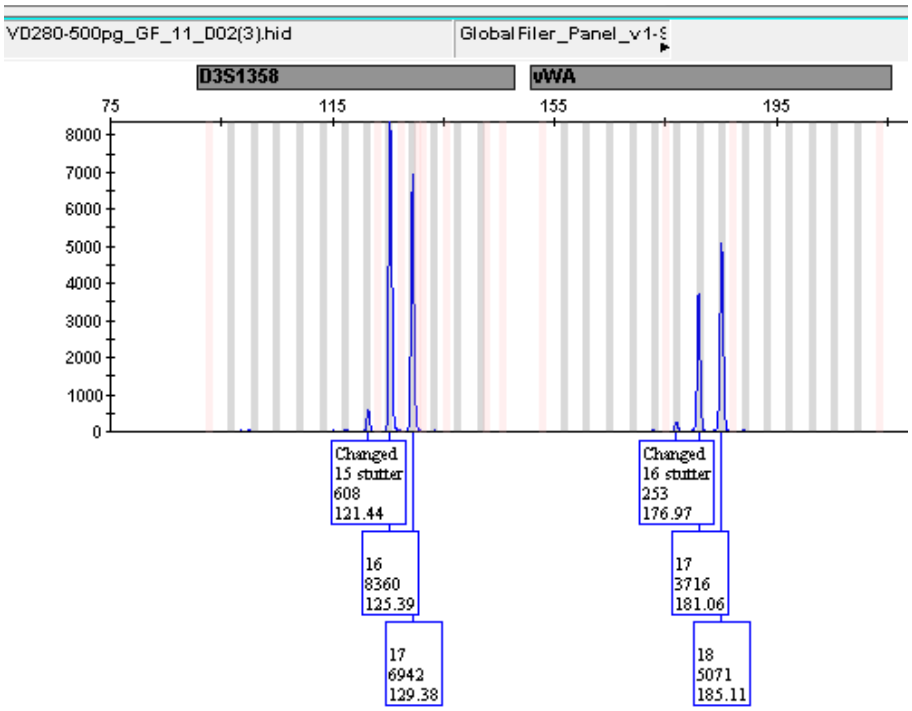

Figure 7: Example of stutter in VD280 at 0.5ng on the D3S1358 and vWA loci.

Mixture study

There were some markers on the mixed samples that did not produce any alleles. These were not interrogated further, as peak height ratio could not be calculated. For each mixture ratio that did produce alleles, the peak height ratios were calculated between major and minor contributors. The reference sample profiles are shown in Table 13 The average peak height ratio was calculated for each mixture ratio, along with the standard deviation. The trend for the average peak height ratio is that the ratio goes up until the amount of DNA in VD160 becomes greater than that of the other reference samples. This is because there was less DNA in VD160 than the other references to begin with, and therefore makes the 1:1, 1:1:1, and 1:1:1:1 ratios smaller. For the 2 person, the amount of DNA in VD160 becomes greater than the amount of DNA in VD286 when the ratio is 10:1. For the 3 person, the DNA in VD160 becomes greater than the DNA in VD286 and VD173 when the ratio is 5:1:1, and in the 4 person mixture the amount of DNA in VD160 becomes greater than the amount of DNA in

VD286, VD170 and VD300 when the ratio is 10:1:1:1. This is all shown in Tables 14 to 16 below.

Table 13: Reference samples used for the mixtures and their profiles.

Marker	VD160		VD286		VD173		VD300	
D3S1358	11	14	15	17.1	14	17	14	17
vWA	15	18	15	16	17	18	17	
D16S539	10	13	9		12	13	12	
CSF1PO	11	12	11		10	11	9	11
TPOX	8	11	8	11	10	11	8	
Yindel								
AMEL	X		X		X	Y	X	
D8S1179	8	13	12	14	12	14	12	13
D21S11	30.2	32.2	28	33.2	30		30	31.2
D18S51	16	18	16	18	14	19	15	18
DYS391								
D2S441	10	11	11	12	14			
D19S433	13	14	11	14	13	15.2	13	
TH01	7	8	6	7	9	9.3	9	
FGA	24	25	23		21		21	23
D22S1045	15	16	16	17			13	
D5S818	11	12	12	13	10	12	12	
D13S317	8	13	11		8		12	
D7S820	10	11	9	12	8	10	10	
SE33	25.2	27.2	19	29.2	16	18	14	28.2
D10S1248	11	15	15	16	13	14		
D1S1656	15	15.3	12	15	16	16.3	11	14
D12S391	17.3	18	18	20	17	19	21	
D2S1338	20	25	17	21	17		23	

Table 14: Two person mixture ratios with PHR, Average PHR and Standard Deviation.

Marker	1:1	2:1	3:1	5:1	10:1
D3S1358	0.33	0.49	0.68	0.86	0.40
vWA	0.46	0.62	0.83	0.54	0.28
D16S539	0.16	0.23	0.37	0.45	0.85
D8S1179	0.38	0.44	0.61	0.89	0.55
D21S11	0.46	0.58	0.75	0.88	0.35
D2S441	0.36	0.56	0.78	0.90	0.33
D19S433	0.33	0.43	0.74	0.62	0.43
TH01	0.23	0.39	0.41	0.80	0.59
FGA	0.64	0.67	0.67	0.70	0.34
D22S1045	0.44	0.68	0.84	0.77	0.33
D5S818	0.60	0.82	1.00	0.74	0.25
D13S317	0.25	0.36	0.37	0.77	0.61
D7S820	0.48	0.61	0.71	0.89	0.32
SE33	0.35	0.56	0.60	0.90	0.38
D10S1248	0.31	0.12	0.60	0.77	0.40
D1S1656	0.27	0.59	0.73	0.68	0.40
D12S391		0.53	0.57	0.94	0.37
D2S1338	0.26	0.46	0.65	0.89	0.38
Average Peak Height ratio	0.37	0.51	0.66	0.78	0.42
Standard Deviation	0.13	0.17	0.16	0.14	0.14

Table 15: Three person mixture ratios with PHR, Average PHR and Standard Deviation.

Marker	1:1:1	2:1:1	3:1:1	5:1:1	10:1:1
vWA	0.35	0.34	0.71	0.95	0.32
D16S539	0.32	0.36	0.37	1.35	0.52
CSF1PO	0.90	0.28	0.74	0.31	0.25
D21S11	0.44	0.51	0.73	0.34	0.34
D2S441	0.34	0.33	0.47	0.21	0.25
D19S433	0.20	0.19	0.25	0.27	0.09
TH01	0.28	0.40	0.65	0.39	0.23
FGA	0.58	0.31	0.59	0.46	0.34
D22S1045	0.19	0.22	0.32	0.21	0.23
D5S818	0.42	0.36	0.59	0.29	0.28
D13S317	0.18	1.00	0.43	0.52	0.78
D7S820	0.51	0.38	0.64	0.49	0.39
SE33	0.45	0.40	0.63	0.37	0.30
D10S1248	0.24	0.27	0.38	0.33	0.24
D1S1656	0.41	0.30	0.50	0.35	0.35
D12S391	0.35	0.22	0.30	0.37	0.40
D2S1338	0.28	0.65	0.75	0.42	0.66
Average Peak Height ratio	0.38	0.38	0.53	0.45	0.35
Standard Deviation	0.17	0.19	0.16	0.28	0.16

Table 16: Four person mixture ratios with PHR, Average PHR and Standard Deviation.

Marker	1:1:1:1	2:1:1:1	3:1:1:1	5:1:1:1	10:1:1:1
TH01	0.27	0.46	0.47	0.53	0.43
SE33	0.44	0.44	0.47	0.54	0.33
D1S1656	0.39	0.39	0.48	0.42	0.31
D12S391	0.42	0.31	0.31	0.32	0.27
D2S1338	0.38	0.40	0.43	0.45	0.33
Average Peak Height ratio	0.07	0.06	0.07	0.09	0.06
Standard Deviation	0.27	0.46	0.47	0.53	0.43

Degradation Study

Each sample before degradation had 140 ng/µl of DNA. The sample that was in the autoclave for 30 minutes (VD435), had an average of 126.301ng/µl of DNA, the 60 minute sample (VD205) had an average of 115.161 ng/µl of DNA, and the sample that was degraded for 90 minutes (VD441) had an average of 66.123 ng/µl of DNA. This shows that the DNA in each sample was degraded with time. The amount of DNA in each replicate is shown in Table 17. Nevertheless, there was enough DNA in each sample to provide a full profile. Each profile was concordant with their corresponding reference samples as shown in Tables 18 to 20 below.

Table 17: Table of each replicate sample, the amount of DNA in each, and the average amount of DNA.

Time in Autoclave (minutes)	Sample	Amount of DNA: Replicate 1 (ng/µl)	Amount of DNA: Replicate 2 (ng/µl)	Amount of DNA: Replicate 3 (ng/µl)	Average amount of DNA(ng/µl)
30	VD435	126.04	126.24	126.63	126.30
60	VD205	115.02	115.14	115.31	115.16
90	VD441	66.03	66.10	66.24	66.12

Table 18: Degradation sample VD435 compared with the reference sample VD435 in IdentiFiler Plus.

Marker	Reference (VD435 ID Plus)		VD435 (30 min)	
D3S1358	16	18	16	18
vWA	14	16	14	16
D16S539	11	14	11	14
CSF1PO	11		11	
TPOX	8		8	
Yindel				
AMEL	X		X	
D8S1179	11	13	11	13
D21S11	29	31.2	29	31.2
D18S51	14	20	14	20
DYS391				
D2S441			10	14
D19S433	14		14	
TH01	9.3		9.3	
FGA	23		23	
D22S1045			15	17
D5S818	11	12	11	12
D13S317	11	12	11	12
D7S820	8	10	8	10
SE33			19	20
D10S1248			14	
D1S1656			12	17.3
D12S391			16	26
D2S1338	17	20	17	20

Table 19: Degradation Sample VD205 compared with the reference sample VD205.

Marker	Reference (VD205)		VD205 (60 min)	
D3S1358	14		14	
vWA	13	18	13	18
D16S539	9	11	9	11
CSF1PO	8	10	8	10
TPOX	11		11	
Yindel	2			
AMEL	X	Y	X	Y
D8S1179	14		14	
D21S11	28		28	
D18S51	16	17	16	17
DYS391	10		10	
D2S441	11	14	11	14
D19S433	13	14	13	14
TH01	7	9.3	7	9.3
FGA	23	26	23	26
D22S1045	17	18	17	18
D5S818	11	13	11	13
D13S317	12		12	
D7S820	10	11	10	11
SE33	21.2	25.2	21.2	25.2
D10S1248	13	15	13	15
D1S1656	14	16	14	16
D12S391	19		19	
D2S1338	19	22	19	22

Table 20: Degradation sample VD441 compared with reference sample VD441 in IdentiFiler Plus.

Marker	Reference (VD 441 ID Plus)		VD441	
D3S1358	16	18	16	18
vWA	17	18	17	18
D16S539	9	13	9	13
CSF1PO	10	12	10	12
TPOX	8		8	
Yindel			2	
AMEL	X	Y	X	Y
D8S1179	8	13	8	13
D21S11	29	31	29	31
D18S51	13	15	13	15
DYS391			11	
D2S441			14	15
D19S433	13	15	13	15
TH01	8	9.3	8	9.3
FGA	21	23	21	23
D22S1045			15	16
D5S818	11	13	11	13
D13S317	10	12	10	12
D7S820	9	12	9	12
SE33			13.2	23.2
D10S1248			13	15
D1S1656			16.3	17.3
D12S391			22	23
D2S1338	20	25	20	25

Known and non-probative evidence samples or mock evidence samples

All the samples were analyzed with GeneMapper™ ID-X Client Version 1.4. Samples 1A1-NS and 11A1-NS were observed as single source profiles and were concordant with the reference samples as seen in Tables 21 and 23. Samples 1A1-S and 11A1-S were also concordant, but were observed to have minor contributors as seen in Tables 22 and 24. Each allele sourced to the minor contributors however, can be traced to the same allele on the same loci to their corresponding NS fractions. Both the 2 saliva samples and 2 blood samples were observed as single source samples, and were concordant with the reference samples as seen in Tables 25 to 28. The organically extracted saliva sample VD472 did not have sufficient DNA to generate a profile and was excluded from the final analysis. However, the organically-extracted blood sample VD83 in Table 30 was concordant with the QIAgen extracted reference sample VD83. The touch DNA swab samples SK-Phone and DY-keyboard had the same alleles as seen in the references obtained from sample donors and were seen with a minor contributor, which was expected, due to the fact that the phone and the keyboard are communally owned by the Genetics Laboratory. This is seen in Tables 29 and 30. SK-Cup was observed as a single source profile and was concordant with the reference sample shown in Table 29.

Table 21: Profile of sample 1A1-NS compared with the reference sample VD425 in IdentiFiler Plus.

Marker	Reference (VD425 ID Plus)		1A1-NS	
D3S1358	15		15	
vWA	14	17	14	17
D16S539	9	12	9	12
CSF1PO	12		12	13
TPOX	8	11	8	11
Yindel				
AMEL	X		X	
D8S1179	12	14	12	14
D21S11	30	32.2	30	32.2
D18S51	14	20	14	20
DYS391				
D2S441			11	11.3
D19S433	13	15	13	15
TH01	6	8	6	8
FGA	19	21	19	21
D22S1045			16	
D5S818	11		11	12
D13S317	10	12	10	12
D7S820	9		9	
SE33			16	27.2
D10S1248			14	
D1S1656			14	16.3
D12S391			18	23
D2S1338	16	21	16	21

Table 22: Profile of sample 1A1-S compared with the reference sample VD455 in IdentiFiler Plus.

Marker	Reference (VD455 ID Plus)		1A1-S		
D3S1358	15	16	15	16	
vWA	17	19	17	19	
D16S539	10	11	10	11	
CSF1PO	10	12	10	12	
TPOX	8	11	8	11	
Yindel			2		
AMEL	X	Y	X	Y	
D8S1179	10		10		
D21S11	28	30	28	30	**32.2**
D18S51	12	15	12	15	
DYS391			11		
D2S441			10	14	
D19S433	14	15	14	15	
TH01	6	9	6		
FGA	20	23.2	20	23.2	
D22S1045			11	15	
D5S818	11	13	11	13	
D13S317	8	11	8	11	
D7S820	13	12	11	12	
SE33			16		
D10S1248			13	14	
D1S1656			13	16	
D12S391			18	21	
D2S1338	17	20	17	20	

Table 23: Profile of sample 11A1-NS compared to the reference sample VD470.

Marker	Reference (VD470)		11A1-NS	
D3S1358	14	18	14	18
vWA	16		16	
D16S539	12	13	12	13
CSF1PO	11		11	
TPOX	8	11	8	11
Yindel				
AMEL	X		X	
D8S1179	11	14	11	14
D21S11	31	33.2	31	33.2
D18S51	12	18	12	18
DYS391				
D2S441	11	14	11	14
D19S433	14		12	14
TH01	6		3	6
FGA	22	24	22	24
D22S1045	11	15	11	15
D5S818	11	12	11	12
D13S317	9	12	9	12
D7S820	8	10	8	10
SE33	18	31.2	18	31.2
D10S1248	14	15	14	15
D1S1656	15	18	15	18
D12S391	15	20	15	20
D2S1338	16	17	16	17

Table 24: Profile of sample 11A1-S compared to the reference sample VD455 in IdentiFiler Plus.

Marker	Reference (VD 455 ID Plus)		11A1-S			
D3S1358	15	16	15	16	**18**	
vWA	17	19	17	19		
D16S539	10	11	10	11		
CSF1PO	10	12	10	12		
TPOX	8	11	8	11		
Yindel			2			
AMEL	X	Y	X	Y		
D8S1179	10		10		**11**	**14**
D21S11	28	30	28	30		
D18S51	12	15	12	15		
DYS391						
D2S441			10	14		
D19S433	14	15	14	15		
TH01	6	9	6	9		
FGA	20	23.2	20	23.2		
D22S1045			11	15		
D5S818	11	13	11	13		
D13S317	8	11	8	11		
D7S820	13	12	11	12		
SE33			16	17		
D10S1248			13	14		
D1S1656			13	16		
D12S391			21			
D2S1338	17	20	17	20		

Table 25: Profile of sample Q-479 compared to the reference sample VD475 in IdentiFiler Plus.

Marker	Reference (VD475 ID Plus)		Q-479 (Saliva)	
D3S1358	14		14	
vWA	17	20	17	20
D16S539	19	21	19	21
CSF1PO	8	9.3	8	9.3
TPOX	12		12	
Yindel	12	13	12	13
AMEL	8		8	
D8S1179			17	27.2
D21S11	8	13	8	13
D18S51	9	12	9	12
DYS391	12		12	
D2S441			15	
D19S433				
TH01	X		X	
FGA	13	14	13	14
D22S1045	31.2	34.2	31.2	34.2
D5S818	13	16	13	16
D13S317				
D7S820			11	14
SE33	13	14.2	13	14.2
D10S1248			13	16
D1S1656			15	17
D12S391			18	
D2S1338	17		17	

Table 26: Sample VD474 compared with reference sample.

Marker	Reference (VD474)		VD474 (Saliva)	
D3S1358	16	17	16	17
vWA	15	17	15	17
D16S539	11	13	11	13
CSF1PO	12		12	
TPOX	9		9	
Yindel				
AMEL	X		X	
D8S1179	13	15	13	15
D21S11	30	32.2	30	32.2
D18S51	14	15	14	15
DYS391				
D2S441	11.3	15	11.3	15
D19S433	13	14	13	14
TH01	8	9.3	8	9.3
FGA	22	23	22	23
D22S1045	15	16	15	16
D5S818	11	13	11	13
D13S317	8	12	8	12
D7S820	11	12	11	12
SE33	24.2	29.2	24.2	29.2
D10S1248	14		14	
D1S1656	12	13	12	13
D12S391	18	24	18	24
D2S1338	18		18	

Table 27: Profile of sample K9 compared to the reference VD90 in IdentiFiler Plus.

Marker	Reference (VD90 ID Plus)		K9	
D3S1358	16	18	16	18
vWA	15	17	15	17
D16S539	9	13	9	13
CSF1PO	11	12	11	12
TPOX	8	10	8	10
Yindel				
AMEL	X		X	
D8S1179	13	14	13	14
D21S11	27	28	27	28
D18S51	14	17	14	17
DYS391				
D2S441			12	14
D19S433			14	15
TH01	6	7	6	7
FGA	20	22	20	22
D22S1045			16	
D5S818	12		12	
D13S317	11		11	
D7S820	11	12	11	12
SE33			17	20.2
D10S1248			14	
D1S1656			14	16
D12S391			17	21
D2S1338			19	20

Table 28: Profile of sample K10 compared to the reference sample VD69 in IdentiFiler Plus.

Marker	Reference (VD 69 ID Plus)		K10	
D3S1358	16		16	
vWA	17	18	17	18
D16S539	10	11	10	11
CSF1PO	11	12	11	12
TPOX	8		8	
Yindel				
AMEL	X		X	
D8S1179	10	13	10	13
D21S11	29	31	29	31
D18S51	14	16	14	16
DYS391				
D2S441			11	14
D19S433			13	14
TH01	7		7	
FGA	21	22	21	22
D22S1045			12	18
D5S818	12	13	12	13
D13S317	12		12	13
D7S820	8	9	8	9
SE33			18	27.2
D10S1248			14	15
D1S1656			15.3	
D12S391			17	19
D2S1338			17	22

Table 29: Case-type samples SK-Cup and SK-phone compared with the reference sample. The alleles in bold were also found in the sample SK-Phone show a possible mixture.

Marker	Reference (VD483)		SK-Cup		SK-Phone		
D3S1358	15	16	15	16	15	16	
vWA	15	17	15	17	15	17	
D16S539	11	14	11	14	11	14	
CSF1PO	12		12		12		
TPOX	8	11	8	11	8	11	
Yindel	2		2		2		
AMEL	X	Y	X	Y	X	Y	
D8S1179	12	14	12	14	12	14	
D21S11	28	29	28	29	28	29	**25.3**
D18S51	13	19	13	19	13	19	
DYS391	10		10		10		
D2S441	10	14	10	14	10	14	
D19S433	13	14	13	14	13	14	
TH01	6	9.3	9.3		6	9.3	
FGA	21	24	21	24	21	24	**28**
D22S1045	11	14	11	14	11	14	**17**
D5S818	11	13	11	13	11	13	
D13S317	11	12	11	12	11	12	
D7S820	10		10		10		
SE33	23.2	24.2	23.2	24.2	23.2	24.2	
D10S1248	15	16	15	16	15	16	
D1S1656	16	17.3	16	17.3	16	17.3	
D12S391	18	22	22		18	22	
D2S1338	19	23	19	23	19	23	

Table 30: Case-type samples DY-Keyboard and VD83 compared with the reference sample. The alleles in bold found in sample DY-Keyboard show a mixture.

Marker	Reference (VD83)		DY-keyboard					VD83 (organic blood)	
D3S1358	15	16	15	16				15	16
vWA	19		19		**16**			19	
D16S539	12		12					12	
CSF1PO	10	11			**12**			10	11
TPOX	8	11	8	11				8	11
Yindel	1		1		**2**			1	
AMEL	X	Y	X	Y				X	Y
D8S1179	11	14	11	14				11	14
D21S11	30	32.2	30					30	32.2
D18S51	16	17			**15**			16	17
DYS391	10		10					10	
D2S441	9.1	10	9.1	10	**11**	**11.3**	**14**	9.1	10
D19S433	14	14.2	14		**13**	**15**	**15.2**	14	14.2
TH01	7		7		**9.3**			7	
FGA	22	23.2						22	23.2
D22S1045	16	18	16					16	18
D5S818	10	12	12					10	12
D13S317	9	12						9	12
D7S820	11		11		**10**	**12**		11	
SE33	20	29.2	20		**17**	**27.2**		20	29.2
D10S1248	13		13		**14**	**16**		13	
D1S1656	16		16					16	
D12S391	17	22	17	22				17	22
D2S1338	17	24	17		**23**			17	24

Contamination assessment

Three sets of negatives and reagent blanks were analyzed. The first set had contamination possibly due to the reagents and consumables used. The second set had contamination due to possible lack of cleaning of the block in the instrument. The third set, using both clean buffer and after a weekly clean of the capillaries, had no contamination. Therefore, it is recommended that the reagents and consumables used for samples be replaced every day and the block washed weekly instead of the manufacturers recommended bi-weekly wash The Figures 8 to 10 highlight the importance of this recommendation.

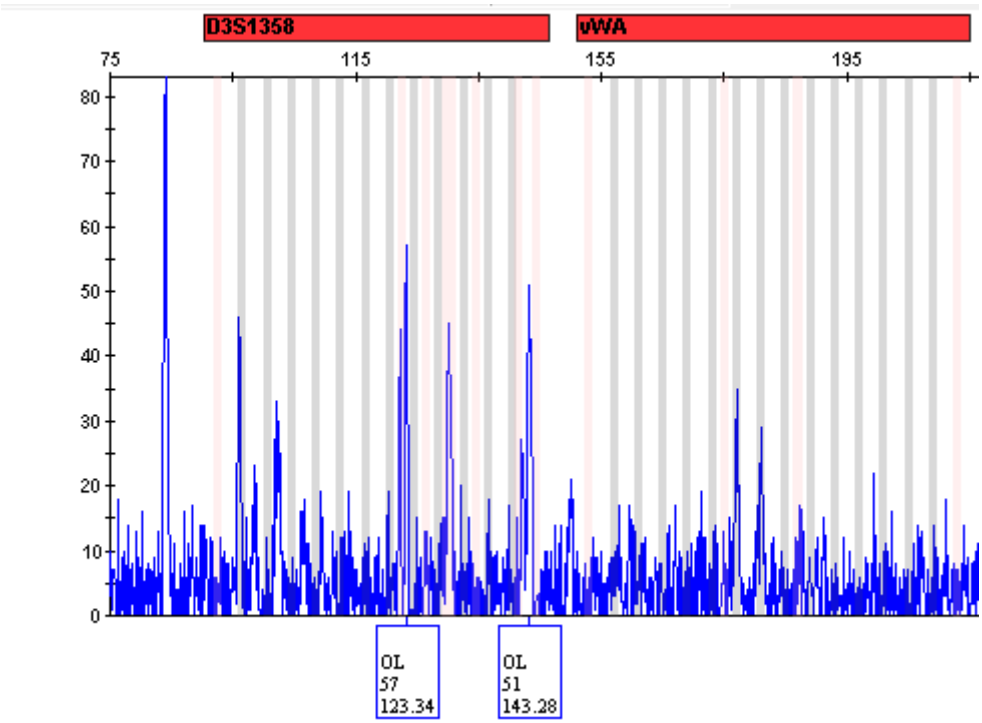

Figure 8: Example of possible contaminated TE-4 buffer with bi-weekly washing of capillaries (first run).

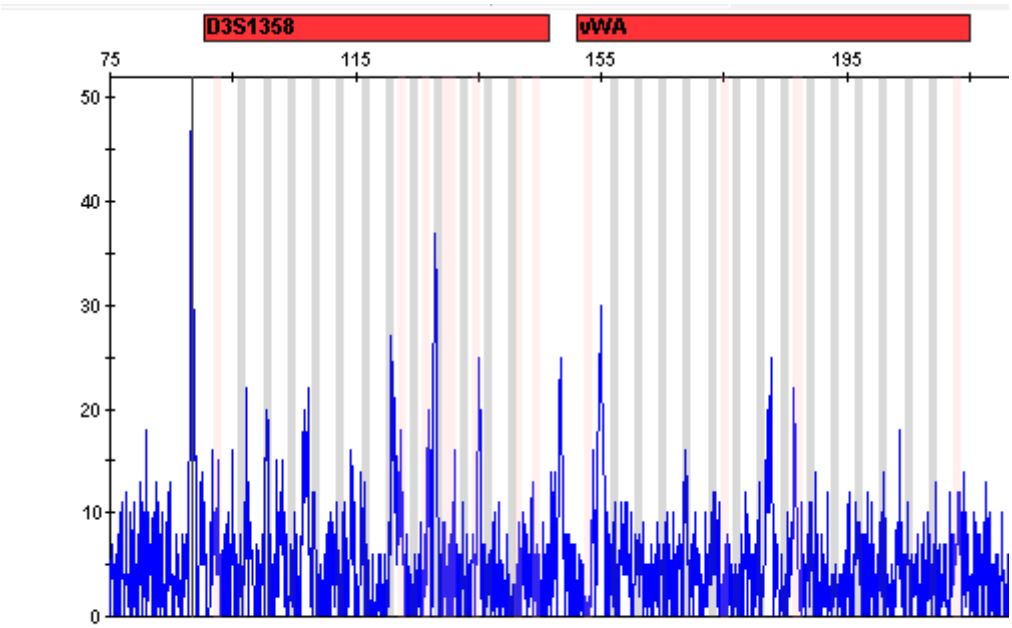

Figure 9: Example of uncontaminated TE^{-4} buffer with bi-weekly washing of capillaries (second run).

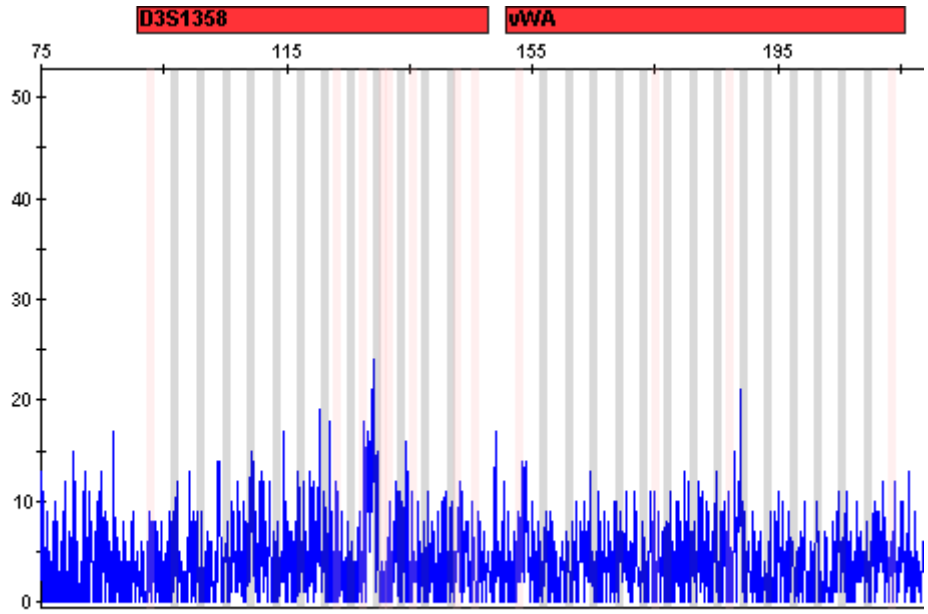

Figure 10: Example of uncontaminated TE^{-4} buffer with weekly washing of capillaries (third run).

Discussion and Conclusion

With the use of the Applied Biosystems™ 3500xL Genetic Analyzer all the studies required in an internal validation were completed. All DNA profiles were typed accurately and precisely after elimination of artifacts and over a wide range of input target DNA amounts. Based on the fact that each study proved the reliability of the instrument and Applied Biosystems™ GlobalFiler™ PCR Amplification Kit, several specific settings and protocols were incorporated into the Standard Operating Procedure (SOP) of the Harris County Institute of Forensic Sciences. First, it was found that 28 cycles on the Applied Biosystems Veriti™ 96- Well Thermal Cycler and a 24 second injection time on the Applied Biosystems™ 3500xL Genetic Analyzer were the optimal settings, as 28 cycles exhibited less blowout and artifacts than the other cycle number, while a 24 second injection time presented better resolution of peaks also with less artifacts than the other injection times. While the manufacturer recommends 29 to 30 cycles, [6] and has not performed a subsequent study recommending the use of 28 cycles on the Applied Biosystems™ 3500xL Genetic Analyzer; there have been other laboratories that have used 28 cycles with success [8,9]. The injection time was not changed from the manufactures recommendations. [10] A new set of analytical threshold RFU settings were made for each dye channel; 50 for blue, 65 for yellow, 45 for green, 55 for red and 60 for purple. Typically, one value is used across all dye channels, as seen in Flores, S. et al, in which the highest RFU value is rounded to the nearest 5 [9]. However, all the dye channels have different baselines, and therefore it was prudent to create separate analytical thresholds for each dye, as the laboratory needs to be as specific as possible in casework. These values were inputted into GeneMapper™ ID-X, and a stochastic threshold was found to assist in the determination of homozygous and heterozygous peaks. Each threshold, analytical and stochastic, will be different between each instrument, a Linear Regression plot is the principal method of finding the stochastic threshold, and in through this

study it was determined to be 400 RFU. [12]. Along with the thresholds, an optimal amount of DNA was found to be 0.5ng, which is widely used in forensic laboratories and is manufacturer's recommendation [6, 8, 9, 12]. At 0. 5ng, one is expected to see peak heights from approximately 1000 to 9000 RFUs. Laboratory specific stutter ratios and mixture interpretation guidelines were also updated in the SOP to include stutter ratios not corresponding to the manufactures guidelines. It was also determined that it is possible to identify mixtures from 2 person mixtures at a ratio of 1:1 to four person mixtures at a 10:1:1:1 ratio. This study does not take into account the use of the Yindel and DYS391 markers found in the Applied Biosystems™ GlobalFiler™ PCR Amplification Kit to aid in mixture deconvolution between mixed male samples, however, this is merely a preliminary study focusing on major and minor contributor ratios. Future studies will be performed in mind of typical casework samples and will include more than one male sample. Further studies will be completed on both stutter and mixture interpretation, as the laboratory will also be conducting an internal validation of STRmix on the Applied Biosystems™ 3500xL Genetic Analyzer. Based on the case-type samples and contamination studies, the SOP regarding regular cleaning of the instrument and use of TE^{-4} buffer will stay the same. In conclusion, the GlobalFiler™ PCR amplification Kit on the Applied Biosystems™ 3500xL Genetic Analyzer is recommended for use in future casework based on the internal validation studies, and the recommendation of Standard Operation Procedures developed.

Appendix A

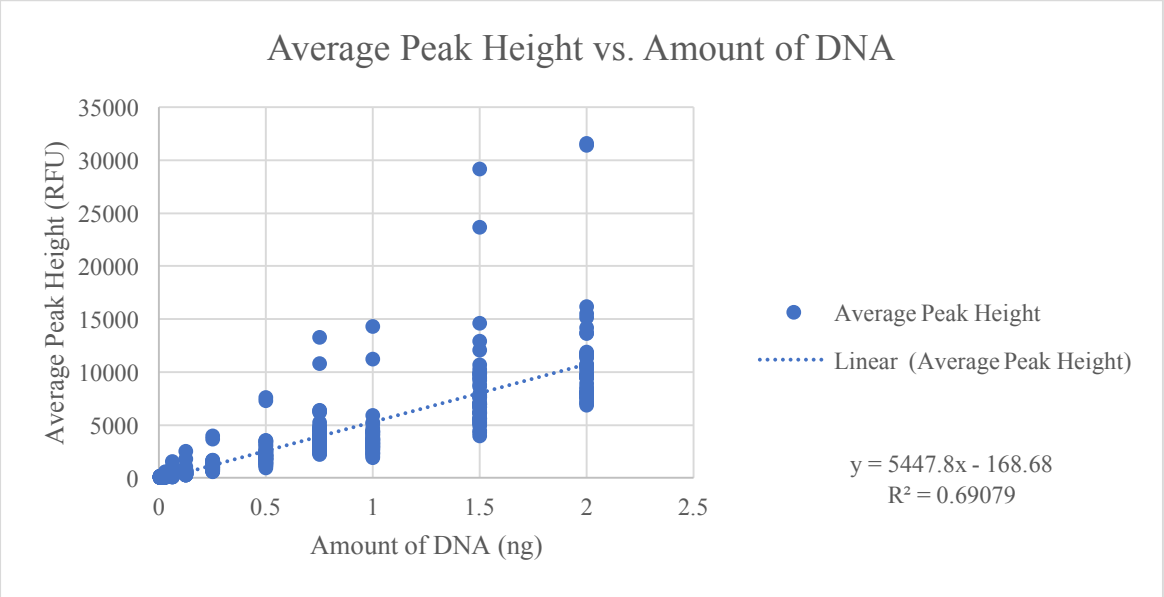

Figure S 1: Peak height vs amount of DNA for VD280 at 28 cycles for 22 seconds.

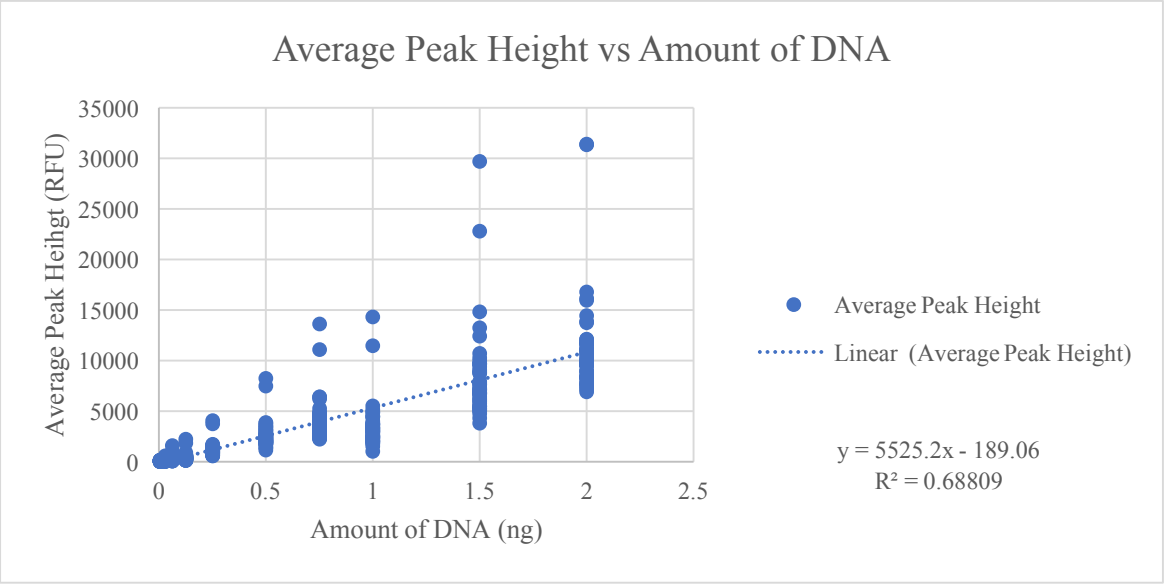

Figure S 2: Peak height vs amount of DNA for VD280 at 28 cycles injected for 23 seconds.

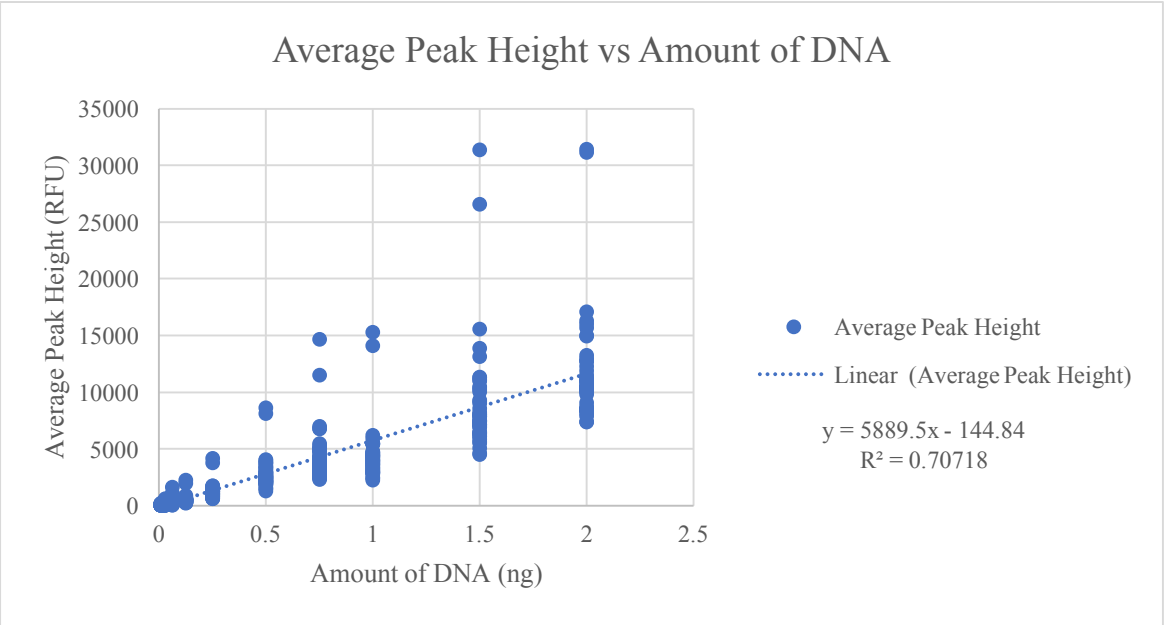

Figure S 3: Peak height vs Amount of DNA for VD280 at 28 cycles injected for 24 seconds.

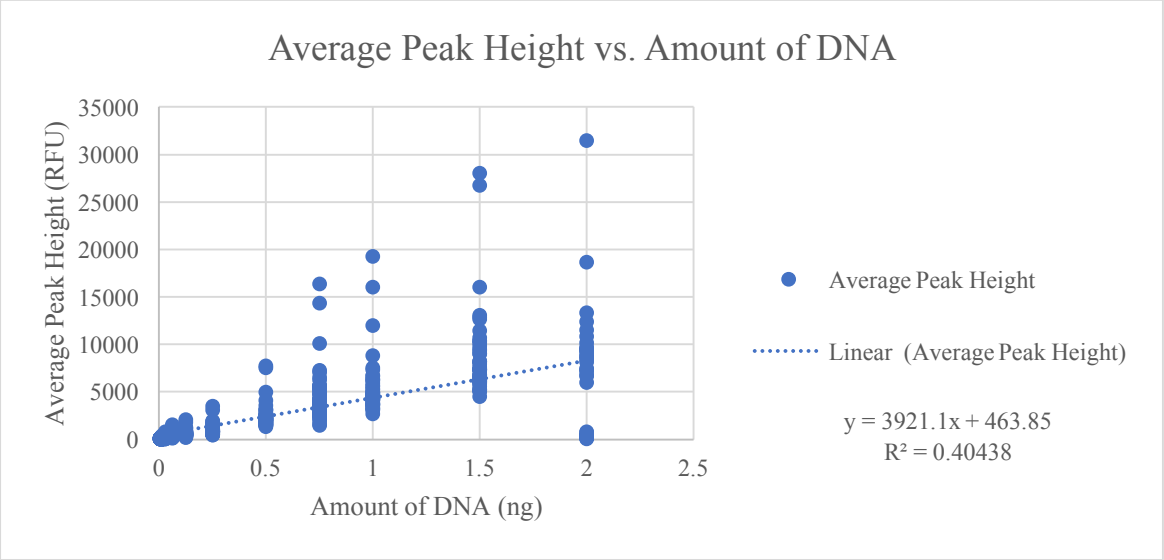

Figure S 4: Peak height vs amount of DNA for VD285 at 28 cycles injected for 22 seconds.

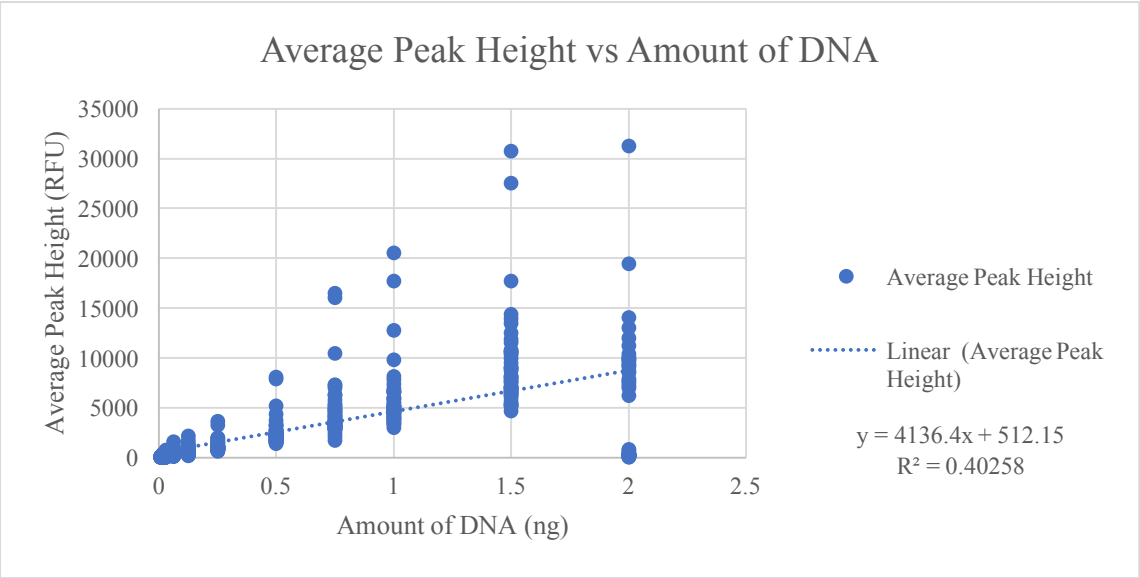

Figure S 5: Peak height vs Amount of DNA for VD285 at 28 cycles injected for 23 seconds.

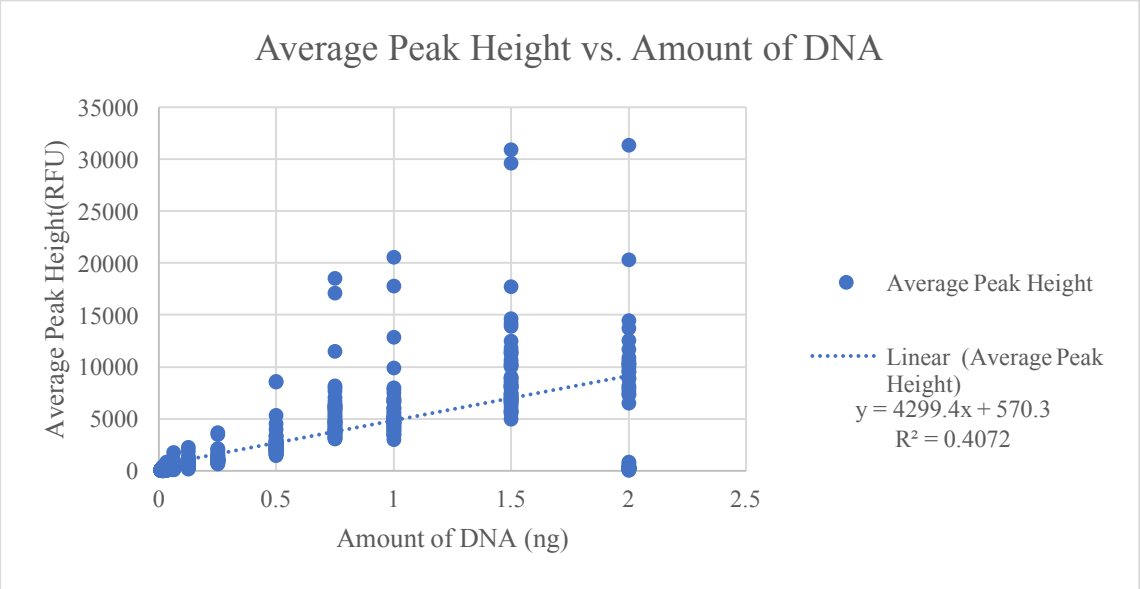

Figure S 6: Peak Height vs. Amount of DNA for VD285 at 28 cycles injected for 24 seconds.

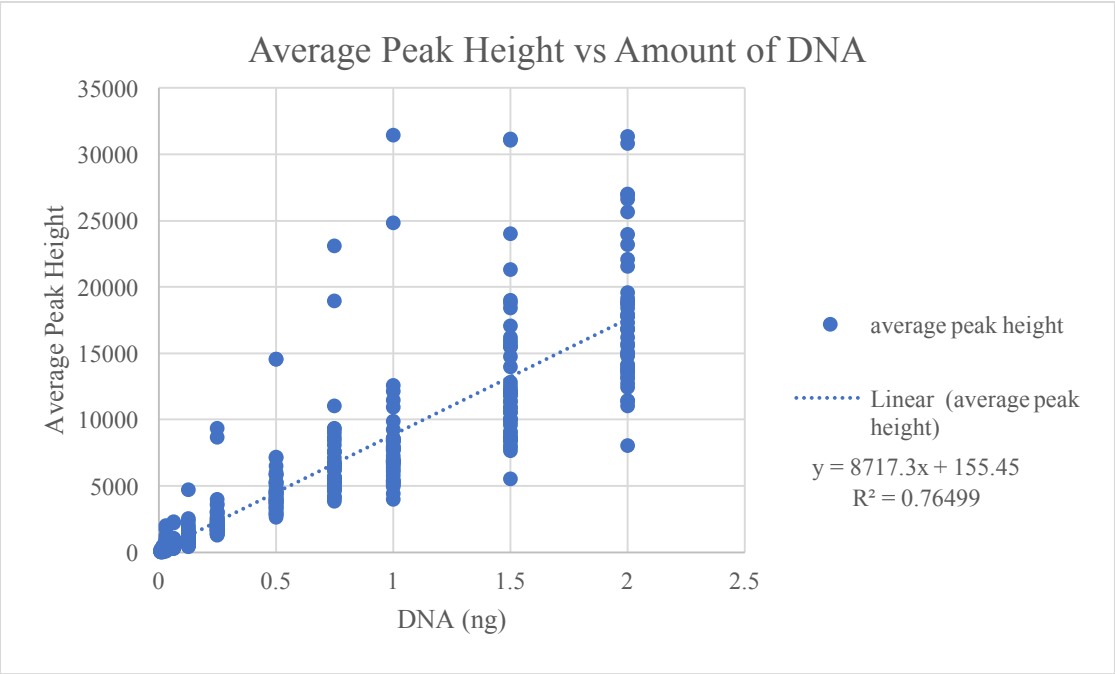

Figure S 7: Peak height vs. Amount of DNA for VD280 at 29 cycles injected for 22 seconds.

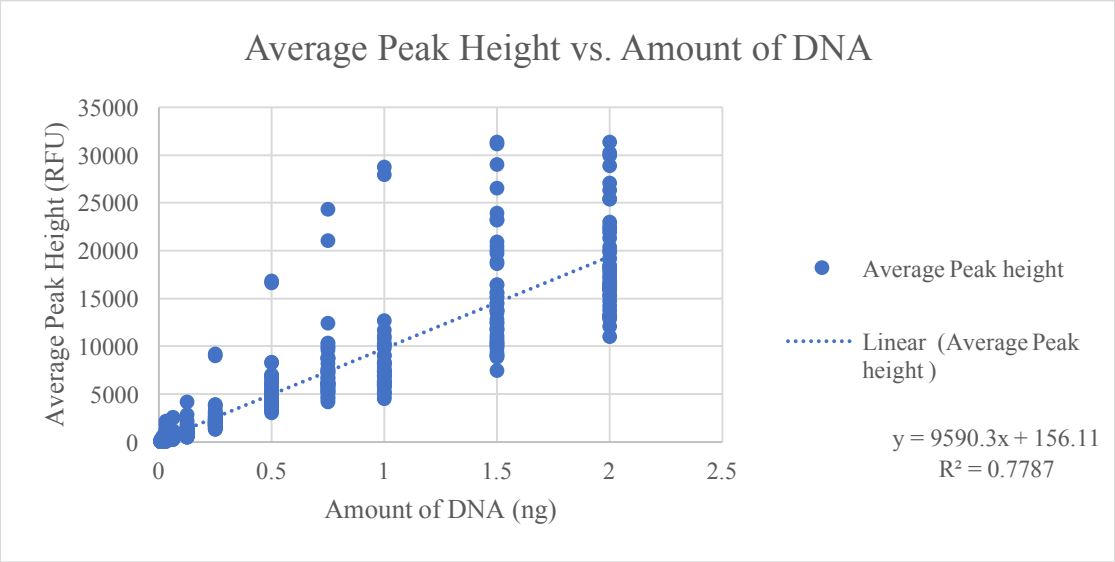

Figure S 8: Peak height vs. amount of DNA for VD280 at 29 cycles injected for 23 seconds.

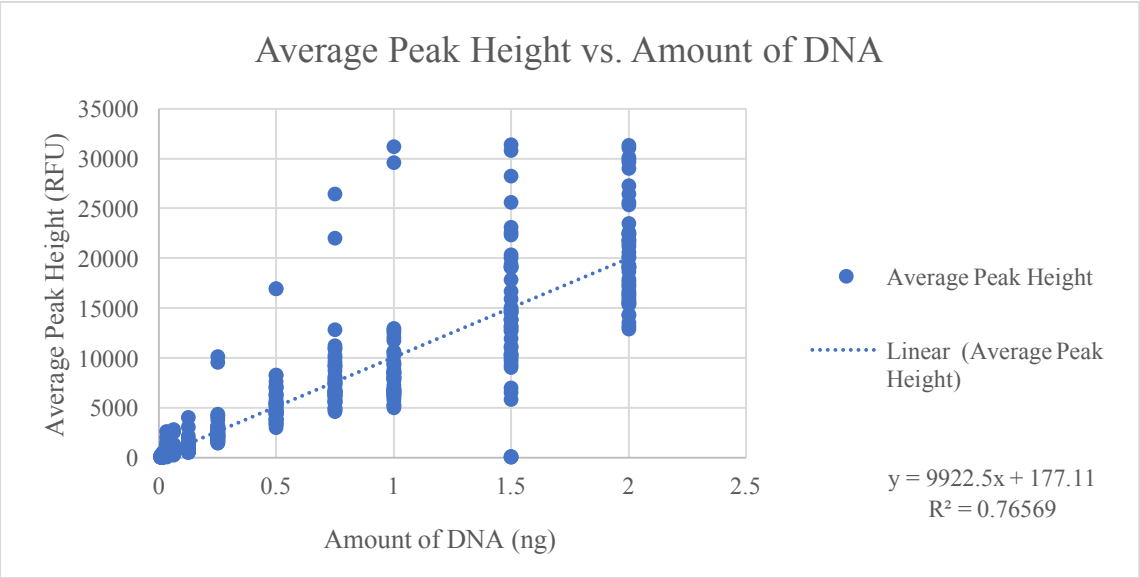

Figure S 9: Peak height vs amount of DNA for VD280 at 29 cycles injected for 24 seconds.

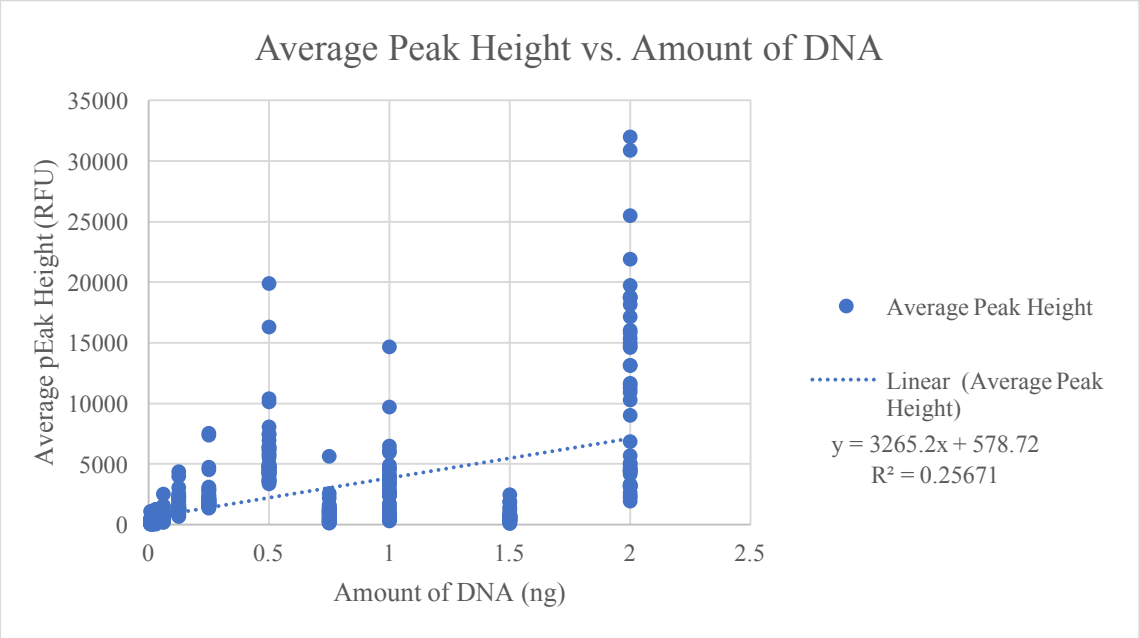

Figure S 10: Peak height vs. Amount of DNA for VD285 at 29 cycles injected for 22 seconds.

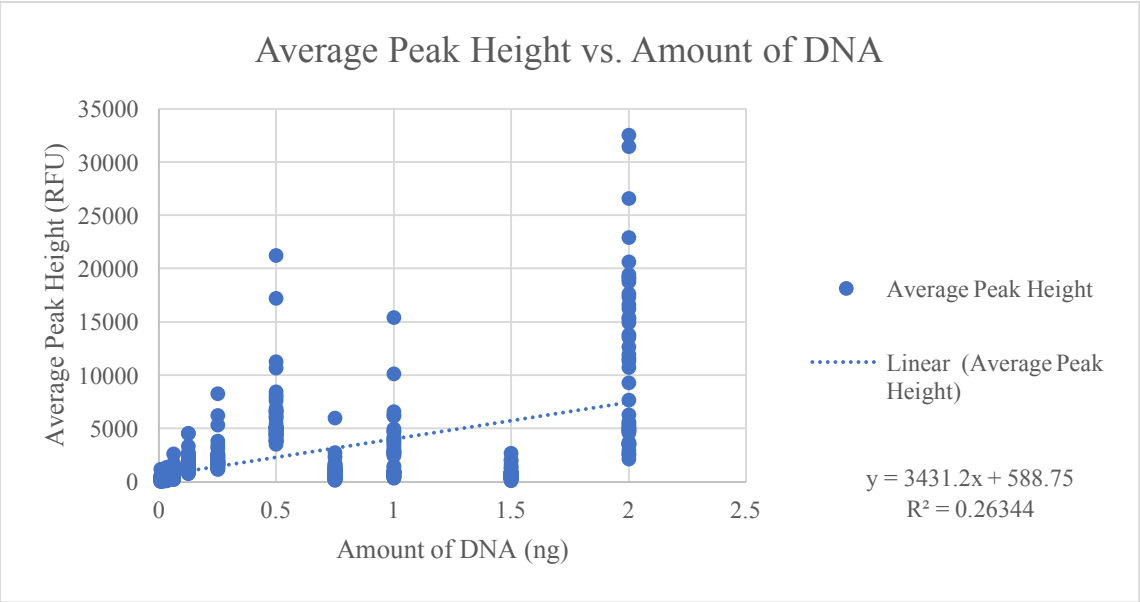

Figure S 11: Peak height vs amount of DNA for VD285 at 29 cycles injected for 23 seconds.

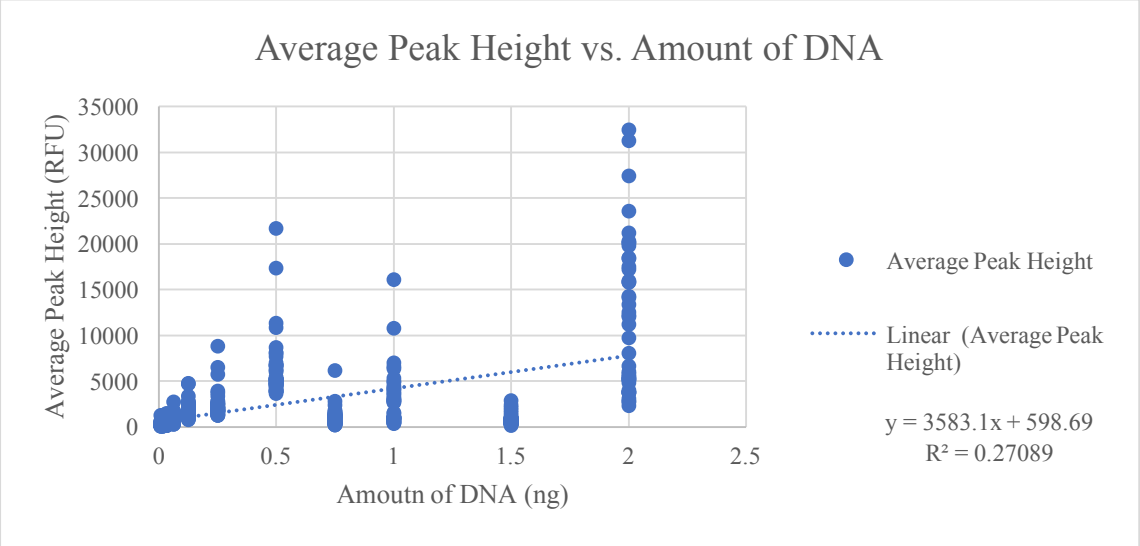

Figure S 12: Peak height vs amount of DNA for VD285 at 29 cycles injected for 24 seconds.

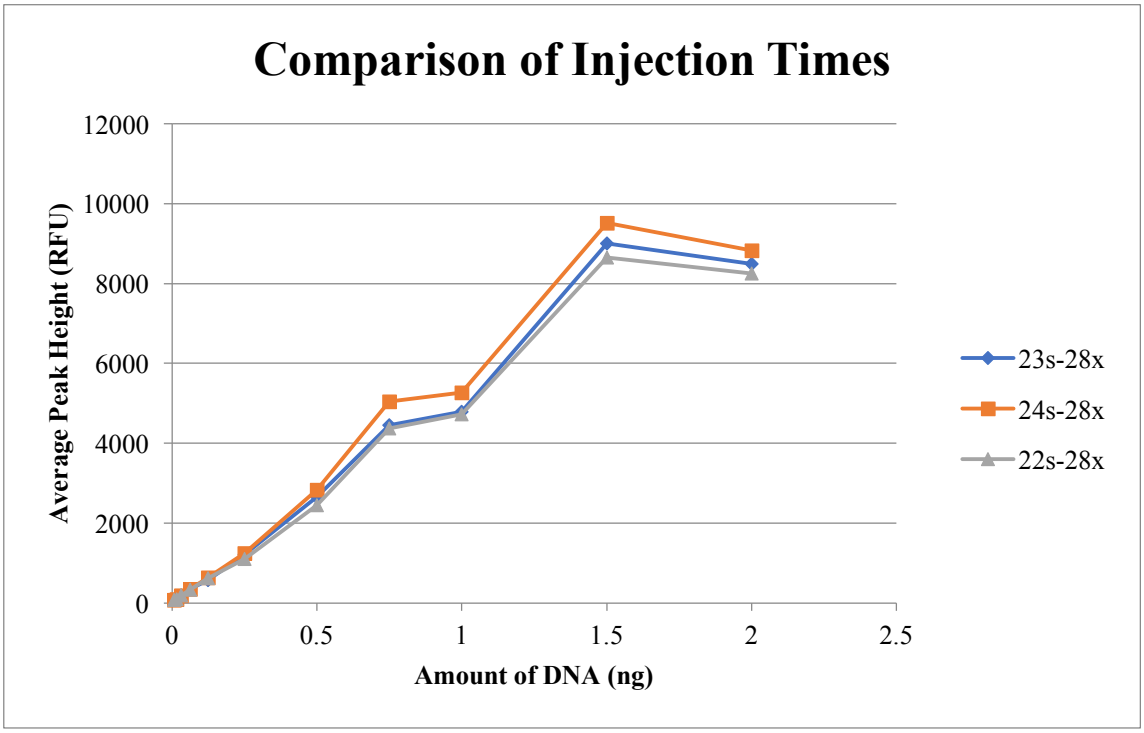

Figure S 13: Peak heights for DNA target at 28 cycles injected for 22, 23, and 24 seconds.

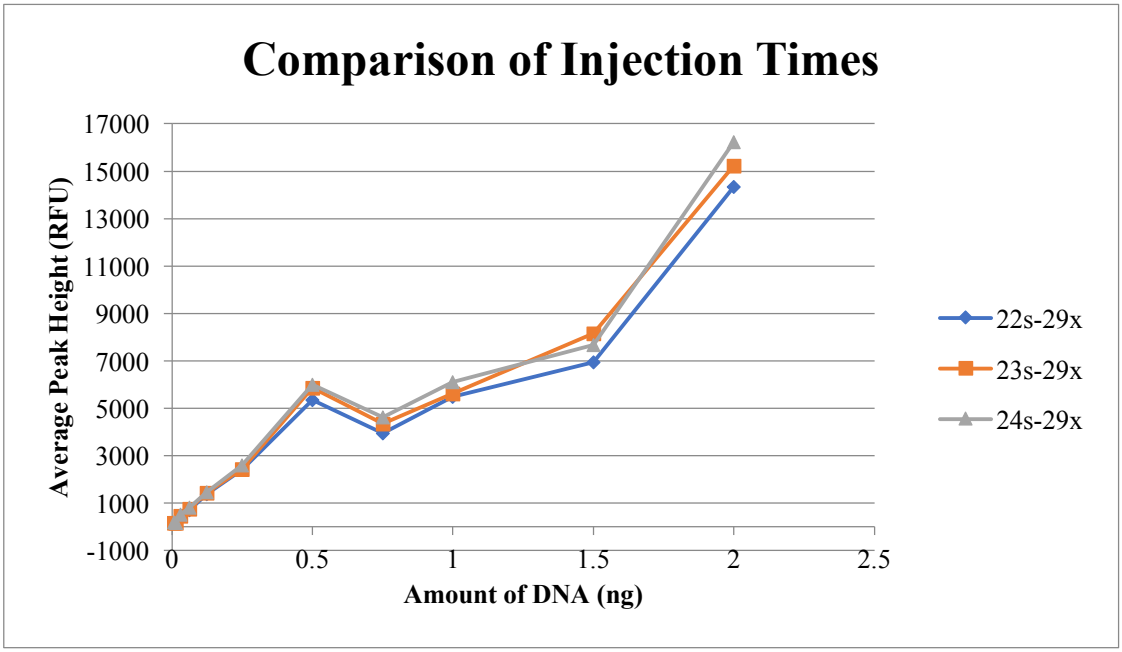

Figure S 14: Peak heights for DNA target at 29 cycles injected for 22, 23, and 24 seconds.

Appendix B

Table S 1: Number of alleles and percent profile detected for VD280 at 28 cycles injected for 22 seconds.

Amplification Target (ng)	Average Alleles Detected	Percent Profile Detected
2	43	100%
1.5	43	100%
1	43	100%
0.75	43	100%
0.5	43	100%
0.25	43	100%
0.125	43	100%
0.062	43	100%
0.031	38.5	90%
0.015	24.5	57%
0.007	21.5	50%

Table S 2: Number of alleles and percent profile detected for VD280 at 28 cycles injected for 23 seconds.

Amplification Target (ng)	Average Alleles Detected	Percent Profile Detected
2	43	100%
1.5	43	100%
1	43	100%
0.75	43	100%
0.5	43	100%
0.25	43	100%
0.125	43	100%
0.062	43	100%
0.031	40	93%
0.015	23	53%
0.007	21	49%

Table S 3: Number of alleles and percentage profile detected for VD280 at 28 cycles injected for 24 seconds.

Amplification Target (ng)	Average Alleles Detected	Percent Profile Detected
2	43	100%
1.5	43	100%
1	43	100%
0.75	43	100%
0.5	43	100%
0.25	43	100%
0.125	43	100%
0.062	43	100%
0.031	39.5	92%
0.015	26	60%
0.007	23	53%

Table S 4: Number of alleles and percent profile detected for VD285 at 28 cycles injected for 22 seconds.

Amplification Target (ng)	Average Alleles Detected	Percent Profile Detected
2	41.5	99%
1.5	42	100%
1	42	100%
0.75	42	100%
0.5	42	100%
0.25	42	100%
0.125	42	100%
0.062	42	100%
0.031	39	93%
0.015	29.5	70%
0.007	21.5	51%

Table S 5: Number of alleles and percent profile detected for VD285 at 28 cycles injected for 23 seconds.

Amplification Target (ng)	Average Alleles Detected	Percent Profile Detected
2	41.5	99%
1.5	42	100%
1	42	100%
0.75	42	100%
0.5	42	100%
0.25	42	100%
0.125	42	100%
0.062	42	100%
0.031	39	93%
0.015	29	69%
0.007	17.5	42%

Table S 6: Number of alleles and percentage profile detected for VD285 at 28 cycles injected for 24 seconds.

Amplification Target (ng)	Average Alleles Detected	Percent Profile Detected
2	41.5	99%
1.5	42	100%
1	42	100%
0.75	42	100%
0.5	42	100%
0.25	42	100%
0.125	42	100%
0.062	42	100%
0.031	39	93%
0.015	30.5	73%
0.007	20.5	49%

Table S 7: Number of alleles and percentage profile detected for VD280 at 29 cycles injected for 22 seconds.

Amplification Target (ng)	Average Alleles Detected	Percent Profile Detected
2	43	100%
1.5	42.5	99%
1	43	100%
0.75	42.5	99%
0.5	43	100%
0.25	43	100%
0.125	43	100%
0.0625	41.5	97%
0.03125	41.5	97%
0.015625	35.5	83%
0.007813	26.5	62%

Table S 8: Number of alleles and percent profile detected for VD280 at 29 cycles injected for 23 seconds.

Amplification Target (ng)	Average Alleles Detected	Percent Profile Detected
2	42	100%
1.5	42	100%
1	42	100%
0.75	42	100%
0.5	41.5	99%
0.25	42	100%
0.125	42	100%
0.0625	42	100%
0.03125	41.5	99%
0.015625	34	81%
0.0078125	13	31%

Table S 9: Number of alleles and percent profile detected for VD280 at 29 cycles injected for 24 seconds.

Amplification Target (ng)	Average Alleles Detected	Percent Profile Detected
2	42	100%
1.5	39	93%
1	42	100%
0.75	42	100%
0.5	42	100%
0.25	42	100%
0.125	42	100%
0.0625	42	100%
0.03125	41.5	99%
0.015625	34	81%
0.0078125	13	31%

Table S 10: Number of alleles and percent profile detected for VD285 at 29 cycles injected for 22 seconds.

Amplification Target (ng)	Average Alleles Detected	Percent Profile Detected
2	42	100%
1.5	42	100%
1	42	100%
0.75	42	100%
0.5	42	100%
0.25	42	100%
0.125	42	100%
0.0625	42	100%
0.03125	41.5	99%
0.015625	33	79%
0.0078125	12.5	30%

Table S 11: number of alleles and percent profile detected for VD285 at 29 cycles injected for 23 seconds.

Amplification Target (ng)	Average Alleles Detected	Percent Profile Detected
2	41	100%
1.5	40.5	99%
1	41	100%
0.75	40.5	99%
0.5	41	100%
0.25	41	100%
0.125	41	100%
0.0625	41	100%
0.03125	39.5	96%
0.015625	33.5	82%
0.0078125	26	63%

Table S 12: Number of alleles and percent profile detected for VD285 at 29 cycles injected for 24 seconds.

Amplification Target (ng)	Average Alleles Detected	Percent Profile Detected
2	41	100%
1.5	40.5	99%
1	40	98%
0.75	40.5	99%
0.5	41	100%
0.25	41	100%
0.125	41	100%
0.0625	41	100%
0.03125	39.5	96%
0.015625	33.5	82%
0.0078125	25.5	62%

Table S 13: Logistical regression table showing the percent dropout for 10 to 500 RFU.

RFU	DO %	RFU	DO %	RFU	DO %
10	0.78801	**180**	0.19115	**350**	0.01480
20	0.75967	**190**	0.16733	**360**	0.01262
30	0.72884	**200**	0.14595	**370**	0.01075
40	0.69565	**210**	0.12688	**380**	0.00915
50	0.66029	**220**	0.10998	**390**	0.00780
60	0.62305	**230**	0.09509	**400**	0.00664
70	0.58429	**240**	0.08203	**410**	0.00565
80	0.54446	**250**	0.07062	**420**	0.00481
90	0.50406	**260**	0.06070	**430**	0.00409
100	0.46360	**270**	0.05209	**440**	0.00348
110	0.42362	**280**	0.04464	**450**	0.00296
120	0.38461	**290**	0.03822	**460**	0.00252
130	0.34703	**300**	0.03269	**470**	0.00214
140	0.31127	**310**	0.02793	**480**	0.00182
150	0.27762	**320**	0.02385	**490**	0.00155
160	0.24631	**330**	0.02035	**500**	0.00132
170	0.21747	**340**	0.01736		

Table S 14: Precision and accuracy study of VD230 with average, 3 times standard deviation, minimum and maximum peak size.

Sample	Marker	Allele 1	Allele 2	Avg 1	SD1	3*SD 1	Min1	Max1	Avg 2	SD2	3*SD2	Min 2	Max 2
VD320	AMEL	X	Y	98.82	0.04	0.13	98.74	98.84	104.89	0.04	0.13	104.85	104.95
	CSF1PO	11		303.11	0.01	0.04	303.09	303.12					
	D10S1248	13	15	106.11	0.02	0.07	106.09	106.13	114.23	0.05	0.16	114.19	114.29
	D12S391	19	23	236.51	0.05	0.16	236.45	236.58	252.58	0.06	0.17	252.51	252.64
	D13S317	11	13	223.18	0.05	0.16	223.14	223.27	231.11	0.04	0.12	231.05	231.16
	D16S539	11	12	252.30	0.01	0.03	252.29	252.31	256.27	0.04	0.13	256.20	256.31
	D18S51	18	19	305.72	0.03	0.08	305.71	145.67	309.74	0.05	0.14	309.71	153.69
	D19S433	13	15	145.66	0.01	0.04	145.64	145.67	153.60	0.06	0.19	153.51	153.69
	D1S1656	15	17.3	184.25	0.05	0.15	184.16	184.29	195.41	0.04	0.11	195.39	195.47
	D21S11	28	30.2	97.46	0.06	0.18	199.68	199.79	209.62	0.09	0.26	209.50	209.74
	D22S1045	11	16	179.30	0.04	0.13	97.38	97.48	112.53	0.01	0.02	112.53	112.54
	D2S1338	16	17	301.37	0.06	0.17	301.33	301.44	305.25	0.02	0.07	305.21	305.26
	D2S441	10	14	84.98	0.05	0.16	84.92	85.02	101.31	0.04	0.12	101.28	101.38
	D3S1358	14	17	117.41	0.04	0.13	117.33	117.43	129.51	0.02	0.07	129.47	129.53
	D5S818	11	12	155.08	0.05	0.14	155.05	155.16	159.18	0.04	0.13	159.16	159.26
	D7S820	9	12	274.66	0.03	0.10	274.60	274.68	286.69	0.06	0.17	286.64	286.77
	D8S1179	13	14	146.93	0.04	0.12	146.88	146.99	151.08	0.04	0.12	151.02	151.13
	DYS391	10		377.55	0.06	0.17	377.45	377.58					
	FGA	20	24	251.70	0.05	0.15	251.65	251.74	267.58	0.01	0.04	267.56	267.60
	SE33	16	17	354.17	0.05	0.16	354.11	354.23	358.24	0.06	0.19	358.19	358.32
	TH01	6	9.3	187.37	0.02	0.05	187.36	187.40	202.53	0.01	0.02	202.52	202.53
	TPOX	8	11	351.09	0.03	0.09	351.06	351.12	363.21	0.06	0.17	363.11	363.24
	vWA	16	17	177.08	0.05	0.14	177.06	177.17	181.15	0.04	0.13	181.07	181.17
	Yindel	2		86.71	0.05	0.14	86.63	86.74					

Table S 15: Precision and accuracy study of VD344 with average. 3 times standard deviation, minimum and maximum peak size.

Sample	Marker	Allele 1	Allele 2	Avg 1	SD1	3*SD 1	Min1	Max1	Avg 2	SD2	3*SD2	Min 2	Max 2
VD344	AMEL	X		98.82	0.04	0.12	98.75	98.84					
	CSF1PO	10	13	299.19	0.05	0.15	299.10	299.21	311.03	0.07	0.21	310.97	311.11
	D10S1248	13	17	106.13	0.05	0.15	106.08	106.18	122.02	0.01	0.02	122.01	122.02
	D12S391	15	19	220.58	0.06	0.18	220.54	220.65	236.51	0.05	0.14	236.47	236.59
	D13S317	10	13	218.99	0.05	0.16	218.90	219.02	231.13	0.03	0.08	231.10	231.17
	D16S539	9	12	244.16	0.05	0.14	244.11	244.22	256.27	0.03	0.08	256.25	256.31
	D18S51	12	16	281.81	0.05	0.14	281.78	141.74	297.82	0.06	0.17	297.75	143.69
	D19S433	12	12.2	141.70	0.05	0.15	141.64	141.74	143.68	0.01	0.03	143.67	143.69
	D1S1656	13	16	176.08	0.04	0.12	176.04	176.12	188.33	0.05	0.15	188.26	188.38
	D21S11	30	31.2	112.52	0.02	0.07	207.71	207.76	213.66	0.04	0.13	213.58	213.68
	D22S1045	16		188.69	0.04	0.12	112.45	112.54					
	D2S1338	19	20	313.25	0.07	0.20	313.18	313.31	317.40	0.05	0.16	317.35	317.47
	D2S441	10	11	84.92	0.04	0.12	84.90	84.99	89.12	0.05	0.15	89.07	89.17
	D3S1358	16	18	125.25	0.04	0.13	125.20	125.30	133.57	0.04	0.13	133.52	133.62
	D5S818	12		159.14	0.04	0.13	159.06	159.16					
	D7S820	11	12	282.70	0.04	0.13	282.67	282.78	286.64	0.06	0.18	286.57	286.72
	D8S1179	10	14	134.50	0.02	0.05	134.48	134.51	151.10	0.03	0.10	151.06	151.13
	DYS391												
	FGA	19	22	247.67	0.08	0.25	247.55	247.76	259.59	0.05	0.14	259.56	259.67
	SE33	15	16	350.12	0.03	0.10	350.09	350.16	354.23	0.07	0.20	354.15	354.33
	TH01	7	8	191.38	0.04	0.11	191.35	191.43	195.44	0.05	0.15	195.40	195.50
	TPOX	8	11	351.08	0.03	0.10	351.05	351.12	363.23	0.05	0.14	363.21	363.32
	vWA	17	18	181.13	0.06	0.18	181.06	181.17	185.27	0.06	0.19	185.19	185.34
	Yindel												

Table S 16: Precision and accuracy study of VD396 with average, 3 times standard deviation, minimum and maximum peak size.

Sample	Marker	Allele 1	Allele 2	Avg 1	SD1	3*SD 1	Min1	Max1	Avg 2	SD2	3*SD2	Min 2	Max 2
VD396	AMEL	X		98.83	0.00	0.00	98.83	98.83					
	CSF1PO	11		303.14	0.02	0.05	303.12	303.15					
	D10S1248	14	15	110.26	0.03	0.09	110.20	110.27	114.27	0.04	0.13	114.19	114.29
	D12S391	19	20	236.57	0.01	0.03	236.57	236.59	240.57	0.00	0.00	240.57	240.57
	D13S317	11		223.10	0.06	0.17	223.06	223.17					
	D16S539	13		260.22	0.00	0.00	260.22	260.22					
	D18S51	12	15	281.79	0.05	0.15	281.70	149.68	293.84	0.04	0.13	293.77	0.00
	D19S433	14		149.64	0.03	0.10	149.62	149.68					
	D1S1656	11	15.3	168.03	0.04	0.12	167.98	168.09	187.30	0.02	0.05	187.29	187.33
	D21S11	28	29.2	109.57	0.00	0.01	199.67	199.68	205.67	0.03	0.08	205.62	205.69
	D22S1045	15	16	181.66	0.03	0.09	109.51	109.58	112.53	0.00	0.01	112.53	112.54
	D2S1338	17	19	305.31	0.04	0.13	305.26	305.38	313.27	0.05	0.15	313.18	313.30
	D2S441	10	11	84.97	0.04	0.12	84.94	85.04	89.10	0.05	0.16	89.06	89.16
	D3S1358	15	16	121.47	0.04	0.12	121.45	121.54	125.49	0.05	0.16	125.43	125.53
	D5S818	13		163.15	0.06	0.17	163.10	163.22					
	D7S820	8	10	270.64	0.06	0.17	270.55	270.69	278.70	0.06	0.18	278.63	278.75
	D8S1179	11	14	138.52	0.06	0.17	138.47	138.58	151.08	0.04	0.12	151.03	151.14
	DYS391												
	FGA	19	24	247.69	0.06	0.19	247.62	247.74	267.57	0.07	0.20	267.50	267.64
	SE33	17	24.2	358.30	0.01	0.03	358.29	358.31	388.47	0.07	0.22	388.41	388.55
	TH01	6	7	187.35	0.06	0.18	187.29	187.41	191.41	0.05	0.15	191.37	191.49
	TPOX	11	12	363.25	0.01	0.04	363.23	363.26	367.23	0.02	0.07	367.19	367.24
	vWA	17		181.12	0.06	0.18	181.07	181.18					
	Yindel												

Table S 17: Precision and accuracy study of VD457 with average, 3 times standard deviation, minimum and maximum peak size.

Sample	Marker	Allele 1	Allele 2	Avg 1	SD1	3*SD 1	Min1	Max1	Avg 2	SD2	3*SD2	Min 2	Max 2
VD457	AMEL	X	Y	98.78	0.044721	0.16	98.74	98.84	104.88	0.04	0.09	104.85	104.93
	CSF1PO	12		307.04	0.013416	0.11	307.00	307.08					
	D10S1248	13	16	106.12	0.021909	0.08	106.08	106.14	118.10	0.05	0.05	118.09	118.13
	D12S391	16	18	224.60	0.054129	0.18	224.53	224.66	232.59	0.06	0.27	232.45	232.70
	D13S317	11	12	223.06	0.052154	0.16	223.02	223.15	227.10	0.04	0.20	227.02	227.16
	D16S539	9	12	244.12	0.010954	0.13	244.05	244.16	256.21	0.04	0.09	256.20	256.27
	D18S51	14	18	289.84	0.026833	0.14	289.81	145.72	305.77	0.05	0.31	305.65	0.00
	D19S433	13		145.71	0.014142	0.09	145.65	145.72		0.06			
	D1S1656	13	16	176.18	0.0498	0.16	176.14	176.25	188.32	0.04	0.13	188.28	188.39
	D21S11	27	30	109.54	0.058566	0.05	195.60	195.63	207.65	0.09	0.15	207.59	207.73
	D22S1045	15	16	178.39	0.044721	0.21	109.44	109.62	112.52	0.01	0.12	112.45	112.55
	D2S1338	17		305.32	0.057619	0.33	305.21	305.47		0.02			
	D2S441	11	15	89.11	0.054772	0.12	89.07	89.17	105.43	0.04	0.08	105.39	105.45
	D3S1358	14	15	117.40	0.044721	0.13	117.33	117.43	121.38	0.02	0.15	121.34	121.44
	D5S818	13		163.19	0.045056	0.04	163.17	163.21		0.04			
	D7S820	10	11	278.78	0.033912	0.14	278.75	278.86	282.71	0.06	0.15	282.67	282.79
	D8S1179	14	15	151.08	0.039749	0.08	151.07	151.13	155.21	0.04	0.03	155.19	155.21
	DYS391	10		377.52	0.056125	0.20	377.45	377.59					
	FGA	22	23	259.57	0.049295	0.01	259.56	259.57	263.58	0.01	0.14	263.55	263.66
	SE33	22	26.2	378.38	0.054955	0.32	378.19	378.44	396.58	0.06	0.35	396.40	396.68
	TH01	7	9	191.43	0.017889	0.13	191.37	191.47	199.51	0.01	0.17	199.46	199.57
	TPOX	8		351.06	0.031305	0.13	351.00	351.12		0.06			
	vWA	17	18	181.11	0.04827	0.17	181.06	181.17	185.23	0.04	0.14	185.20	185.31
	Yindel	2		86.71	0.046043	0.13813	86.63	86.74					

Table S 18: Precision and accuracy study of VD474 with average, 3 times standard deviation, minimum and maximum peak size.

Sample	Marker	Allele 1	Allele 2	Avg 1	SD1	3*SD 1	Min1	Max1	Avg 2	SD2	3*SD2	Min 2	Max 2
VD474	AMEL	X		98.80	0.05	0.15	98.74	98.84					
	CSF1PO	12		307.04	0.05	0.16	306.96	307.08					
	D10S1248	14		110.21	0.04	0.11	110.18	110.27					
	D12S391	18	24	232.55	0.07	0.22	232.50	232.68	256.35	0.03	0.10	256.31	256.38
	D13S317	8	12	210.88	0.09	0.26	210.78	210.99	227.09	0.06	0.19	227.04	227.20
	D16S539	11	13	252.24	0.05	0.15	252.20	252.29	260.26	0.06	0.18	260.22	260.33
	D18S51	14	15	289.83	0.03	0.09	289.81	145.67	293.87	0.06	0.17	293.77	149.68
	D19S433	13	14	145.65	0.01	0.04	145.64	145.67	149.62	0.05	0.16	149.57	149.68
	D1S1656	12	13	172.05	0.02	0.06	172.04	172.09	176.19	0.05	0.16	176.12	176.23
	D21S11	30	32.2	109.54	0.05	0.16	207.63	207.78	217.73	0.06	0.18	217.66	217.81
	D22S1045	15	16	188.09	0.05	0.16	109.49	109.61	112.52	0.05	0.14	112.43	112.54
	D2S1338	18		309.25	0.02	0.05	309.24	309.28					
	D2S441	11.3	15	92.30	0.04	0.11	92.24	92.34	105.46	0.05	0.16	105.38	105.50
	D3S1358	16	17	125.52	0.01	0.04	125.51	125.54	129.53	0.02	0.05	129.52	129.56
	D5S818	11	13	155.05	0.05	0.14	154.97	155.08	163.16	0.06	0.17	163.10	163.21
	D7S820	11	12	282.72	0.04	0.13	282.70	282.80	286.69	0.05	0.15	286.65	286.76
	D8S1179	13	15	146.93	0.05	0.16	146.88	146.99	155.22	0.05	0.16	155.18	155.29
	DYS391												
	FGA	22	23	259.56	0.01	0.02	259.56	259.57	263.57	0.07	0.20	263.47	263.66
	SE33	24.2	29.2	388.50	0.07	0.22	388.42	388.62	408.56	0.09	0.26	408.43	408.64
	TH01	8	9.3	195.46	0.04	0.13	195.39	195.50	202.50	0.04	0.12	202.44	202.53
	TPOX	9		355.13	0.05	0.15	355.07	355.17					
	vWA	15	17	173.07	0.05	0.16	172.98	173.12	181.13	0.05	0.16	181.07	181.17
	Yindel												

Table S 19: Precision study of VD477 with average, standard deviation, 3 times standard deviation, minimum and maximum peak size.

Sample	Marker	Allele 1	Allele 2	Avg 1	SD1	3*SD 1	Min1	Max1	Avg 2	SD2	3*SD2	Min 2	Max 2
VD477	AMEL	X		98.82	0.04	0.13	98.74	98.84					
	CSF1PO	10	11	299.14	0.06	0.18	299.10	299.21	303.09	0.08	0.23	303.02	303.20
	D10S1248	14	18	110.19	0.04	0.12	110.12	110.22	125.93	0.04	0.13	125.88	125.98
	D12S391	18	23	232.56	0.01	0.03	232.56	232.58	252.62	0.00	0.01	252.61	252.62
	D13S317	11	13	223.19	0.06	0.17	223.13	223.24	231.18	0.05	0.14	231.15	231.26
	D16S539	11	13	252.27	0.05	0.15	252.18	252.29	260.20	0.05	0.15	260.11	260.22
	D18S51	12	16	281.81	0.05	0.14	281.78	145.72	297.82	0.06	0.18	297.75	153.66
	D19S433	13	15	145.69	0.04	0.13	145.64	145.72	153.62	0.06	0.18	153.52	153.66
	D1S1656	16	17	188.34	0.06	0.17	188.27	188.38	192.36	0.06	0.18	192.31	192.42
	D21S11	30		97.47	0.08	0.24	207.54	207.71					
	D22S1045	11	15	185.63	0.05	0.14	97.39	97.50	109.53	0.02	0.05	109.51	109.54
	D2S1338	18	20	309.20	0.06	0.18	309.15	309.27	317.43	0.05	0.15	317.35	317.47
	D2S441	11	14	89.12	0.05	0.14	89.07	89.17	101.33	0.05	0.15	101.27	101.38
	D3S1358	14	17	117.39	0.05	0.16	117.33	117.43	129.54	0.04	0.13	129.48	129.59
	D5S818	11		155.10	0.02	0.05	155.08	155.11					
	D7S820	10	11	278.70	0.06	0.18	278.65	278.76	282.70	0.04	0.13	282.67	282.78
	D8S1179	13	14	146.94	0.04	0.11	146.88	146.98	151.07	0.04	0.11	151.02	151.12
	DYS391												
	FGA	20	24	251.72	0.05	0.15	251.63	251.75	267.59	0.06	0.17	267.55	267.67
	SE33	15	20	350.18	0.03	0.09	350.16	350.22	370.37	0.03	0.09	370.32	370.39
	TH01	7	9.3	191.36	0.00	0.00	191.36	191.36	202.53	0.01	0.03	202.51	202.53
	TPOX	8		351.07	0.05	0.16	351.00	351.12					
	vWA	17	19	181.14	0.04	0.13	181.06	181.16	189.29	0.05	0.16	189.23	189.33
	Yindel												

Table S 20: Reproducibility of VD320 with each marker of each allele compared with each analyst.

Sample		Analyst 1		Analyst 2	
VD320	Marker	Allele 1	Allele 2	Allele 1	Allele 2
	AMEL	X	Y	X	Y
	CSF1PO	11		11	
	D10S1248	13	15	13	15
	D12S391	19	23	19	23
	D13S317	11	13	11	13
	D16S539	11	12	11	12
	D18S51	18	19	18	19
	D19S433	13	15	13	15
	D1S1656	15	17.3	15	17.3
	D21S11	28	30.2	28	30.2
	D22S1045	11	16	11	16
	D2S1338	16	17	16	17
	D2S441	10	14	10	14
	D3S1358	14	17	14	17
	D5S818	11	12	11	12
	D7S820	9	12	9	12
	D8S1179	13	14	13	14
	DYS391	10		10	
	FGA	20	24	20	24
	SE33	16	17	16	17
	TH01	6	9.3	6	9.3
	TPOX	8	11	8	11
	vWA	16	17	16	17
	Yindel	2		2	

Table S 21: Reproducibility of VD344 with each marker of each allele compared with each analyst.

Sample		**Analyst 1**		**Analyst 2**	
VD344	Marker	Allele 1	Allele 2	Allele 1	Allele 2
	AMEL	X		X	
	CSF1PO	10	13	10	13
	D10S1248	13	17	13	17
	D12S391	15	19	15	19
	D13S317	10	13	10	13
	D16S539	9	12	9	12
	D18S51	12	16	12	16
	D19S433	12	12.2	12	12.2
	D1S1656	13	16	13	16
	D21S11	30	31.2	30	31.2
	D22S1045	16		16	
	D2S1338	19	20	19	20
	D2S441	10	11	10	11
	D3S1358	16	18	16	18
	D5S818	12		12	
	D7S820	11	12	11	12
	D8S1179	10	14	10	14
	DYS391				
	FGA	19	22	19	22
	SE33	15	16	15	16
	TH01	7	8	7	8
	TPOX	8	11	8	11
	vWA	17	18	17	18
	Yindel				

Table S 22: Reproducibility of VD396 with each marker of each allele compared with each analyst.

Sample		**Analyst 1**		**Analyst 2**	
VD396	Marker	Allele 1	Allele 2	Allele 1	Allele 2
	AMEL	X		X	
	CSF1PO	11		11	
	D10S1248	14	15	14	15
	D12S391	19	20	19	20
	D13S317	11		11	
	D16S539	13		13	
	D18S51	12	15	12	15
	D19S433	14		14	
	D1S1656	11	15.3	11	15.3
	D21S11	28	29.2	28	29.2
	D22S1045	15	16	15	16
	D2S1338	17	19	17	19
	D2S441	10	11	10	11
	D3S1358	15	16	15	16
	D5S818	13		13	
	D7S820	8	10	8	10
	D8S1179	11	14	11	14
	DYS391				
	FGA	19	24	19	24
	SE33	17	24.2	17	24.2
	TH01	6	7	6	7
	TPOX	11	12	11	12
	vWA	17		17	
	Yindel				

Table S 23: Reproducibility of VD457 with each marker of each allele compared with each analyst.

Sample		Analyst 1		Analyst 2	
VD457	Marker	Allele 1	Allele 2	Allele 1	Allele 2
	AMEL	X	Y	X	Y
	CSF1PO	12		12	
	D10S1248	13	16	13	16
	D12S391	16	18	16	18
	D13S317	11	12	11	12
	D16S539	9	12	9	12
	D18S51	14	18	14	18
	D19S433	13		13	
	D1S1656	13	16	13	16
	D21S11	27	30	27	30
	D22S1045	15	16	15	16
	D2S1338	17		17	
	D2S441	11	15	11	15
	D3S1358	14	15	14	15
	D5S818	13		13	
	D7S820	10	11	10	11
	D8S1179	14	15	14	15
	DYS391	10		10	
	FGA	22	23	22	23
	SE33	22	26.2	22	26.2
	TH01	7	9	7	9
	TPOX	8		8	
	vWA	17	18	17	18
	Yindel	2		2	

Table S 24: Reproducibility of VD474 with each marker of each allele compared with each analyst.

Sample		**Analyst 1**		**Analyst 2**	
VD474	Marker	Allele 1	Allele 2	Allele 1	Allele 2
	AMEL	X		X	
	CSF1PO	12		12	
	D10S1248	14		14	
	D12S391	18	24	18	24
	D13S317	8	12	8	12
	D16S539	11	13	11	13
	D18S51	14	15	14	15
	D19S433	13	14	13	14
	D1S1656	12	13	12	13
	D21S11	30	32.2	30	32.2
	D22S1045	15	16	15	16
	D2S1338	18		18	
	D2S441	11.3	15	11.3	15
	D3S1358	16	17	16	17
	D5S818	11	13	11	13
	D7S820	11	12	11	12
	D8S1179	13	15	13	15
	DYS391				
	FGA	22	23	22	23
	SE33	24.2	29.2	24.2	29.2
	TH01	8	9.3	8	9.3
	TPOX	9		9	
	vWA	15	17	15	17
	Yindel				

Table S 25: Reproducibility of VD477 with each marker of each allele compared with each analyst.

Sample		**Analyst 1**		**Analyst 2**	
VD477	Marker	Allele 1	Allele 2	Allele 1	Allele 2
	AMEL	X		X	
	CSF1PO	10	11	10	11
	D10S1248	14	18	14	18
	D12S391	18	23	18	23
	D13S317	11	13	11	13
	D16S539	11	13	11	13
	D18S51	12	16	12	16
	D19S433	13	15	13	15
	D1S1656	16	17	16	17
	D21S11	30		30	
	D22S1045	11	15	11	15
	D2S1338	18	20	18	20
	D2S441	11	14	11	14
	D3S1358	14	17	14	17
	D5S818	11		11	
	D7S820	10	11	10	11
	D8S1179	13	14	13	14
	DYS391				
	FGA	20	24	20	24
	SE33	15	20	15	20
	TH01	7	9.3	7	9.3
	TPOX	8		8	
	vWA	17	19	17	19
	Yindel				

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