MISSISSIPPI FORENSICS LABORATORY

Validation studies

YFiler Plus PCR Amplification Kit, cat no. 4484678, 4482730.

Applied Biosystems 3500 Genetic Analyzer, SN 27124-180

ProFlex Thermal Cycler, SN 297803672

3500 Data Collection Software, v. 3

GeneMapper ID-X, v 1.5 Software

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Biosciences/ DNA Section

Validation studies performed by Mary Jones Dukes, MS, MBA

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YFiler Plus PCR Amplification Kit Validation Summary

BACKGROUND

In February 2016, Applied Biosystems by Thermo Fisher Scientific was employed by the Mississippi Forensics Laboratory (MSFL) to validate the YFiler Plus PCR Amplification kit. The comprehensive validation summary was received at the MSFL, but the chemistry was never released for forensic DNA casework operations. Given the potential for manufacturer's changes to chemistry, capillary electrophoresis data collection software upgrades, inclusion of multiple ABI 3500 instruments, and new revision of the Quality Assurance Standards (QAS) for Forensic DNA testing laboratories (revision July 1, 2020), the Yfiler Plus PCR amplification kit validation was repeated.

Validation studies were drafted by Mary Jones Dukes, MS, MBA (CSSGB) and approved by the DNA Technical Leader, Alexandria Bradley BS, MFS, ABC-CC. Validation studies began in September 2022 and were performed and analyzed by Mary Jones Dukes, MS, MBA (CSSGB).

In accordance with the FBI's Quality Assurance Standards (QAS) for Forensic DNA testing laboratories (effective July 1, 2020), the following categories should be addressed in an internal validation:

1. Known & non-probative, or mock evidence: QAS 8.3.1(1)
2. Precision and accuracy studies QAS 8.3.1(2)
3. Sensitivity & Stochastic: QAS 8.3.1(3)
4. Mixture Studies: QAS 8.3.1(4)
5. Contamination assessment studies: QAS 8.3.1 (5)
6. Certified Reference Material: QAS 8.4
7. Concordance/Comparison to Original Procedures: QAS 8.5

This document serves as the YFiler Plus PCR Amplification kit validation summary. Methods performed and the results obtained are presented within this summary. Supporting data will be maintained at the MSFL.

METHODS

The methods outlined in this validation summary were manually prepared or aliquoted. Assisted pipetting was occasionally employed using the QIAgility liquid handling instrument where feasible, and is considered as an extension of manual methods for the purpose of the YFiler Plus PCR kit validation.

DNA Sources

EZ1 Extracted DNA

Fresh, liquid saliva was collected in sterile, 50 mL tubes from anonymous males (M01, M02) and anonymous female donors (F01, F02). Two (2) mL aliquots of saliva from each donor were prepared in duplicate using 2.0 mL tubes. The 2.0 mL tubes were centrifuged at 10,000rpm for 5 minutes. Supernatant was decanted from pelleted cells. Additional saliva was subsequently added to a maximum volume in the 2.0mL tubes; the tubes were centrifuged as before; and, the liquid was decanted from the pelleted cells. The pelleted material was utilized for DNA extraction using 440 µL buffer G2 (QIAGEN), 20 µL Proteinase K (QIAGEN; 20 mg/mL), and 40 µL, 1.0M Dithiothreitol (DTT) per sample. All samples were incubated in a Thermal shaker at 56°C for 60 minutes using 700rpm. Following incubation, the samples were prepared according to the QIAGEN EZ1 DNA Investigator handbook (rev. 04/2009) and purified using the "Large Volume" protocol. Samples were eluted using 40 µL of TE-4. Following purification, the samples were pooled into appropriate single donor tubes.

A neat male (M03) blood sample was diluted with TE-4 (190.11 µL DNA + 309.89 µL TE-4) to create dilution A which was serially diluted in a four-fold dilution series (125 µL DNA + 375 µL TE-4) to yield a total of eight serial dilutions (A-H). All samples were incubated in a Thermal shaker at 56°C for 60 minutes using 700rpm. Following incubation, the samples were prepared according to the QIAGEN EZ1 DNA Investigator handbook (rev. 04/2009) and purified using the "Large Volume" protocol. Samples were eluted using 40 µL of TE-4.

Commercially Available DNA

DNA Control 007 utilized in the studies was provided in the YFiler Plus PCR amplification kit (cat no. 4484678, 4482730).

Quantification using Quantiplex Pro

DNA samples and serial dilutions of male control DNA were prepared for quantification according to the Investigator Quantiplex Pro Handbook (rev. April, 2017) using the QIAGEN Quantiplex Pro real-time PCR kit. The master mix was prepared to include 9.0 µL of reaction mix and 9.0 µL primer mix per sample. Eighteen (18) µL of master mix were aliquoted per well and either 2 µL of each standard or unknown were added to deliver a final volume of 20 µL per reaction. Real-time PCR was conducted using an ABI 7500 instrument (SN 275006241) and HID Real-Time PCR Analysis Software, v. 1.2.

DNA samples were quantified in duplicate or triplicate and the average values used for calculations. Resultant data were analyzed using manufacturer suggested protocols. The autosomal DNA quantification result for sample F01_091922 was determined to be ~14.5 ng/µL. The autosomal DNA quantification result for sample M01_091922 was determined to be ~12.3 ng/µL. The "M01" EZ1 DNA extracted sample was used for Sensitivity study 2.

Quantification using Quantifiler Trio

DNA samples were setup for quantification and results analyzed according to the MSFL Bioscience Section DNA STANDARD OPERATING PROCEDURES, 18.23.

Amplification using the YFiler Plus PCR Amplification kit

DNA samples were amplified on the ProFlex 96-well PCR System (Cat no. 4484075; 297803672) following steps outlined in the YFiler Plus PCR amplification Kit User Guide (Publication Number 4485610; Revision C). Master mix was prepared to allow 10.0 µL master mix and 5.0 µL primer set per sample. Fifteen (15) µL of master mix were aliquoted per well and either 10 µL of DNA template or nuclease-free water were added to deliver a final volume of 25 µL. per well per reaction.

The cycling conditions were according to extracted DNA and were as follows:

Table 1

Initial incubation step	Denature	Anneal/Extend	Final Extension	Final hold
HOLD	CYCLE (30)	CYCLE (30)	HOLD	HOLD

95 ºC, 1 min	94 ºC, 4 sec	61.5 ºC, 1 min	60 ºC, 22 minutes	4 ºC, ∞

The Table 1 above is a quick reference to the cycling parameters used in the YFiler Plus PCR amplification kit validation.

Capillary Electrophoresis (CE) of YFiler Plus Amplicon

Unless stated otherwise, sample amplicons were processed on the Applied Biosystems 3500 genetic according to the Yfiler Plus PCR Amplification Kit User Guide. Briefly, Size Standard GS600_LIZ (60-460), Partial size ranges (60bp- 460bp), Local Southern Method, Baseline Window Pts 30, and Peak window size 13 were used for the 3500 QC protocol, and 0.4 µL of GeneScan 600 Size Standard 2.0, 9.6 µL Hi-Di Formamide, and 1 µL sample amplicon or allelic ladder to appropriate wells. Following this, plates were denatured for 3 minutes at 95°C and snap chilled at ~ 4°C for 3 minutes. Samples were injected using as recommended for the 3500/3500xl instrument protocol parameters of 1.2 kV/ 12 seconds, dye set J6, and run module HID36_POP4.

A quick summary of the validation chemistry and conditions may be referenced below:

Table 2

This validation plan is prepared according to the FBI Quality Assurance Standards for Forensic DNA Testing Laboratories (effective July 1, 2020; Standard 8.3.1)

CHEMISTRY	
Extraction	QIAGEN EZ1 DNA Investigator chemistry & instruments
Quantification	QIAGEN Quantiplex PRO Quantifiler Trio DNA Quantification Kit
Amplification	Yfiler™ Plus PCR Amplification Kit
INSTRUMENTS	
Real-time PCR system	ABI 7500 Real-Time PCR System; SN: 275006241
Thermal Cycler	ProFlex PCR System Thermal Cycler; SN: 297803672
Genetic Analyzer	ABI 3500 Genetic Analyzer; SN: 27124-180
SOFTWARE	
Quantification	HID Real-Time PCR Analysis Software, v. 1.2
Data Collection	3500 Series Data Collection Software, v. 3
Analysis	GeneMapper ID-X Software, v.1.5
TEST PARAMETERS	
Quantification	40 cycles
Amplification	30 cycles
Injection	1.2 kV; 12 seconds

The Table 2 above is a quick reference to the chemistry, instruments, software, and test parameters used in the YFiler Plus PCR amplification kit validation.

GeneMapper ID-X, v. 1.5 Analysis

The GeneMapper ID-X Software was setup for analysis according to the Yfiler Plus PCR Amplification Kit User Guide. The version 4 panels, bins, and stutter files were used for analysis. Data was exported from the GMID-X software and analyzed using Microsoft Office Professional Plus 2019 Excel, version 1808 (Build 10392.20029).

QAS STUDIES

Minimum Threshold & Contamination Assessment (QAS 8.3.1.5)

Amplification blanks (AB) and extraction blanks (EB) co-extracted alongside donor DNA samples were amplified with the YFiler PCR amplification kit using maximum volume (10 uL) of either diluent or eluate. These samples were used to mimic the MSFL current forensic DNA casework procedures. The blank controls were amplified concurrently with the Sensitivity study samples. These samples were used to verify presence or absence of male DNA in amplification processes and to determine the minimum threshold for analysis as determined by baseline (noise).

The minimum threshold for analysis was determined using a GeneMapper ID-X, 1.5 analysis setting of 1 RFU and accessing the background detected. The average RFU detected across EB and AB samples was determined and the AVG, SD, and CV

was calculated. Plus/Minus 3 sigma and plus/minus 5 sigma were calculated and evaluated in determining the minimum threshold for analysis.

Sensitivity & Stochastic QAS 8.3.1(1)-Study 1
Serial dilutions of Yfiler™ Plus DNA Control (2 ng/uL) were prepared to final template DNA of 0.004pg. Dilutions were prepared to allow for 10 uL of DNA into the reaction. Each template quantity was amplified in triplicate. (N=36) The average Total RFU, locus peak balance for 385I/II and 389I/II, and % Y-STR haplotype for each dilution series was evaluated.

Sensitivity & Stochastic QAS 8.3.1(1)- Study 2
Serial dilutions of EZ1 extracted male DNA normalized to 2 ng/uL stock DNA were prepared to final template DNA of 0.004pg. Dilutions were prepared to allow for 10 uL of DNA into the reaction. Each template quantity was amplified in triplicate. (N=36) The average Total RFU, locus peak balance for 385I/II and 389I/II, and % Y-STR haplotype for each dilution series was evaluated.

Sensitivity & Stochastic QAS 8.3.1(1)- Study 3
Each dilution was extracted and quantified in duplicate. After quantification, the replicates were pooled and again quantified prior to amplification then genotyped. Serially diluted DNA samples were manually setup for quantification using both Quantiplex PRO and Quantifiler Trio. Following quantification, either 1.0 ng in 10 uL sample input, or maximum volume of 10 uL if a total of 1.0ng was unavailble, was used for YFIler Plus amplification. The % of alleles detected (or profile completion) was compared and the average peak height for replicate samples was calculated.

Mixture Studies QAS 8.3.1(4)

Study 1 – 2 persons (female 1: male 1)
Resultant quantification values from the EZ1/2 extracted male DNA and female DNA stock samples were used to prepare samples for the Mixture study 1. The following mixture ratios (F1:M1) were created and amplified in duplicate: 99:1, 49:1, 29:1, 19:1, 14:1, 9:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:9, 1:14, 1:19, 1:29, 1:49, and 1:99. For this study, the male DNA was held constant at 1 ng. A total of 19 mixture samples (Mix 1, Mix 2, Mix 3, Mix 4,……Mix 19) were amplified in duplicate with inclusion of both a positive control (DNA 007) and amplification blank. The mixture samples in study 1 were first analyzed using 75 RFU with the GeneMapper ID-X, v 5 software. Data was exported and analyzed via Excel. Additional analysis using 100 RFU was performed given the results of the 75 RFU analysis.

Study 2 – 2 persons (male 1: male 2)
Resultant quantification values from the EZ1 extracted male DNA stock samples will be used to prepare samples for the Mixture, study 2. The following mixture ratios (M:M) to be created and amplified: 99:1, 49:1, 29:1, 19:1, 14:1, 9:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:9, 1:14, 1:19, 1:29, 1:49, and 1:99.Male DNA will be held constant at 1 ng per reaction, with each male DNA calculated in respective ratios. A total of 19 mixture samples (Mix2.1, Mix 2.2, Mix 2.3, Mix 2.4,……Mix 2.19) amplified in duplicate. The mixture samples in study 1 were first analyzed using 75 RFU with the GeneMapper ID-X, v 5 software. Data was exported and analyzed via Excel. Additional analysis using 100 RFU was performed given the results of the 75 RFU analysis.

Study 3- 3 persons (female 1: male 1: male 2)
Resultant quantification values from the EZ1 extracted male DNA stock samples will be used to prepare samples for the Mixture, study 2. The following mixture ratios (M:M) to be created and amplified: 99:1, 49:1, 29:1, 19:1, 14:1, 9:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:9, 1:14, 1:19, 1:29, 1:49, and 1:99. Male DNA will be held constant at 1 ng per reaction, with each male DNA calculated in respective ratios. A background of female DNA will be added to each sample, resulting in 25 ng total in each sample amplified. A total of 19 mixture samples (Mix3.1, Mix 3.2, Mix 3.3, Mix 3.4,……Mix 3.19) amplified in duplicate.

Known & Mock Evidence QAS 8.3.1(1)

Mock evidence samples will be quantified using Quantiplex PRO and Quantifiler Trio, and will be amplified at a target of 1.0ng or maximum template if less than 1.0ng is available from the sample. Resultant Y-STR profiles will be evaluated according to the established and analytical thresholds.

Accuracy: NIST 2391d: QAS 8.4

The Standard Reference Material® 2391d PCR Based DNA Profiling Standard (components B, C, D) will be amplified, each in duplicate. Resultant Y-STR profiles will be compared against those published for the Reference Material® 2391d Certified Haplotypes, 28 Y-STR Loci publication, located in Table 5.

Concordance/Comparison to Original Procedures: QAS 8.5

Previously processed samples to be used and compared to new procedure. NIST SRM 2391d (components B, C, D) used for this study. Resultant Y-STR profiles will be compared to those previously obtained using the AmpFlSTR Yfiler PCR amplification kit.

RESULTS

Minimum Threshold & Contamination Assessment QAS 8.3.1 (5)

Nine samples were concurrently amplified with the sensitivity studies using the Yfiler Plus PCR amplification kit. Capillary electrophoresis was performed according to manufacturer's recommendation of 1.2kV for 12 seconds (Appendix A) and employed a GeneMapper ID-X, v 1.5 analysis threshold of 1 RFU. The signal of detection for all 5 dyes (B, G, P, R, and Y) were exported to Excel, and the average RFU, Standard Deviation, and +3 and +6 Sigma calculations were determined. Results are depicted below in Table 1.

Table 3

	Minimum Threshold (RFU)					
# samples * (loci/dye); (N=9)	Dye	Avg RFU	Std Dev.	+3 ð	+6 ð	Chosen
45	B	9.54	6.09	27.82	46.10	75
54	G	18.53	6.08	36.76	54.99	75
45	P	17.99	5.48	34.44	50.89	75
36	R	15.77	5.10	31.08	46.39	75
45	Y	9.28	3.20	18.87	28.46	75

Table 3 above defines the average RFU, Standard Deviation, +3 sigma, +6 sigma for each dye set when nine samples were amplified with the Yfiler Plus PCR amplification kit. The detected signal (across all loci) for the blue (B), green (G), purple (P), red (R) and (Y) dye channels were used to determine the minimum threshold for analysis and contamination assessment. A 75 RFU was chosen for analysis and was employed for GMID-X analysis of the Sensitivity studies.

Sensitivity & Stochastic QAS 8.3.1(3)- Study 1

The sensitivity and stochastic study (study 1) was performed using the YFiler Plus PCR amplification control DNA 007. Serial dilutions of the sample were performed. The results are defined in the figures and tables below.

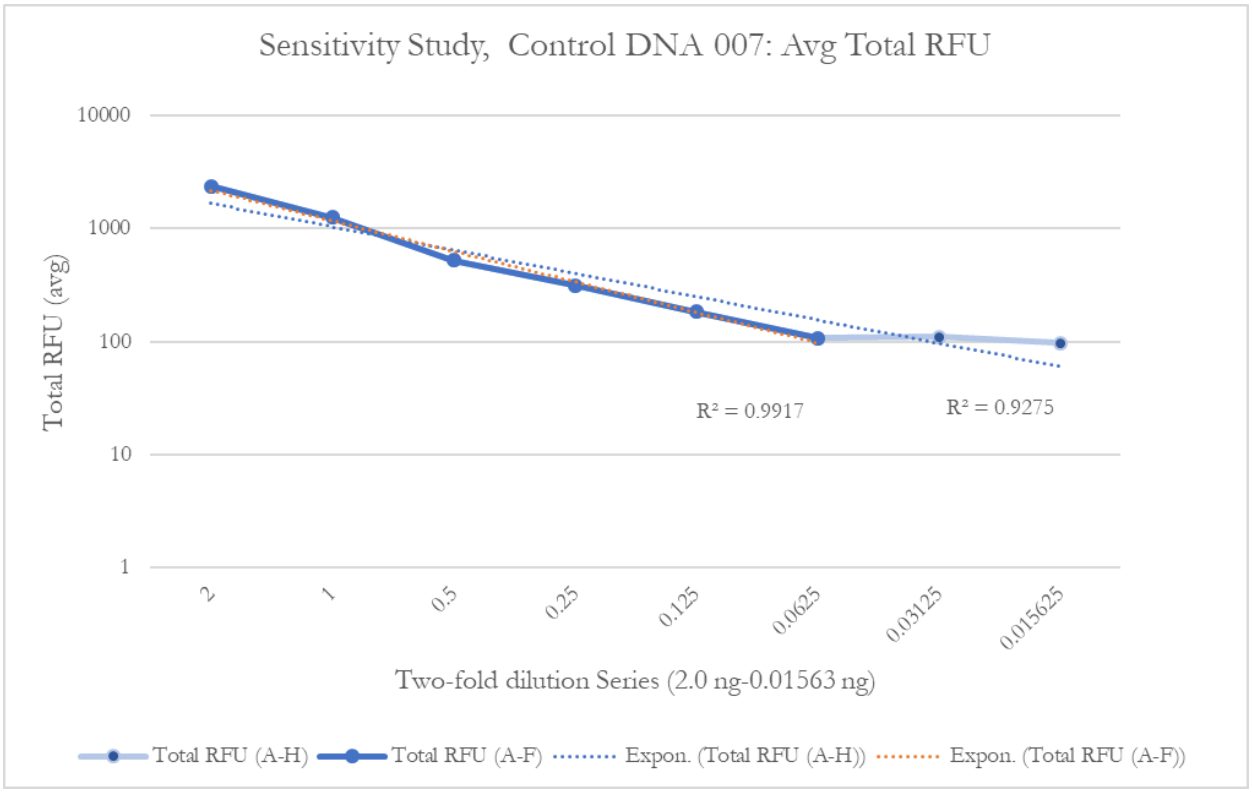

Figure 1 depicts a line chart of above the average total RFU and R^2 values for all dyes detected when a two-fold dilution series of Control DNA 007 was amplified using the AmpFlSTR Yfiler Plus PCR amplification kit. Two-fold dilutions ranging from 2.0ng to 0.01563 ng were performed and amplified using the AmpFlSTR YFiler Plus PCR amplification kit. The resultant R^2 value =0.9917 for dilutions from 2.0ng to 0.0625 ng (A-F); the resultant R^2 = 0.9275 for dilutions A-H. Dilution I (0.0078 ng) was undetected for all triplicate reactions. Analysis was performed using 75 RFU and the GeneMapper ID-X, version 1.5 software.

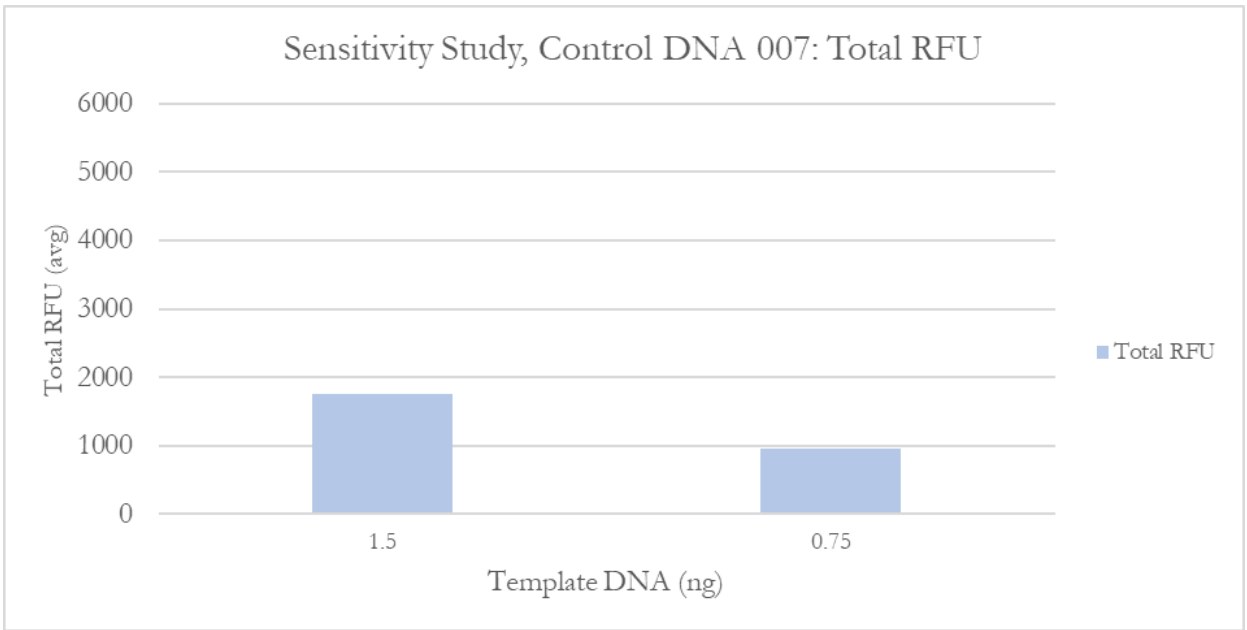

Figure 2 depicts a bar chart of the average total RFU for all dyes detected when 1.5ng and 0.75 ng template DNA (Control DNA 007) was amplified using the AmpFlSTR Yfiler Plus PCR amplification kit. A two-fold dilution between the two samples was performed and the samples were triplicate amplified using the AmpFlSTR YFiler Plus PCR amplification kit. Analysis was performed using 75 RFU and the GeneMapper ID-X, version 1.5 software.

Table 4

		DYS576			DYS389I 1			DYS635			DYS389II			DYS627		
		19			13			24			29			21		
Template	Sample ID	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height
2	SENS007_A1	19	109.62	1695	13	163.5	2991	24	227.8	2274	29	285.5	1558	21	363.4	2897
	SENS007_A2	19	109.63	2010	13	163.4	3355	24	227.8	2444	29	285.5	1827	21	363.4	3299
	SENS007_A3	19	109.67	2504	13	163.4	4446	24	227.8	3427	29	285.5	2357	21	363.4	4536
1	SENS007_B1	19	109.64	1386	13	163.5	1857	24	227.9	1236	29	285.5	1057	21	363.4	1916
	SENS007_B2	19	109.69	772	13	163.5	1191	24	227.8	821	29	285.5	654	21	363.4	805
	SENS007_B3	19	109.61	1413	13	163.5	2003	24	227.9	2079	29	285.4	1109	21	363.4	1949
0.5	SENS007_C1	19	109.61	495	13	163.5	537	24	227.8	417	29	285.5	361	21	363.5	618
	SENS007_C2	19	109.78	589	13	163.5	1189	24	228	1035	29	285.4	660	21	363.7	1012
	SENS007_C3	19	109.69	362	13	163.4	443	24	227.9	413	29	285.4	236	21	363.6	650
0.25	SENS007_D1	19	109.73	231	13	163.4	513	24	227.9	383	29	285.5	240	21	363.7	584
	SENS007_D2	19	109.72	318	13	163.4	646	24	227.9	275	29	285.4	386	21	363.7	456
	SENS007_D3	19	109.71	427	13	163.5	426	24	227.9	288	29	285.4	242	21	363.7	359
0.125	SENS007_E1	19	109.71	314	13	163.4	307	24	227.9	222	29	285.4	180	21	363.7	266
	SENS007_E2	19	109.7	95	13	163.4	237	24	227.9	128	29	285.3	146	21	363.7	237
	SENS007_E3	19	109.69	112	13	163.4	170				29	285.4	81	21	363.7	396
0.0625	SENS007_F1	19	109.78	78	13	163.4	89	24	227.8	84				21	363.8	210
	SENS007_F2	19	109.76	87	13	163.4	110							21	363.7	89
	SENS007_F3				13	163.4	96							21	363.7	97
0.03125	SENS007_G1															
	SENS007_G2															
	SENS007_G3															
0.015625	SENS007_H1															
	SENS007_H2															
	SENS007_H3															
0.0078125	SENS007_I1															
	SENS007_I2															
	SENS007_I3															
0.00390625	SENS007_J1															
	SENS007_J2															
	SENS007_J3															
1.5	SENS007_K1	19	109.76	1854	13	163.4	2821	24	227.9	2085	29	285.4	1620	21	363.7	3435
	SENS007_K2	19	109.76	1353	13	163.5	2629	24	227.9	2060	29	285.4	1423	21	363.6	2025
	SENS007_K3	19	109.76	1214	13	163.4	2537	24	227.9	1860	29	285.4	1250	21	363.7	2594
0.75	SENS007_L1	19	109.71	913	13	163.4	1596	24	227.9	1167	29	285.3	920	21	363.8	1551
	SENS007_L2	19	109.75	806	13	163.5	1412	24	228	1154	29	285.4	742	21	363.7	1324
	SENS007_L3	19	109.76	792	13	163.5	1160	24	227.9	806	29	285.4	708	21	363.7	1126

Table 4 shows the blue dye (6-FAM™) channel results in the Sensitivity study 1 for the positive control DNA (007) serially diluted 2-fold and amplified using the YFiler Plus PCR amplification kit. Drop out of alleles began occurring at 125pg. Template amounts tested from the serially diluted samples were 2.0, 1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, and 0.00390625, with corresponding sample ID's of A-J. Additionally, 1.5 and 0.75 ng were also tested (sample identifiers K and L) and alleles detected were measured. Each sample was amplified in triplicate.

Table 5

		DYS460			DYS458			DYS19			YGATAH4			DYS448			DYS391		
		11			17			14			12			19			11		
Template	Sample ID	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height
2	SENS007_A1	11	96.52	1359	17	143.5	2843	15	208.2	2379	13	256.3	1634	19	307.6	1240	11	377.5	1346
	SENS007_A2	11	96.46	1703	17	143.5	2774	15	208.2	2395	13	256.3	1623	19	307.5	1729	11	377.4	2084
	SENS007_A3	11	96.48	2334	17	143.5	3515	15	208.1	3621	13	256.3	2360	19	307.6	2127	11	377.4	2327
1	SENS007_B1	11	96.45	1111	17	143.5	1502	15	208.2	1390	13	256.3	1045	19	307.5	1045	11	377.5	1201
	SENS007_B2	11	96.51	742	17	143.4	1090	15	208.2	948	13	256.3	824	19	307.6	827	11	377.5	877
	SENS007_B3	11	96.5	1028	17	143.5	1971	15	208.1	1787	13	256.2	1094	19	307.6	1443	11	377.4	1205
0.5	SENS007_C1	11	96.5	412	17	143.5	571	15	208.2	628	13	256.3	343	19	307.6	370	11	377.5	415
	SENS007_C2	11	96.43	510	17	143.6	893	15	208.2	795	13	256.3	521	19	307.8	634	11	377.6	361
	SENS007_C3	11	96.44	234	17	143.6	398	15	208.2	439	13	256.3	292	19	307.8	276	11	377.5	232
0.25	SENS007_D1	11	96.46	205	17	143.6	356	15	208.2	233	13	256.4	208	19	307.6	245	11	377.6	231
	SENS007_D2	11	96.39	402	17	143.6	464	15	208.2	336	13	256.3	341	19	307.7	310	11	377.7	137
	SENS007_D3	11	96.46	238	17	143.7	584	15	208.2	381	13	256.3	290	19	307.7	255	11	377.5	368
0.125	SENS007_E1	11	96.38	186	17	143.6	441	15	208.2	302	13	256.2	254	19	307.7	203	11	377.6	293
	SENS007_E2	11	96.51	147	17	143.6	212	15	208.2	155	13	256.4	104	19	307.8	239	11	377.6	111
	SENS007_E3	11	96.46	115	17	143.7	157	15	208.2	144	13	256.2	106	19	307.7	108			
0.0625	SENS007_F1													19	307.7	77	11	377.6	133
	SENS007_F2																		
	SENS007_F3				17	143.6	121							19	307.8	77			
0.03125	SENS007_G1																		
	SENS007_G2													19	307.7	77			
	SENS007_G3																		
0.015625	SENS007_H1																		
	SENS007_H2																11	377.6	130
	SENS007_H3																		
0.0078125	SENS007_I1																		
	SENS007_I2																		
	SENS007_I3																		
0.00390625	SENS007_J1																		
	SENS007_J2																		
	SENS007_J3																		
1.5	SENS007_K1	11	96.42	1371	17	143.6	2564	15	208.2	2006	13	256.4	1535	19	307.6	1699	11	377.6	1685
	SENS007_K2	11	96.45	1338	17	143.6	2260	15	208.3	2011	13	256.3	1414	19	307.7	1441	11	377.6	1487
	SENS007_K3	11	96.36	726	17	143.7	1770	15	208.2	1335	13	256.4	1059	19	307.6	962	11	377.6	1394
0.75	SENS007_L1	11	96.45	798	17	143.7	1524	15	208.3	1396	13	256.3	663	19	307.7	968	11	377.5	769
	SENS007_L2	11	96.36	709	17	143.6	1014	15	208.3	1467	13	256.3	675	19	307.7	682	11	377.6	1221
	SENS007_L3	11	96.43	532	17	143.7	1056	15	208.3	909	13	256.3	376	19	307.7	763	11	377.5	508

Table 5 shows the green dye (VIC™) channel results in the Sensitivity study 1 for the positive control DNA (007) serially diluted 2-fold and amplified using the YFiler Plus PCR amplification kit. Drop out of alleles began occurring at 125pg. Template amounts tested from the serially diluted samples were 2.0, 1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, and 0.00390625, with corresponding sample ID's of A-J. Additionally, 1.5 and 0.75 ng were also tested (sample identifiers K and L) and alleles detected were measured. Each sample was amplified in triplicate.

Table 6

		DYS456			DYS390 1			DYS438 1			DYS392			DYS518 1		
		15			24			12			13			37		
Template	Sample ID	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height
2	SENS007_A1	15	97.37	1794	24	172.5	1451	12	237.9	3299	13	300.8	3368	37	352.8	2725
	SENS007_A2	15	97.39	2549	24	172.6	1762	12	237.9	4059	13	300.8	3490	37	352.7	2694
	SENS007_A3	15	97.39	2417	24	172.5	2369	12	237.9	4301	13	300.8	3589	37	352.8	2739
1	SENS007_B1	15	97.37	1157	24	172.6	1071	12	237.9	1999	13	300.8	1698	37	352.7	1443
	SENS007_B2	15	97.36	903	24	172.5	691	12	237.9	1189	13	300.8	1395	37	352.8	1043
	SENS007_B3	15	97.43	1707	24	172.6	1063	12	238	2158	13	300.8	2466	37	352.7	1630
0.5	SENS007_C1	15	97.43	504	24	172.5	326	12	237.9	745	13	300.8	461	37	352.7	346
	SENS007_C2	15	97.38	1031	24	172.5	445	12	238.1	986	13	300.8	958	37	352.8	929
	SENS007_C3	15	97.39	427	24	172.5	260	12	238.1	690	13	300.8	654	37	352.7	560
0.25	SENS007_D1	15	97.4	251	24	172.5	241	12	238	366	13	300.9	467	37	352.7	320
	SENS007_D2	15	97.33	301	24	172.5	206	12	238	340	13	300.9	278	37	352.7	281
	SENS007_D3	15	97.4	285	24	172.5	243	12	238.1	305	13	300.9	358	37	352.7	300
0.125	SENS007_E1	15	97.4	221	24	172.5	193	12	238	240	13	300.8	274	37	352.8	234
	SENS007_E2	15	97.38	96				12	238	334	13	300.8	325	37	352.7	152
	SENS007_E3	15	97.4	175	24	172.5	87	12	238	145	13	300.8	170	37	352.7	85
0.0625	SENS007_F1				24	172.5	156	12	238	125				37	352.7	157
	SENS007_F2										13	300.9	75			
	SENS007_F3				24	172.4	77	12	238.1	103	13	300.8	123			
0.03125	SENS007_G1															
	SENS007_G2													37	352.6	118
	SENS007_G3															
0.015625	SENS007_H1															
	SENS007_H2															
	SENS007_H3															
0.0078125	SENS007_I1															
	SENS007_I2															
	SENS007_I3															
0.00390625	SENS007_J1															
	SENS007_J2															
	SENS007_J3															
1.5	SENS007_K1	15	97.45	2420	24	172.5	1247	12	238	2968	13	300.9	2842	37	352.7	2388
	SENS007_K2	15	97.39	1567	24	172.5	1227	12	238	2376	13	300.9	2205	37	352.7	1824
	SENS007_K3	15	97.39	1433	24	172.5	1269	12	238	2783	13	300.8	2814	37	352.7	2190
0.75	SENS007_L1	15	97.39	760	24	172.4	708	12	238	1360	13	300.8	1208	37	352.8	1340
	SENS007_L2	15	97.31	882	24	172.4	625	12	238.1	1350	13	300.9	1129	37	352.7	1178
	SENS007_L3	15	97.38	924	24	172.5	571	12	238	956	13	300.9	891	37	352.8	975

Table 6 shows the yellow dye (NED™) channel results in the Sensitivity study 1 for the positive control DNA (007) serially diluted 2-fold and amplified using the YFiler Plus PCR amplification kit. Drop out of alleles began occurring at 125pg. Template amounts tested from the serially diluted samples were 2.0, 1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, and 0.00390625, with corresponding sample ID's of A-J. Additionally, 1.5 and 0.75 ng were also tested (sample identifiers K and L) and alleles detected were measured. Each sample was amplified in triplicate.

Table 7

		DYS570			DYS437			DYS385			DYS385 II			DYS449		
		17			15			11			14			30		
Template	Sample ID	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height
2	SENS007_A1	17	126.36	1448	15	198.4	1893	11	245.6	1237	14	257.7	1290	30	358.1	1776
	SENS007_A2	17	126.3	1714	15	198.4	2790	11	245.7	1730	14	257.7	1544	30	358.1	2357
	SENS007_A3	17	126.27	2104	15	198.4	2806	11	245.7	1830	14	257.7	1669	30	358.1	2674
1	SENS007_B1	17	126.25	1114	15	198.4	1565	11	245.7	1025	14	257.7	811	30	358.1	1272
	SENS007_B2	17	126.32	770	15	198.5	1321	11	245.6	649	14	257.7	706	30	358.1	809
	SENS007_B3	17	126.3	1191	15	198.4	1879	11	245.6	1391	14	257.7	995	30	358.1	1824
0.5	SENS007_C1	17	126.36	470	15	198.5	541	11	245.6	518	14	257.7	398	30	358.2	353
	SENS007_C2	17	126.24	566	15	198.4	787	11	245.5	524	14	257.6	592	30	358.1	911
	SENS007_C3	17	126.32	304	15	198.4	402	11	245.7	222	14	257.6	194	30	358.1	400
0.25	SENS007_D1	17	126.21	159	15	198.5	371	11	245.6	196	14	257.6	87	30	358.2	210
	SENS007_D2	17	126.24	409	15	198.5	486	11	245.6	197	14	257.6	179	30	358.1	307
	SENS007_D3	17	126.3	201	15	198.4	195	11	245.6	211	14	257.6	232	30	358.1	140
0.125	SENS007_E1	17	126.3	159	15	198.5	166	11	245.6	182	14	257.6	203	30	358.2	114
	SENS007_E2	17	126.21	204	15	198.4	128	11	245.6	159	14	257.7	110	30	358.1	252
	SENS007_E3	17	126.3	76	15	198.4	153	11	245.6	124				30	358.1	128
0.0625	SENS007_F1				15	198.5	84									
	SENS007_F2													30	358.1	133
	SENS007_F3				15	198.4	86									
0.03125	SENS007_G1															
	SENS007_G2															
	SENS007_G3															
0.015625	SENS007_H1															
	SENS007_H2															
	SENS007_H3															
0.0078125	SENS007_I1															
	SENS007_I2															
	SENS007_I3															
0.00390625	SENS007_J1															
	SENS007_J2															
	SENS007_J3															
1.5	SENS007_K1	17	126.26	1635	15	198.4	2269	11	245.5	1529	14	257.7	1187	30	358.1	2056
	SENS007_K2	17	126.34	1312	15	198.5	2256	11	245.5	1469	14	257.6	1167	30	358.1	1595
	SENS007_K3	17	126.24	1065	15	198.5	2024	11	245.6	1124	14	257.7	944	30	358.2	1353
0.75	SENS007_L1	17	126.25	986	15	198.4	1294	11	245.6	786	14	257.6	733	30	358.2	821
	SENS007_L2	17	126.26	841	15	198.5	878	11	245.6	852	14	257.6	613	30	358.1	847
	SENS007_L3	17	126.36	884	15	198.5	918	11	245.5	683	14	257.6	693	30	358.1	893

Table 7 shows the red dye (TAZ™) channel results in the Sensitivity study 1 for the positive control DNA (007) serially diluted 2-fold and amplified using the YFiler Plus PCR amplification kit. Drop out of alleles began occurring at 125pg. Template amounts tested from the serially diluted samples were 2.0, 1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, and 0.00390625, with corresponding sample ID's of A-J. Additionally, 1.5 and 0.75 ng were also tested (sample identifiers K and L) and alleles detected were measured. Each sample was amplified in triplicate.

Table 8

		DYS393			DYS439			DYS481			DYF387SI			DYF387SII			DYS533		
		13			13			23			35			35			12		
Template	Sample ID	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height
2	SENS007_A1	13	114.85	1211	12	174.5	1190	22	221.9	2478	35	283.9	2251	37	291.7	1967	13	363	1791
	SENS007_A2	13	114.91	1564	12	174.6	1524	22	221.9	2918	35	283.9	2671	37	291.7	2640	13	363	2009
	SENS007_A3	13	114.9	2104	12	174.5	1741	22	221.9	4244	35	283.9	3166	37	291.6	3213	13	363	2589
1	SENS007_B1	13	114.91	1184	12	174.5	759	22	221.9	2090	35	283.9	2021	37	291.6	1483	13	363	1097
	SENS007_B2	13	114.91	820	12	174.5	678	22	221.9	1230	35	283.9	960	37	291.7	1282	13	363	887
	SENS007_B3	13	114.92	1073	12	174.5	1239	22	221.9	1982	35	283.9	1647	37	291.7	1850	13	363	1429
0.5	SENS007_C1	13	114.92	470	12	174.5	251	22	221.9	734	35	283.9	609	37	291.6	502	13	363	388
	SENS007_C2	13	114.94	440	12	174.6	354	22	221.9	962	35	284.2	739	37	291.8	990	13	363	613
	SENS007_C3	13	114.94	206	12	174.7	154	22	221.9	450	35	284	443	37	291.7	260	13	363	290
0.25	SENS007_D1	13	114.92	245	12	174.6	134	22	221.8	410	35	284.1	348	37	291.9	327	13	363.1	353
	SENS007_D2	13	114.92	298	12	174.6	236	22	221.9	470	35	284	337	37	291.9	514	13	363	255
	SENS007_D3	13	114.86	267	12	174.6	239	22	221.8	574	35	284.1	338	37	291.8	429	13	363.1	147
0.125	SENS007_E1	13	114.94	221	12	174.6	194	22	221.9	464	35	284.1	288	37	291.8	339	13	363	120
	SENS007_E2				12	174.6	95	22	221.8	312	35	284.1	106	37	291.8	192	13	363	141
	SENS007_E3	13	114.94	86	12	174.6	75	22	221.9	156	35	284.1	109	37	291.8	114	13	363	91
0.0625	SENS007_F1							22	221.8	79	37	291.9	158				13	363.1	133
	SENS007_F2				12	174.6	77				35	284.1	121	37	291.8	81			
	SENS007_F3							22	221.9	137							13	363	122
0.03125	SENS007_G1	13	114.92	97				22	221.9	127	35	284.1	140						
	SENS007_G2							22	221.9	102									
	SENS007_G3																		
0.015625	SENS007_H1										37	291.9	78				13	362.9	90
	SENS007_H2																		
	SENS007_H3							22	221.8	77									
0.0078125	SENS007_I1																		
	SENS007_I2																		
	SENS007_I3																		
0.00390625	SENS007_J1																		
	SENS007_J2																		
	SENS007_J3																		
1.5	SENS007_K1	13	114.94	1532	12	174.6	1728	22	222	3145	35	284.1	2502	37	291.9	2094	13	363.1	1969
	SENS007_K2	13	114.87	1521	12	174.6	1230	22	221.8	2376	35	284.1	1925	37	291.9	1973	13	363	1485
	SENS007_K3	13	114.92	1078	12	174.6	900	22	221.9	2643	35	284.1	1363	37	291.9	1679	13	363	1465
0.75	SENS007_L1	13	114.86	919	12	174.6	1036	22	221.9	1463	35	284.1	1489	37	291.8	1216	13	363.1	1069
	SENS007_L2	13	114.94	963	12	174.6	742	22	222	1212	35	284	967	37	291.8	1098	13	363	631
	SENS007_L3	13	114.95	630	12	174.6	827	22	221.9	1063	35	284.1	1031	37	291.8	1163	13	362.9	873

Table 8 shows the purple dye (SID™) channel results in the Sensitivity study 1 for the positive control DNA (007) serially diluted 2-fold and amplified using the YFiler Plus PCR amplification kit. Drop out of alleles began occurring at 125pg. Template amounts tested from the serially diluted samples were 2.0, 1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, and 0.00390625, with corresponding sample ID's of A-J. Additionally, 1.5 and 0.75 ng were also tested (sample identifiers K and L) and alleles detected were measured. Each sample was amplified in triplicate.

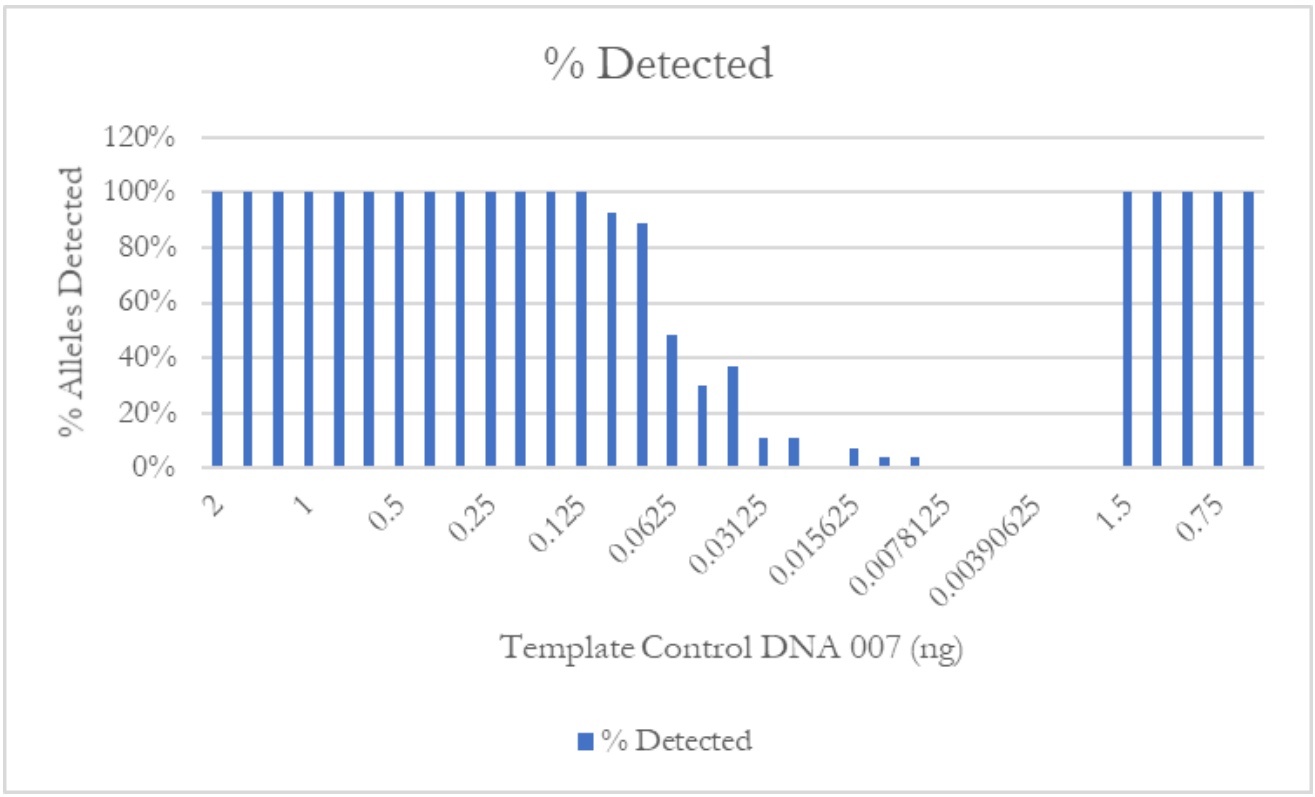

Figure 3 depicts a bar chart of the % alleles detected across all dyes when the serially diluted positive control DNA (007) was amplified using the YFiler Plus PCR amplification kit. The samples were amplified in triplicate and each replicate is shown on the graph. Drop out of alleles began occurring at 125pg. Template amounts tested from the serially diluted samples were 2.0, 1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, and 0.00390625. Additionally, 1.5 and 0.75 ng were included in this study.

Sensitivity & Stochastic QAS 8.3.1(3)- Study 2

The sensitivity and stochastic study (study 2) was performed using male DNA sample extracted using the QIAGEN DNA Investigator kit chemistry. The stock DNA was quantified and serially diluted, and then each sample was quantified using both the Quantiplex PRO and Quantifiler Trio DNA quantification kits to confirm the dilutions performed. The results of the sensitivity study 2 are defined in the figures and tables below.

Table 9

	QUANTIPLEX PRO		QUANTIFILER TRIO					
	HUMAN	MALE	LARGE	SMALL	Y			
M01 Dilution	2 uL	2 uL	2 uL	2 uL	2 uL	Average all values	Target Dilution	Per 10 uL into YFP
A	0.15659	0.37476	0.21000	0.22000	0.24000	0.240	2	2.402687
B	0.07589	0.16971	0.10000	0.11000	0.12000	0.115	1	1.151203
C	0.03401	0.08665	0.04600	0.05100	0.04300	0.052	0.5	0.521335
D	0.01670	0.04838	0.02200	0.01800	0.02300	0.026	0.25	0.256148
E	0.00817	0.02336	0.00550	0.00730	0.00490	0.010	0.125	0.098465
F	0.00488	0.01326	0.00680	0.00580	0.00480	0.007	0.0625	0.071082
G	0.00211	0.00365	0.00190	0.00230	0.00530	0.003	0.03125	0.030529
H	0.00098	0.00393	0.00220	0.00140	0.00140	0.002	0.015625	0.019832
I	0.00054	0.00179	0.00042	0.00034	0.00034	0.001	0.007813	0.006854
J	0.00034	0.00106	0.00000	0.00039	0.00000	0.000	0.003906	0.003589
K	0.12788	0.27974	0.18000	0.19000	0.19000	0.194	1.5	1.935247
L	0.05474	0.16194	0.06900	0.07700	0.07300	0.087	0.75	0.871354

Table 9 defines the averaged quantification results for the serially diluted male DNA sample (M01) used in the sensitivity study 2 and the calculated template for the YFIler Plus PCR amplification. For the quantifications, each sample was quantified in duplicate using both the Quantiplex PRO and Quantifiler TRIO quantification kits. The average from both kits was calculated and subsequently multiplied by 10 to determine the accuracy of the targeted template for amplification. The expected template amounts were 2.0, 1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, and 0.00390625, and 1.5 and 0.75ng. Quantification results showed the samples were accurately serially diluted. Regardless of the quantification value differences, the targeted amount (maximum volume) into the YFiler Plus reaction would not have changed.

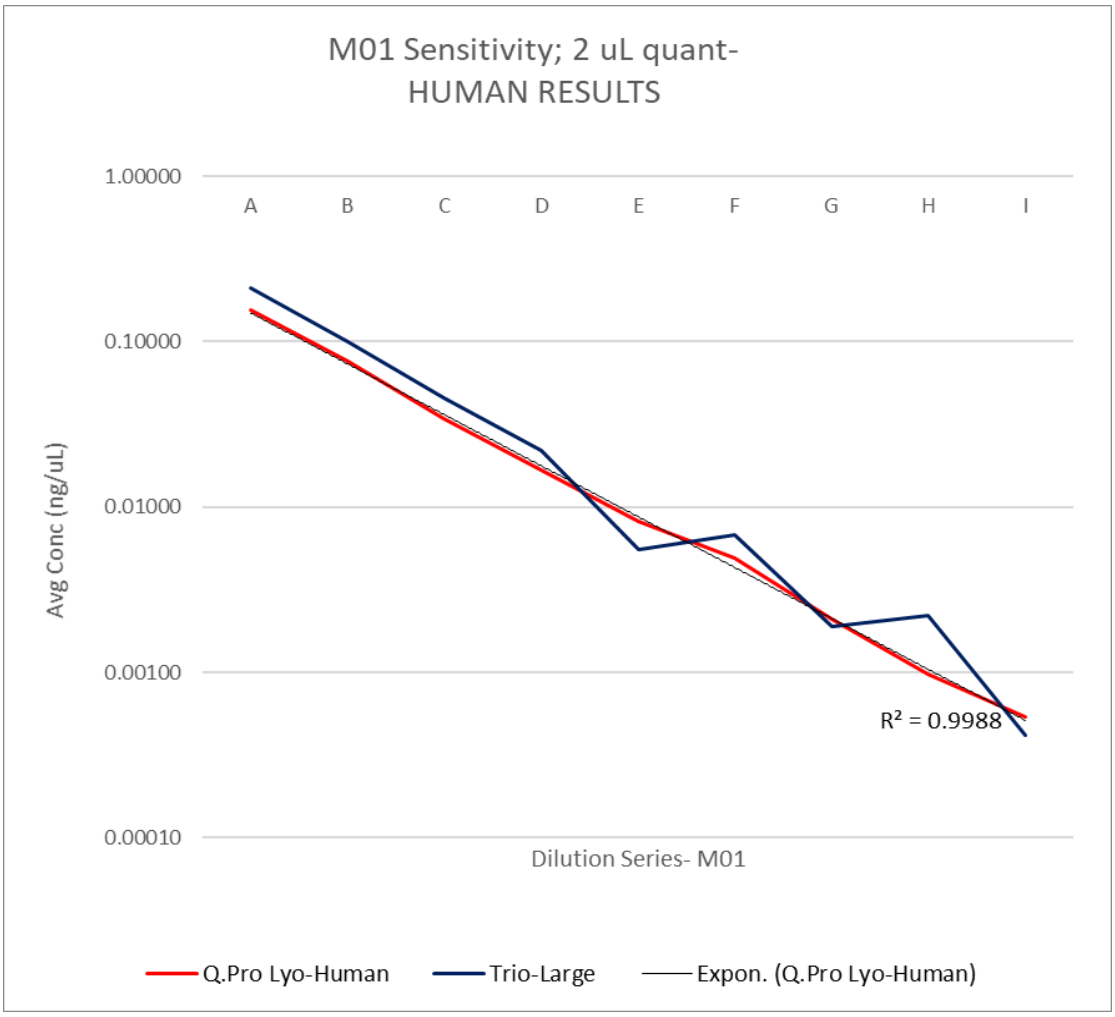

Figure 4 depicts the average concentration of the sensitivity dilution series (M01) for the human quantitification results. Linearity (R^2) of the Quantiplex PRO was 0.9988 for dilutions A-I. Linearity (R^2) of the Trio Large was not calculated, but can be stated as less than 0.99.

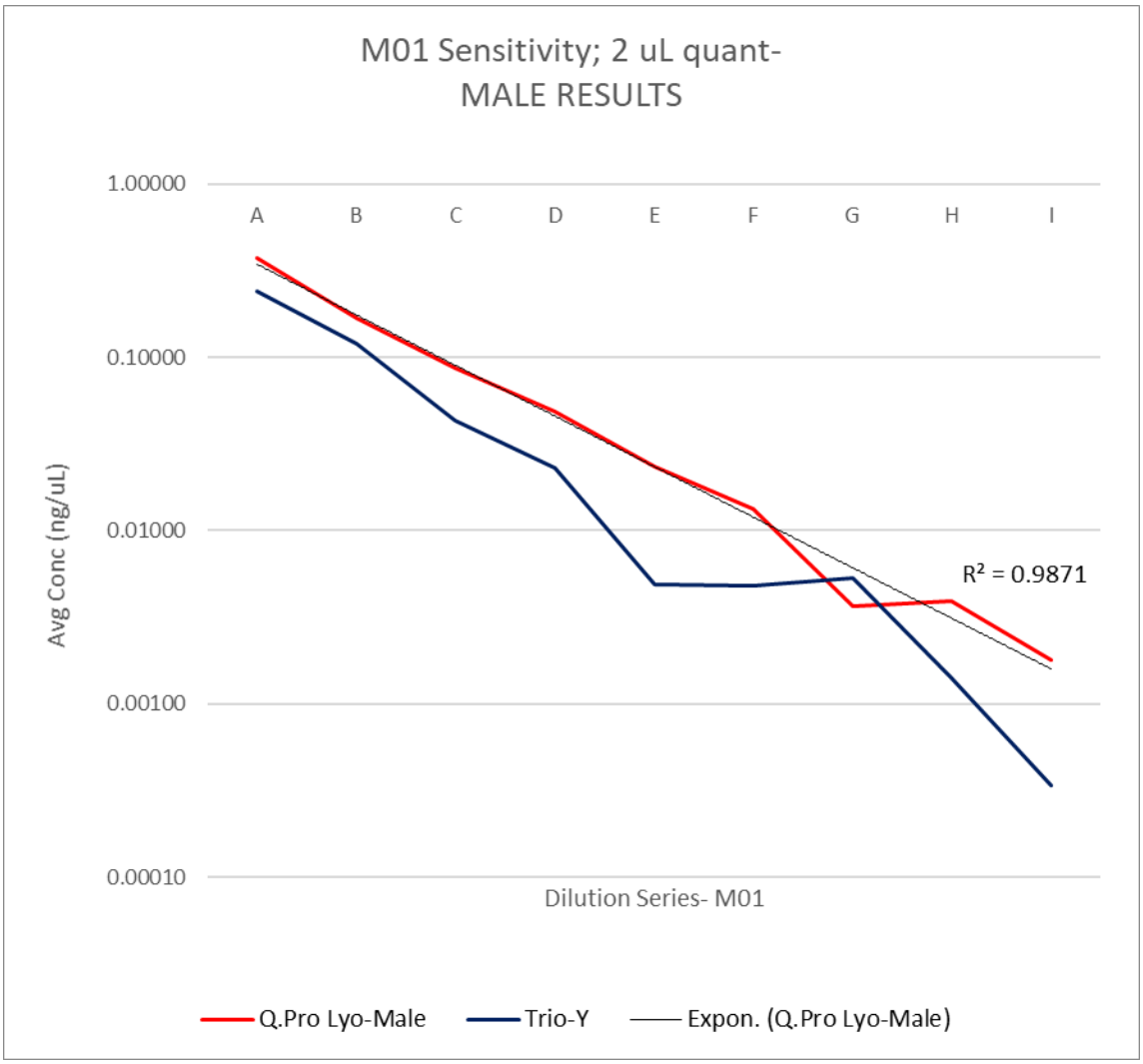

Figure 5 depicts the average concentration of the sensitivity dilution series (M01) for the male quantitification results. Linearity (R^2) of the Quantiplex PRO was 0.9871 for dilutions A-I. Linearity (R^2) of the Trio Large was not calculated, but can be stated as less than 0.99.

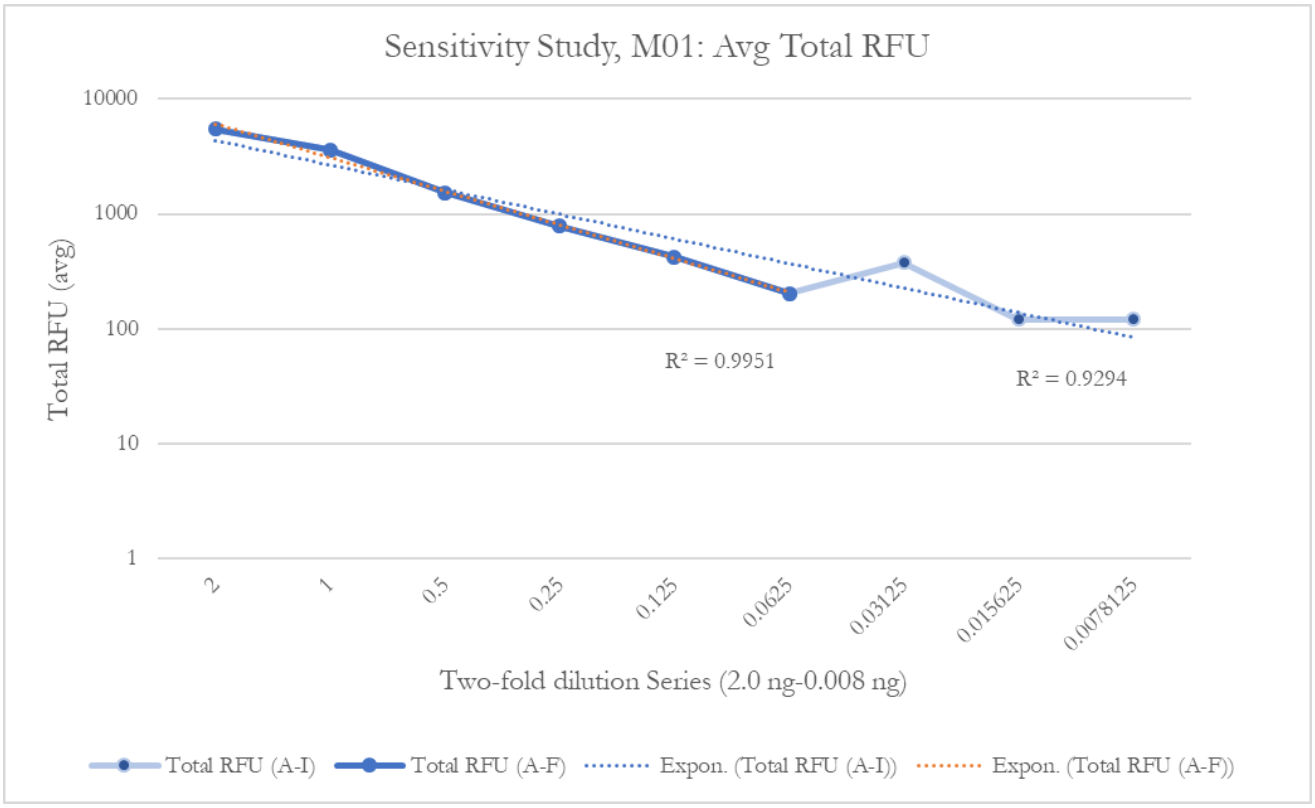

Figure 6 above depicts the total RFU and R^2 values for all dyes detected when a two-fold dilution series was amplified using the AmpFlSTR Yfiler Plus PCR amplification kit. Two-fold dilutions ranging from 2.0ng to 0.008ng were performed and amplified using the AmpFlSTR YFiler Plus PCR amplification kit. The resultant R^2 value =0.9949 for dilutions A-F; the resultant R^2 = 0.9294 for dilutions A-I. Dilution J (0.0031 ng) was undetected for all triplicate reactions.Analysis was performed using 75 RFU and the GeneMapper ID-X, version 1.5 software.

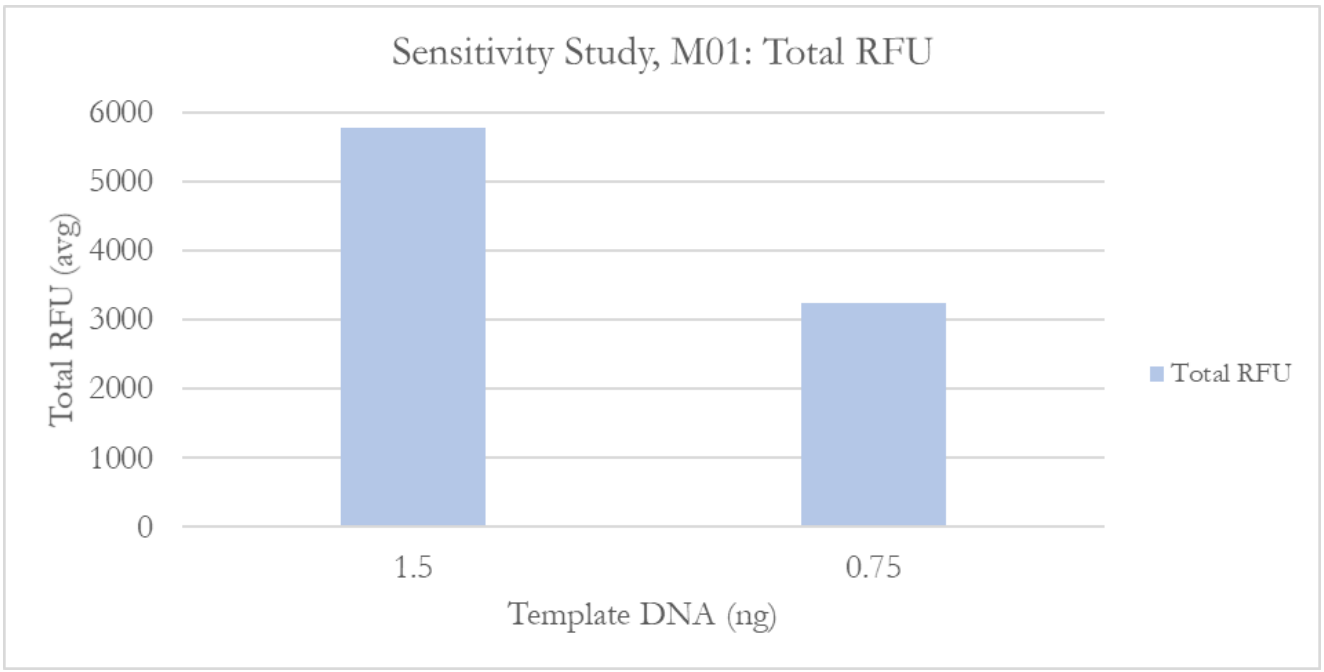

Figure 7 above depicts the average total RFU for all dyes detected when 1.5ng and 0.75 ng template DNA (M01) was amplified using the AmpFlSTR Yfiler Plus PCR amplification kit. A two-fold dilution between the two samples was performed and the samples were triplicate amplified using the AmpFlSTR YFiler Plus PCR amplification kit. Analysis was performed using 75 RFU and the GeneMapper ID-X, version 1.5 software.

Table 10

		DYS576			DYS389I			DYS635			DYS389II			DYS627		
		17			13			26			29			22		
Template	Sample Name	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height
2	SENS-M01.A.1	17	101.83	8812	13	163.31	10800	26	236.01	9563	29	285.2	7700	22	367.9	6470
	SENS-M01.A.2	17	101.68	8833	13	163.33	10092	26	235.87	7389	29	285.28	7123	22	367.6	8013
	SENS-M01.A.3	17	101.75	6976	13	163.32	8638	26	235.87	6894	29	285.28	5949	22	367.63	6868
1	SENS-M01.B.1	17	101.67	4108	13	163.32	4893	26	235.88	3861	29	285.25	3526	22	367.58	4504
	SENS-M01.B.2	17	101.68	5327	13	163.33	4920	26	235.85	4071	29	285.3	3268	22	367.56	5324
	SENS-M01.B.3	17	101.67	3263	13	163.34	3147	26	235.92	1987	29	285.3	1994	22	367.62	2413
0.5	SENS-M01.C.1	17	101.69	1552	13	163.27	2001	26	235.91	1305	29	285.3	1325	22	367.66	2147
	SENS-M01.C.2	17	101.69	1446	13	163.34	1207	26	235.92	1469	29	285.3	887	22	367.66	1775
	SENS-M01.C.3	17	101.8	1342	13	163.33	1580	26	235.91	1141	29	285.17	1064	22	367.75	1535
0.25	SENS-M01.D.1	17	101.79	830	13	163.33	956	26	235.95	903	29	285.24	672	22	367.75	877
	SENS-M01.D.2	17	101.8	722	13	163.24	648	26	235.93	507	29	285.23	415	22	367.85	903
	SENS-M01.D.3	17	101.79	829	13	163.24	849	26	235.95	775	29	285.17	497	22	367.81	1048
0.125	SENS-M01.E.1	17	101.8	412	13	163.34	446	26	235.98	451	29	285.16	311	22	367.79	1069
	SENS-M01.E.2	17	101.8	252	13	163.33	299	26	235.93	207	29	285.26	143	22	367.86	329
	SENS-M01.E.3	17	101.8	415	13	163.25	228	26	235.93	369	29	285.2	134	22	367.79	213
0.0625	SENS-M01.F.1	17	101.8	104	13	163.33	158				29	285.24	102			
	SENS-M01.F.2	17	101.82	197	13	163.36	320	26	235.98	106	29	285.19	201	22	367.82	262
	SENS-M01.F.3	17	101.72	191	13	163.27	101	26	235.92	353	29	285.19	82	22	367.82	77
0.03125	SENS-M01.G.1	17	101.81	730	13	163.36	662	26	236.01	530	29	285.2	432	22	367.93	913
	SENS-M01.G.2	17	101.81	85										22	367.83	285
	SENS-M01.G.3				13	163.29	198	26	235.88	129	29	285.22	127	22	367.77	78
0.015625	SENS-M01.H.1															
	SENS-M01.H.2															
	SENS-M01.H.3	17	101.74	156												
0.0078125	SENS-M01.I.1	17	101.81	79												
	SENS-M01.I.2															
	SENS-M01.I.3															
0.00390625	SENS-M01.J.1															
	SENS-M01.J.2															
	SENS-M01.J.3															
1.5	SENS-M01.K.1	17	101.75	5506	13	163.3	7050	26	235.96	5950	29	285.24	4776			
	SENS-M01.K.2	17	101.82	7453	13	163.28	8276	26	235.97	6378	29	285.22	5709	21.2	365.88	170
	SENS-M01.K.3	17	101.83	6624	13	163.31	8942	26	235.92	8340	29	285.17	7708	21.2	365.8	188
0.75	SENS-M01.L.1	17	101.81	3029	13	163.3	4076	26	235.96	2680	29	285.15	2521	22	367.87	2619
	SENS-M01.L.2	17	101.9	2557	13	163.29	2897	26	235.93	2794	29	285.24	2070	21.2	365.87	85
	SENS-M01.L.3	17	101.82	3505	13	163.29	4863	26	235.95	3532	29	285.25	3279	22	367.9	3657

Table 10 shows the blue dye (6-FAM™) channel results in the Sensitivity study 2 for the extracted male DNA (M01) serially diluted 2-fold and amplified using the YFiler Plus PCR amplification kit. DNA extraction of the sample was performed using the EZ1/2 DNA Investigator kit chemistry. Drop out of alleles began occurring at 62.5pg. Template amounts tested from the serially diluted samples were 2.0, 1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, and 0.00390625, with corresponding sample ID's of A-J. Additionally, 1.5 and 0.75 ng were also tested (sample identifiers K and L) and alleles detected were measured. Each sample was amplified in triplicate.

Table 11

		DYS460			DYS458			DYS19			YGATAH4			DYS448			DYS391		
		10			17			14			12			19			11		
Templat	Sample Name	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height
2	SENS-M01.A.1	10	92.16	9473	17	143.89	14178	14	204.31	12453	12	252.48	11140	19	307.92	10253	11	377.47	8424
	SENS-M01.A.2	10	92.27	9885	17	143.73	12125	14	204.21	9176	12	252.28	9664	19	307.68	9087	11	377.51	8333
	SENS-M01.A.3	10	92.27	6027	17	143.71	9830	14	204.21	8226	12	252.37	8468	19	307.77	7078	11	377.5	7679
1	SENS-M01.B.1	10	92.23	3108	17	143.71	4998	14	204.21	4454	12	252.35	3987	19	307.75	4032	11	377.41	3963
	SENS-M01.B.2	10	92.25	3768	17	143.82	6668	14	204.24	4426	12	252.36	4281	19	307.74	4760	11	377.48	4612
	SENS-M01.B.3	10	92.31	2709	17	143.74	4286	14	204.24	3289	12	252.3	2981	19	307.79	2676	11	377.4	2845
0.5	SENS-M01.C.1	10	92.28	1364	17	143.74	2541	14	204.27	2041	12	252.38	2271	19	307.68	1970	11	377.49	992
	SENS-M01.C.2	10	92.22	1418	17	143.74	2014	14	204.24	1333	12	252.28	923	19	307.79	1398	11	377.49	1219
	SENS-M01.C.3	10	92.23	1125	17	143.83	1047	14	204.27	1495	12	252.35	759	19	307.84	1350	11	377.52	1068
0.25	SENS-M01.D.1	10	92.25	622	17	143.82	712	14	204.25	960	12	252.42	859	19	307.84	1106	11	377.52	550
	SENS-M01.D.2	10	92.17	850	17	143.8	933	14	204.27	428	12	252.33	769	19	307.92	442	11	377.52	654
	SENS-M01.D.3	10	92.27	779	17	143.87	1109	14	204.22	884	12	252.42	849	19	307.88	976	11	377.54	1643
0.125	SENS-M01.E.1	10	92.21	314	17	143.82	881	14	204.27	559	12	252.44	273	19	307.81	509	11	377.51	750
	SENS-M01.E.2	10	92.23	169	17	143.91	167	14	204.25	230	12	252.35	353	19	307.9	369	11	377.53	377
	SENS-M01.E.3	10	92.29	549	17	143.82	546	14	204.26	345	12	252.35	504	19	307.85	311	11	377.51	317
0.0625	SENS-M01.F.1	10	92.13	83	17	143.84	247	14	204.16	92	12	252.34	130	19	307.9	77			
	SENS-M01.F.2	10	92.16	186	17	143.86	252	14	204.23	276	12	252.36	231	19	307.85	473			
	SENS-M01.F.3	10	92.19	305				14	204.24	172	12	252.44	216	19	307.87	218	11	377.6	222
0.0313	SENS-M01.G.1	10	92.19	893	17	143.93	956	14	204.24	426	12	252.44	779	19	307.88	444	11	377.6	655
	SENS-M01.G.2				17	143.91	154	14	204.2	116				19	307.9	265			
	SENS-M01.G.3	10	92.24	98				14	204.23	179	12	252.37	177	19	307.85	105			
0.0156	SENS-M01.H.1																11	377.49	120
	SENS-M01.H.2				17	143.86	76	14	204.23	221									
	SENS-M01.H.3	10	92.16	140	17	143.94	100										11	377.6	109
0.0078	SENS-M01.I.1																		
	SENS-M01.I.2																		
	SENS-M01.I.3																		
0.0039	SENS-M01.J.1	10	92.16	75															
	SENS-M01.J.2																		
	SENS-M01.J.3																		
1.5	SENS-M01.K.1	10	92.2	6366	17	143.87	7262	14	204.22	7135	12	252.39	6079	19	307.91	6517	11	377.57	6957
	SENS-M01.K.2	10	92.2	6413	17	143.87	9291	14	204.22	6759	12	252.37	6857	19	307.93	7026	11	377.59	6292
	SENS-M01.K.3	10	92.18	9180	17	143.87	11414	14	204.26	10121	12	252.39	11656	19	307.91	9864	11	377.47	8259
0.75	SENS-M01.L.1	10	92.22	3509	17	143.87	3469	14	204.22	3359	12	252.37	2910	19	307.91	2882	11	377.49	3894
	SENS-M01.L.2	10	92.2	2810	17	143.89	3371	14	204.26	2859	12	252.39	3035	19	307.91	3279	11	377.48	2472
	SENS-M01.L.3	10	92.22	3099	17	143.87	4990	14	204.23	3256	12	252.39	3724	19	307.85	3990	11	377.57	4126

Table 11 shows the green dye (VIC™) channel results in the Sensitivity study 2 for the extracted male DNA (M01) serially diluted 2-fold and amplified using the YFiler Plus PCR amplification kit. DNA extraction of the sample was performed using the EZ1/2 DNA Investigator kit chemistry. Drop out of alleles began occurring at 62.5pg. Template amounts tested from the serially diluted samples were 2.0, 1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, and 0.00390625, with corresponding sample ID's of A-J. Additionally, 1.5 and 0.75 ng were also tested (sample identifiers K and L) and alleles detected were measured. Each sample was amplified in triplicate.

Table 12

		DYS456			DYS390			DYS438			DYS392			DYS518		
		16			23			12			13			38		
Template	Sample Name	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height
2	SENS-M01.A.1	16	101.67	7123	23	168.3	6363	12	238.14	7224	13	301.02	7511	38	356.67	4895
	SENS-M01.A.2	16	101.6	7190	23	168.34	5949	12	238.06	7162	13	300.88	6470	38	356.74	5076
	SENS-M01.A.3	16	101.67	6745	23	168.33	5177	12	237.98	6753	13	300.88	6533	38	356.65	4858
1	SENS-M01.B.1	16	101.59	3479	23	168.39	2555	12	237.98	3270	13	300.89	3137	38	356.67	2211
	SENS-M01.B.2	16	101.6	3915	23	168.33	3612	12	237.96	3961	13	300.89	4162	38	356.65	3291
	SENS-M01.B.3	16	101.67	2005	23	168.37	2128	12	237.96	2255	13	300.9	2233	38	356.74	1959
0.5	SENS-M01.C.1	16	101.61	1585	23	168.32	1312	12	237.96	1686	13	300.89	1465	38	356.72	1156
	SENS-M01.C.2	16	101.61	1429	23	168.37	1029	12	238.05	1473	13	300.9	1286	38	356.73	658
	SENS-M01.C.3	16	101.64	1194	23	168.29	820	12	238	1365	13	300.91	1728	38	356.66	1200
0.25	SENS-M01.D.1	16	101.63	539	23	168.29	361	12	238.02	815	13	301	519	38	356.65	490
	SENS-M01.D.2	16	101.63	679	23	168.29	603	12	238.01	614	13	300.92	619	38	356.65	621
	SENS-M01.D.3	16	101.62	876	23	168.29	865	12	238.02	633	13	300.99	603	38	356.75	387
0.125	SENS-M01.E.1	16	101.64	314	23	168.32	253	12	238.08	665	13	300.92	528	38	356.63	292
	SENS-M01.E.2	16	101.64	91	23	168.29	265	12	238.1	259	13	301.01	403	38	356.72	146
	SENS-M01.E.3	16	101.64	265	23	168.23	185	12	238.1	214	13	301	244	38	356.64	272
0.0625	SENS-M01.F.1				23	168.39	79									
	SENS-M01.F.2	16	101.57	135	23	168.28	147	12	238.08	217	13	300.92	178	38	356.62	203
	SENS-M01.F.3	16	101.64	113	23	168.27	202	12	238	172	13	301.02	87	38	356.62	103
0.03125	SENS-M01.G.1	16	101.56	710	23	168.36	605	12	238.09	623	13	301.01	641	38	356.72	627
	SENS-M01.G.2	16	101.65	76							13	301.01	142			
	SENS-M01.G.3				23	168.32	90				13	301.01	124			
0.015625	SENS-M01.H.1				23	168.27	75									
	SENS-M01.H.2							12	238.07	131						
	SENS-M01.H.3															
0.007813	SENS-M01.I.1															
	SENS-M01.I.2							12	238.07	83						
	SENS-M01.I.3															
0.003906	SENS-M01.J.1															
	SENS-M01.J.2															
	SENS-M01.J.3															
1.5	SENS-M01.K.1	16	101.58	5377	23	168.26	3930	12	238.07	5609	13	301.02	5443	38	356.69	3888
	SENS-M01.K.2	16	101.66	5784	23	168.31	4753	12	238.08	5120	13	300.93	5680	38	356.69	3644
	SENS-M01.K.3	15	97.38	813	23	168.3	5191	12	238.05	6195	13	301.02	5760	38	356.69	4555
0.75	SENS-M01.L.1	16	101.65	2617	23	168.26	1939	12	238.07	3033	13	300.93	2470	38	356.69	2613
	SENS-M01.L.2	16	101.66	2302	23	168.23	1789	12	238.06	2034	13	301.02	2909	38	356.69	1832
	SENS-M01.L.3	16	101.65	3141	23	168.32	2584	12	238.07	3543	13	301.01	3247	38	356.6	2413

Table 12 shows the yellow dye (NED™) channel results in the Sensitivity study 2 for the extracted male DNA (M01) serially diluted 2-fold and amplified using the YFiler Plus PCR amplification kit. DNA extraction of the sample was performed using the EZ1/2 DNA Investigator kit chemistry. Drop out of alleles began occurring at 62.5pg. Template amounts tested from the serially diluted samples were 2.0, 1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, and 0.00390625, with corresponding sample ID's of A-J. Additionally, 1.5 and 0.75 ng were also tested (sample identifiers K and L) and alleles detected were measured. Each sample was amplified in triplicate.

Table 13

		DYS393			DYS439			DYS481			DYF387S1			DYS533		
		13			13			23			35			12		
Templat	Sample Name	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height
2	SENS-M01.A.1	13	114.88	10808	13	178.76	10281	23	224.86	10969	35	284.54	25661	12	358.99	12230
	SENS-M01.A.2	13	114.92	8557	13	178.65	7361	23	224.9	11734	35	284.3	27139	12	358.94	9812
	SENS-M01.A.3	13	114.92	8270	13	178.65	5101	23	224.87	9185	35	284.39	23069	12	358.95	8583
1	SENS-M01.B.1	13	114.85	2941	13	178.66	3099	23	224.88	4744	35	284.36	13325	12	358.86	4383
	SENS-M01.B.2	13	114.92	3958	13	178.57	3372	23	224.88	4927	35	284.32	14682	12	358.85	5132
	SENS-M01.B.3	13	114.94	2854	13	178.65	2608	23	224.82	2655	35	284.31	10240	12	358.94	3460
0.5	SENS-M01.C.1	13	114.92	1885	13	178.64	1607	23	224.9	2596	35	284.31	6533	12	358.94	1749
	SENS-M01.C.2	13	114.92	1633	13	178.65	893	23	224.91	1548	35	284.31	4992	12	358.94	1407
	SENS-M01.C.3	13	114.87	1158	13	178.7	630	23	224.82	1760	35	284.43	5060	12	358.92	1935
0.25	SENS-M01.D.1	13	114.87	888	13	178.7	449	23	224.91	710	35	284.51	2732	12	358.92	851
	SENS-M01.D.2	13	114.87	722	13	178.7	632	23	224.8	814	35	284.4	2924	12	358.92	576
	SENS-M01.D.3	13	114.87	736	13	178.7	511	23	224.91	1262	35	284.44	2770	12	359.02	1082
0.125	SENS-M01.E.1	13	114.95	326	13	178.7	392	23	224.84	672	35	284.43	1590	12	358.91	739
	SENS-M01.E.2	13	114.87	207	13	178.7	399	23	224.93	345	35	284.43	1587	12	359.01	131
	SENS-M01.E.3	13	114.87	378	13	178.7	251	23	224.93	513	35	284.46	1450	12	358.91	293
0.0625	SENS-M01.F.1	13	114.87	154	13	178.71	79	23	224.95	251	35	284.41	344			
	SENS-M01.F.2	13	114.88	181	13	178.69	108	23	224.89	265	35	284.54	737	12	359.01	100
	SENS-M01.F.3	13	114.8	163	13	178.69	191	23	224.88	262	35	284.54	584	12	359.01	119
0.0313	SENS-M01.G.1	13	114.88	732	13	178.78	651	23	224.97	810	35	284.55	2916	12	359.01	572
	SENS-M01.G.2				13	178.7	135	23	224.96	232	35	284.55	661			
	SENS-M01.G.3	13	114.88	150				23	224.87	116	35	284.47	393	12	358.9	92
0.0156	SENS-M01.H.1															
	SENS-M01.H.2	13	114.88	147							35	284.47	83			
	SENS-M01.H.3										35	284.47	149			
0.0078	SENS-M01.I.1							23	224.79	158	35	284.59	92			
	SENS-M01.I.2										35	284.56	147			
	SENS-M01.I.3										35	284.5	138			
0.0039	SENS-M01.J.1															
	SENS-M01.J.2															
	SENS-M01.J.3															
1.5	SENS-M01.K.1	13	114.88	5402	13	178.77	5004	23	224.91	8473	35	284.59	19736	12	359	6535
	SENS-M01.K.2	13	114.88	6981	13	178.69	4927	23	224.92	8302	35	284.56	21310	12	359	7364
	SENS-M01.K.3	13	114.88	11053	13	178.76	8844	23	224.82	10838	35	284.51	26792	12	358.89	12753
0.75	SENS-M01.L.1	13	114.89	3620	13	178.68	2233	23	224.91	5080	35	284.49	12959	12	359	4104
	SENS-M01.L.2	13	114.88	3270	13	178.69	2290	23	224.83	3271	35	284.59	10697	12	359	3942
	SENS-M01.L.3	13	114.88	3405	13	178.69	3489	23	224.81	3587	35	284.5	13022	12	358.9	3599

Table 13 shows the purple dye (SID™) channel results in the Sensitivity study 2 for the extracted male DNA (M01) serially diluted 2-fold and amplified using the YFiler Plus PCR amplification kit. DNA extraction of the sample was performed using the EZ1/2 DNA Investigator kit chemistry. Drop out of alleles began occurring at 62.5pg. Template amounts tested from the serially diluted samples were 2.0, 1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, and 0.00390625, with corresponding sample ID's of A-J. Additionally, 1.5 and 0.75 ng were also tested (sample identifiers K and L) and alleles detected were measured. Each sample was amplified in triplicate.

Table 14

		DYS570			DYS437			DYS385			DYS449		
		17			15			11			30		
Template	Sample Name	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height	Allele	Size	Height
2	SENS-M01.A.1	17	126.13	9216	15	198.56	7883	11	245.38	17952	30	357.88	8087
	SENS-M01.A.2	17	126.24	8981	15	198.45	7761	11	245.45	14895	30	357.98	7354
	SENS-M01.A.3	17	126.19	6137	15	198.44	7582	11	245.41	13648	30	357.9	6853
1	SENS-M01.B.1	17	126.17	3344	15	198.46	3661	11	245.45	7191	30	357.91	3536
	SENS-M01.B.2	17	126.21	3536	15	198.44	4332	11	245.47	7756	30	357.9	4254
	SENS-M01.B.3	17	126.28	2547	15	198.52	2288	11	245.41	4608	30	357.99	2751
0.5	SENS-M01.C.1	17	126.22	1472	15	198.44	1449	11	245.46	3156	30	357.97	1857
	SENS-M01.C.2	17	126.2	1119	15	198.52	711	11	245.5	2567	30	357.89	1162
	SENS-M01.C.3	17	126.2	1106	15	198.49	1057	11	245.38	2184	30	357.93	1235
0.25	SENS-M01.D.1	17	126.18	693	15	198.5	896	11	245.38	1642	30	357.83	583
	SENS-M01.D.2	17	126.24	776	15	198.5	727	11	245.38	1463	30	357.93	426
	SENS-M01.D.3	17	126.24	907	15	198.5	984	11	245.47	1486	30	357.93	503
0.125	SENS-M01.E.1	17	126.18	296	15	198.49	225	11	245.47	762	30	357.92	293
	SENS-M01.E.2	17	126.2	249	15	198.49	410	11	245.47	529	30	357.91	231
	SENS-M01.E.3	17	126.2	563	15	198.57	466	11	245.38	769	30	357.82	562
0.0625	SENS-M01.F.1	17	126.09	105				11	245.42	219			
	SENS-M01.F.2	17	126.24	221	15	198.48	290	11	245.37	496	30	357.91	408
	SENS-M01.F.3	17	126.16	104	15	198.57	426	11	245.43	149	30	357.81	202
0.03125	SENS-M01.G.1	17	126.24	801	15	198.58	730	11	245.43	1477	30	357.91	424
	SENS-M01.G.2							11	245.47	117			
	SENS-M01.G.3							11	245.33	206	30	357.9	105
0.015625	SENS-M01.H.1							11	245.43	113			
	SENS-M01.H.2												
	SENS-M01.H.3												
0.007813	SENS-M01.I.1							11	245.39	109			
	SENS-M01.I.2												
	SENS-M01.I.3												
0.003906	SENS-M01.J.1												
	SENS-M01.J.2												
	SENS-M01.J.3												
1.5	SENS-M01.K.1	17	126.2	6128	15	198.56	6517	11	245.38	11012	30	357.89	6109
	SENS-M01.K.2	17	126.17	6689	15	198.47	5268	11	245.43	11094	30	357.89	5980
	SENS-M01.K.3	17	126.14	8983	15	198.47	8194	11	245.38	17982	30	357.89	8219
0.75	SENS-M01.L.1	17	126.19	3231	15	198.47	3434	11	245.39	6510	30	357.89	2593
	SENS-M01.L.2	17	126.17	2901	15	198.47	3064	11	245.33	6125	30	357.89	3001
	SENS-M01.L.3	17	126.15	2278	15	198.48	4368	11	245.33	6787	30	357.8	3032

Table 10 shows the red dye (TAZ™) channel results in the Sensitivity study 2 for the extracted male DNA (M01) serially diluted 2-fold and amplified using the YFiler Plus PCR amplification kit. DNA extraction of the sample was performed using the EZ1/2 DNA Investigator kit chemistry. Drop out of alleles began occurring at 62.5pg. Template amounts tested from the serially diluted samples were 2.0, 1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, and 0.00390625, with corresponding sample ID's of A-J. Additionally, 1.5 and 0.75 ng were also tested (sample identifiers K and L) and alleles detected were measured. Each sample was amplified in triplicate.

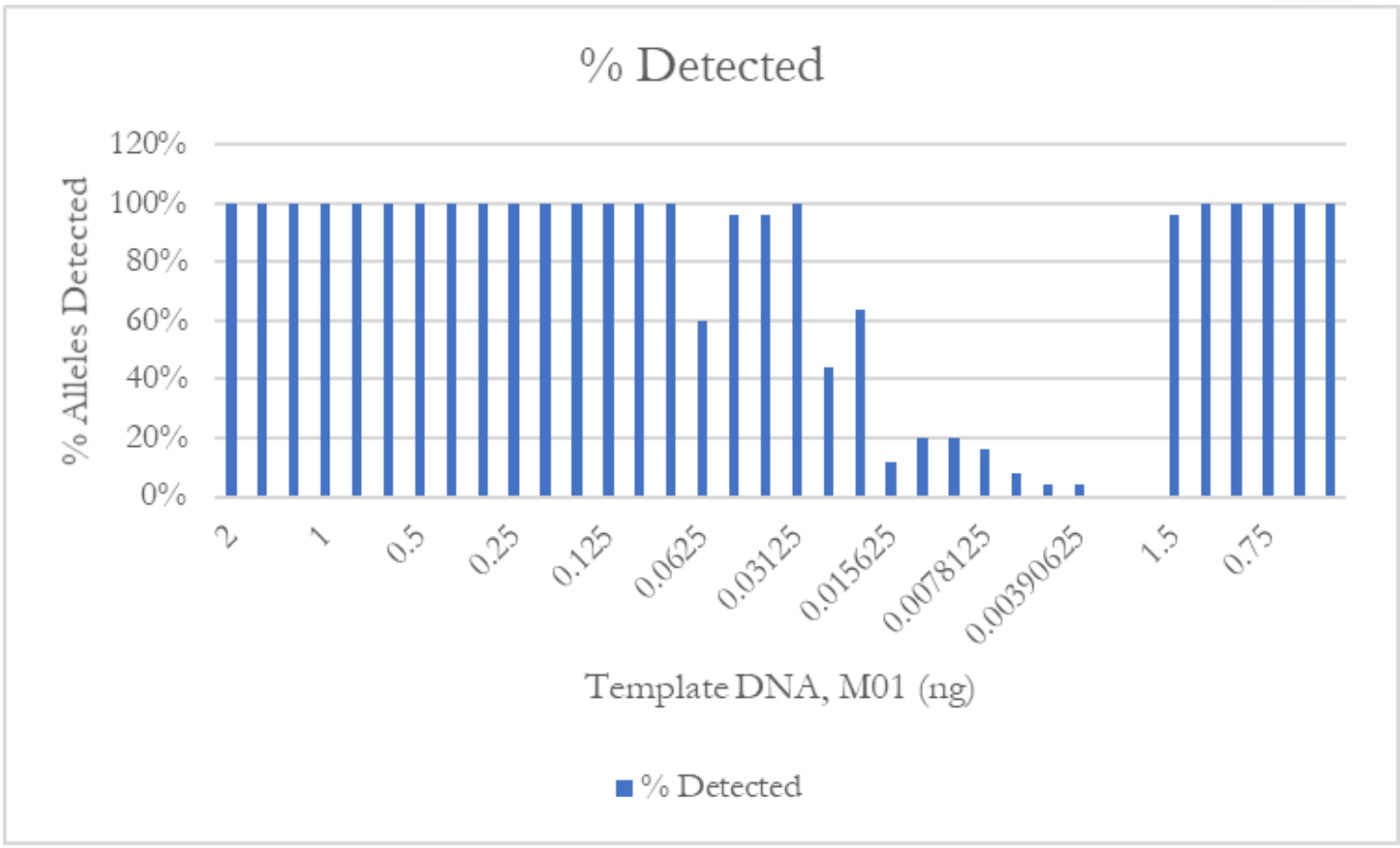

Figure 8 depicts a bar chart of the % alleles detected across all dyes when the serially diluted extracted male DNA (M01) was amplified using the YFiler Plus PCR amplification kit. The samples were amplified in triplicate and each replicate is shown on the graph. Drop out of alleles began occurring at 125pg. Template amounts tested from the serially diluted samples were 2.0, 1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, and 0.00390625. Additionally, 1.5 and 0.75 ng were included in this study.

Table 15

Dilution	Template DNA (M01)	DYS389I (avg)	DYS389II (avg)	% Balance (DYS389I:DYS389II)	Dilution	Control DNA (007)	DYS389I (avg)	DYS389II (avg)	% Balance (DYS389I:DYS389II)
A	2.0	9985.3	10958.0	91%	A	2	3597.3	1914.0	188%
B	1.0	4320.0	4893.0	88%	B	1	1683.7	940.0	179%
C	0.5	1646.0	2001.0	82%	C	0.5	723.0	419.0	173%
D	0.25	817.7	956.0	86%	D	0.25	528.3	289.3	183%
E	0.125	324.3	446.0	73%	E	0.125	238.0	135.7	175%
F	0.0625	193.0	158.0	122%	F	0.0625	98.3	0.0	
G	0.03125	286.7	662.0	43%	G	0.03125	0.0	0.0	
H	0.015625	0.0	0.0		H	0.015625	0.0	0.0	
I	0.0078125	0.0	0.0		I	0.0078125	0.0	0.0	
J	0.00390625	0.0	0.0		J	0.00390625	0.0	0.0	
K	1.5	8089.3	7050.0	115%	K	1.5	2662.3	1431.0	186%
L	0.75	3945.3	4076.0	97%	L	0.75	1389.3	790.0	176%

Table 15 shows the average peak heights at DYS389I and DYS389II , and the corresponding peak height ratio (balance) for the sensitivity studies using DNA control 007 and the extracted male DNA (M01). For the control DNA 007, the DYS389II allele was undetected in the 62.5pg sample. For the extracted male DNA (M01), the peak height balance dropped to 43% at the 31.25pg sample. The YFiler Plus PCR amplification kit shows more sensitive results for the EZ1 DNA Investigator kit extracted DNA than it does for the Control DNA 007 provided in the kit.

The table above depicts the balance ratio between DYS 389I and DYS 389II loci for EZ1 extracted male DNA (M01) and Control DNA 007 sensitivity samples amplified using the AmpFlSTR Yfiler Plus PCR amplification kit. The average total RFU for the DYS 389I and DYS 389II loci was calculated for the two-fold serially diluted samples (range 2.0 ng - 0.004 ng) amplified in triplicate. Data analysis was performed using 75 RFU and the GeneMapper ID-X, version 1.5 software.

The data shows peak imbalance exceeding 30% tolerance when 0.031 ng of EZ1 extracted male DNA (M01) was amplified. Such peak imbalance corresponds to the 0.063ng limit of detection as determined in the Linearity calculations for the EZ1 extracted male DNA (M01) sensitivity series. The extracted DNA from the QIAGEN EZ1 DNA Investigator kit (QIAGEN catalog no. 952034) yielded better results than the positive control provided in the YFiler Plus PCR amplification kit. Such observation is not atypical and it is well understood in the forensic community that QIAGEN extracted DNA is more easily amplified than others.

Sensitivity & Stochastic QAS 8.3.1(3)- Study 3

The serially diluted blood samples purified on the EZ2® Connect Fx instruments resulted in a linear trend when quantified with Investigator® Quantiplex® Pro. Instrument EZ2A recovered DNA from dilutions A-H with the lowest recovery at 0.00028 ng/µL and the lowest recovery for EZ2B was 0.00050 ng/µL.

Serial dilutions A - H ranged in concentration from 1.35812 ng/µL to 0.00014 ng/µL for the male target on EZ2A and 100% alleles were detected with Yfiler™ Plus for dilutions A-D after amplification. Allelic dropout was observed starting in dilution E, which had a male concentration of 0.00526 ng/µL with 92% alleles detected. Serial dilutions A - H ranged in concentration from 1.69069 ng/µL to 0.00038 ng/µL for the male target on EZ2B and 100% alleles were detected with Yfiler™ Plus for dilutions A-D after amplification. Allelic dropout was observed starting in dilution E, which had a male concentration of 0.00435 ng/µL with 96% alleles detected (Figure 15).

As the total amount of amplified DNA decreases both the average percent of alleles detected and the average peak height decrease.

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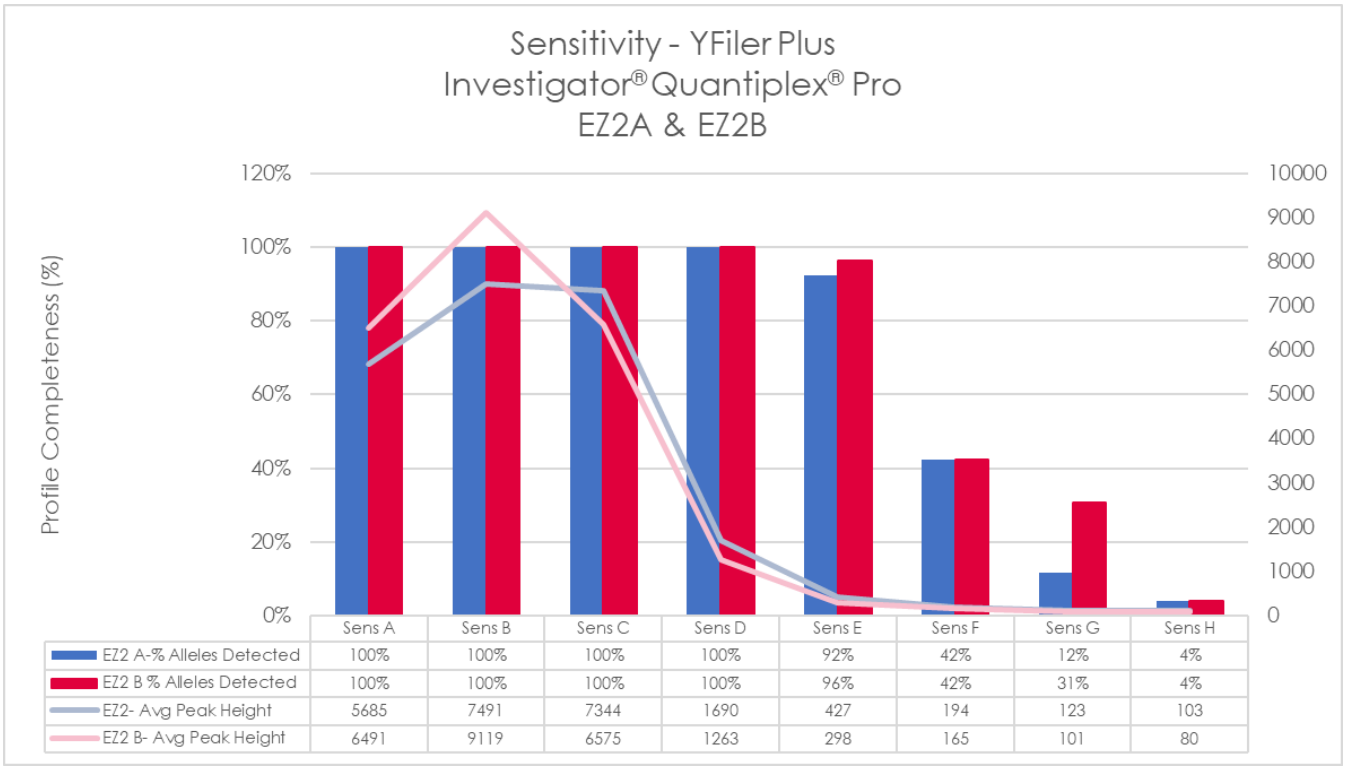

	Sens A	Sens B	Sens C	Sens D	Sens E	Sens F	Sens G	Sens H
EZ2 A-% Alleles Detected	100%	100%	100%	100%	92%	42%	12%	4%
EZ2 B % Alleles Detected	100%	100%	100%	100%	96%	42%	31%	4%
EZ2- Avg Peak Height	5685	7491	7344	1690	427	194	123	103
EZ2 B- Avg Peak Height	6491	9119	6575	1263	298	165	101	80

Figure 9 defines the % alleles detected and the average peak height when amplifying two sets of serially diluted blood samples extracted on the EZ2 FX instrument. Amplification was performed using the YFiler Plus PCR amplification kit. This sample set was quantified using the Quantiplex PRO quantification kit. The average template for both EZ2 A and EZ2 B instruments is reflected in the figure

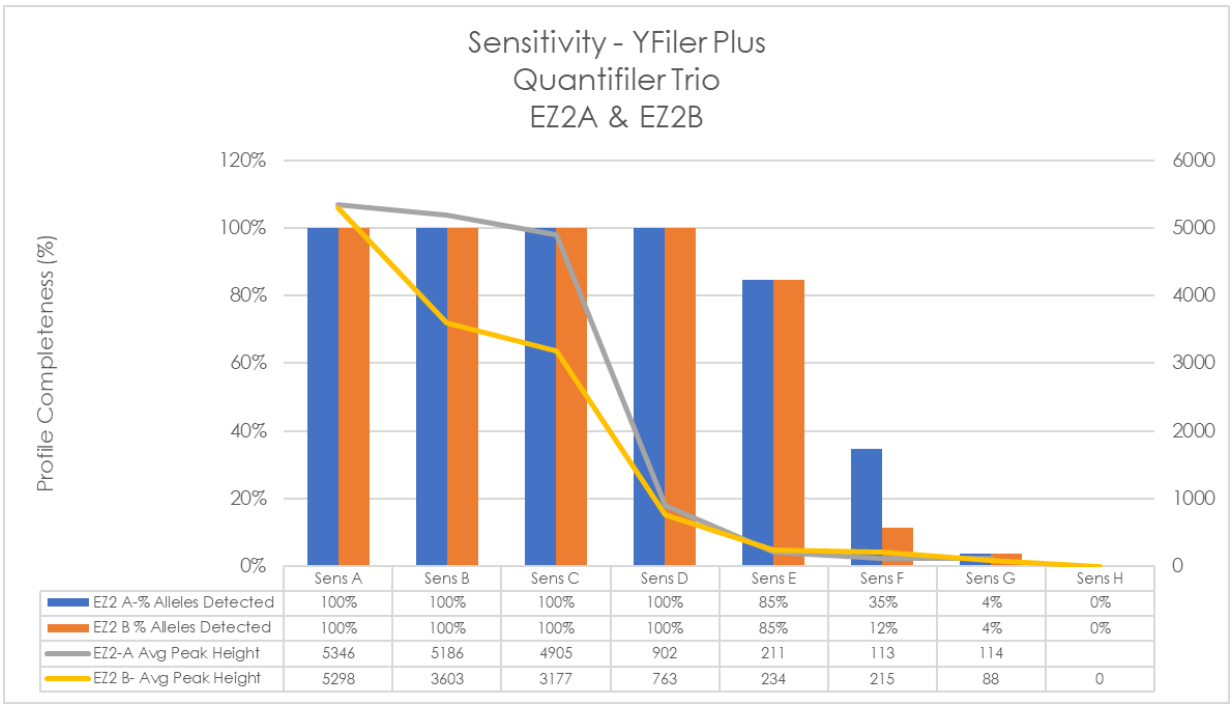

Figure 10 defines the % alleles detected and the average peak height when amplifying two sets of serially diluted blood samples extracted on the EZ2 FX instruments. Amplification was performed using the YFiler Plus PCR amplification kit. This sample set was quantified using the Quantifiler Trio quantification kit. The average template for both EZ2 A and EZ2 B instruments is reflected in the figure.

Mixture Studies QAS 8.3.1 (4)-Study 1

2 persons (female 1: male 1)

The DNA template per contributor into the mixture samples prepared in Mixture Study 1 may be referenced in the table below. Samples were prepared to allow for the respective quantities of template DNA to be added in 10 uL sample volume into each PCR reaction.

Table 16

Sample	Ratio (Female: Male)	Female Donor (ng)	Male Donor (ng)
Mixture 1	99:1	99	1.00
Mixture 2	49:1	49	1.00
Mixture 3	29:1	29	1.00
Mixture 4	19:1	19	1.00
Mixture 5	14:1	14	1.00
Mixture 6	9:1	9	1.00
Mixture 7	4:1	4	1.00
Mixture 8	3:1	3	1.00
Mixture 9	2:1	2	1.00
Mixture 10	1:1	1	1.00
Mixture 11	1:2	0.5	1.00
Mixture 12	1:3	0.33	1.00
Mixture 13	1:4	0.25	1.00
Mixture 14	1:9	0.11	1.00
Mixture 15	1:14	0.07	1.00
Mixture 16	1:19	0.053	1.00
Mixture 17	1:29	0.034	1.00
Mixture 18	1:49	0.02	1.00
Mixture 19	1:99	0.01	1.00

Table 16 defines the ratio of female: male template DNA for mixture studies prepared. The male donor was held constant at 1.0ng in Mixture study 1 while the female DNA was altered to the defined ratio. Each sample was amplified in duplicate.

The results of the Mixture study 1 show that 100% of the donor haplotype was detected when 1.0ng of male DNA was amplified in various amounts of background female DNA. Samples analyzed at 75 RFU show that for 975 intended alleles in the 38 mixtures samples in study 1, an additional 7% of non-intended alleles were detected. Reanalysis of the mixture samples in study 1 using 100 RFU decreased the observation of unintended alleles from 7% to 2 %. Therefore, it is recommended that the threshold for GMIDX analysis be raised to RFU >100.

The 7% of the unintended alleles were attributed to 68 additional alleles. Some loci had more non-intended alleles detected than present in other loci when compared to the known haplotype of the sample. The Table defines which loci had the greatest occurrences of non-intended alleles. Locus specific results are defined in the Table. Briefly, DYS 391 had an additional 21 alleles detected versus the 39 alleles expected. This equates to the haplotype detection of 154%. Other loci are as follows: DYS448 was observed at 138%, and 131% at YGATAH4; 110% at DYS19 and DYS460; 108% at DYS385; 105% at DYS518; 103% at DYS627, DYS 456, DYS449, DYS393, DYS439, DYS533; and, 101% at DYS387.

Table 17

Locus	DYS576 1	DYS389I 1	DYS389II 1	DYS627 1
Expected Alleles	39	39	39	39
Observed Alleles	39	39	39	40
% Haplotype	100%	100%	100%	103%

Locus	DYS460 1	DYS458 1	DYS19 1	YGATAH4	DYS448 1	DYS391 1
Expected Alleles	39	39	39	39	39	39
Observed Alleles	43	39	43	51	54	60
% Haplotype	110%	100%	110%	131%	138%	154%

Locus	DYS456	DYS 390	DYS 438	DYS 392	DYS 518
Expected Alleles	39	39	39	39	39
Observed Alleles	40	39	39	39	41
% Haplotype	103%	100%	100%	100%	105%

Locus	DYS 570	DYS 437	DYS 385	DYS 449
Expected Alleles	39	39	39	39
Observed Alleles	39	39	42	40
% Haplotype	100%	100%	108%	103%

Locus	DYS393	DYS439	DYS481	DYF387SI/II	DYS533
Expected Alleles	39	39	39	78	39
Observed Alleles	40	40	39	79	40
% Haplotype	103%	103%	100%	101%	103%

Table 17 defines the average RFU, standard deviation (ST DEV), and the coefficient of variation (CV) for each locus for the mixture study 1 samples (N=38) and the positive control DNA. The non-specific, off-ladder (OL) alleles were not included in these calculations. One ng of male DNA was the target for each amplification.

Of the 68 additional alleles in the 38 mixture samples and 1 positive control, some loci had more non-intended alleles detected when compared to the known haplotype of the sample. For example, there were an additional 21 alleles detected at DYS 391 versus the 39 alleles detected (or 154%) . For other loci affected with non-intended allele calls, DYS448 was observed at 138%, and 131% at YGATAH4; 110% at DYS19 and DYS460; 108% at DYS385; 105% at DYS518; 103% at DYS627, DYS 456, DYS449, DYS393, DYS439, DYS533; and, 101% at DYS387.

Table 18

	Average (RFU)	ST DEV	CV
DYS576	3241.12	1032.92	32%
DYS389I 1	5024.20	1040.66	21%
DYS635	3917.73	1159.52	30%
DYS389II	3062.10	1012.66	33%
DYS627	4448.60	1090.37	25%
DYS460	2293.67	971.94	42%
DYS458	4619.17	1744.25	38%
DYS19	3998.66	1489.62	37%
YGATAH4	3538.39	1969.96	56%
DYS448	4001.95	1637.66	41%
DYS391	1436.61	704.17	49%
DYS456	3196.93	918.65	29%
DYS390 1	2604.22	744.39	29%
DYS438 1	4207.66	701.15	17%
DYS392	4161.27	608.99	15%
DYS518 1	3891.20	580.37	15%
DYS570	3001.61	1335.05	44%
DYS437	3263.07	1001.06	31%
DYS385 I/II	3402.60	1297.02	38%
DYS449	3640.83	1245.88	34%
DYS393	3246.95	1364.56	42%
DYS439	2589.49	918.62	35%
DYS481	4825.29	1513.04	31%
DYF387SI/II	4811.65	1638.98	34%
DYS533	4443.39	1796.97	40%

Table 18 defines the average RFU for all 19 mixture study samples (Mixture study 1) when male DNA (1.0ng) containing varying amounts of background female DNA was amplified in duplicate. The standard deviation of the peak height and the coefficient of variance (CV) was detected.

Mixture Studies QAS 8.3.1 (4)- Study 2

2 persons (male 1: male 2)

The DNA template per contributor into the mixture samples prepared in Mixture Study 2 are referenced in the table below. The mixture samples for study 2 were prepared from two different male donors to allow for the respective quantities of template DNA to total 1.0ng in 10 uL volume per PCR reaction. Each sample was amplified in duplicate and analyzed at 100 RFU.

Table 19

Sample	Ratio (M1:M2)	Male Donor 1 (ng)	Male Donor 2 (ng)
Mixture 1	99:1	0.99	0.01
Mixture 2	49:1	0.98	0.02
Mixture 3	29:1	0.97	0.03
Mixture 4	19:1	0.95	0.05
Mixture 5	14:1	0.93	0.07
Mixture 6	9:1	0.90	0.10
Mixture 7	4:1	0.80	0.20
Mixture 8	3:1	0.75	0.25
Mixture 9	2:1	0.67	0.33
Mixture 10	1:1	0.50	0.50
Mixture 11	1:2	0.33	0.67
Mixture 12	1:3	0.25	0.75
Mixture 13	1:4	0.25	0.75
Mixture 14	1:9	0.10	0.90
Mixture 15	1:14	0.07	0.93
Mixture 16	1:19	0.05	0.95
Mixture 17	1:29	0.03	0.97
Mixture 18	1:49	0.02	0.98
Mixture 19	1:99	0.01	0.99

Table 19 defines the template DNA per ratio of male 1: male 2 for samples prepared in Mixture study 2. The total male donor template was held constant at 1.0ng. Each sample was amplified in duplicate.

Two male contributors were used in preparation of the mixture samples used in this study. The obligate alleles were determined for each contributor. There were 11 obligate alleles for contributor, M01; there were 13 obligate alleles for the other contributor, M02. Presence or absence of the obligate alleles were determined and a percentage of the total was calculated. Resultant data may be reference in the chart below.

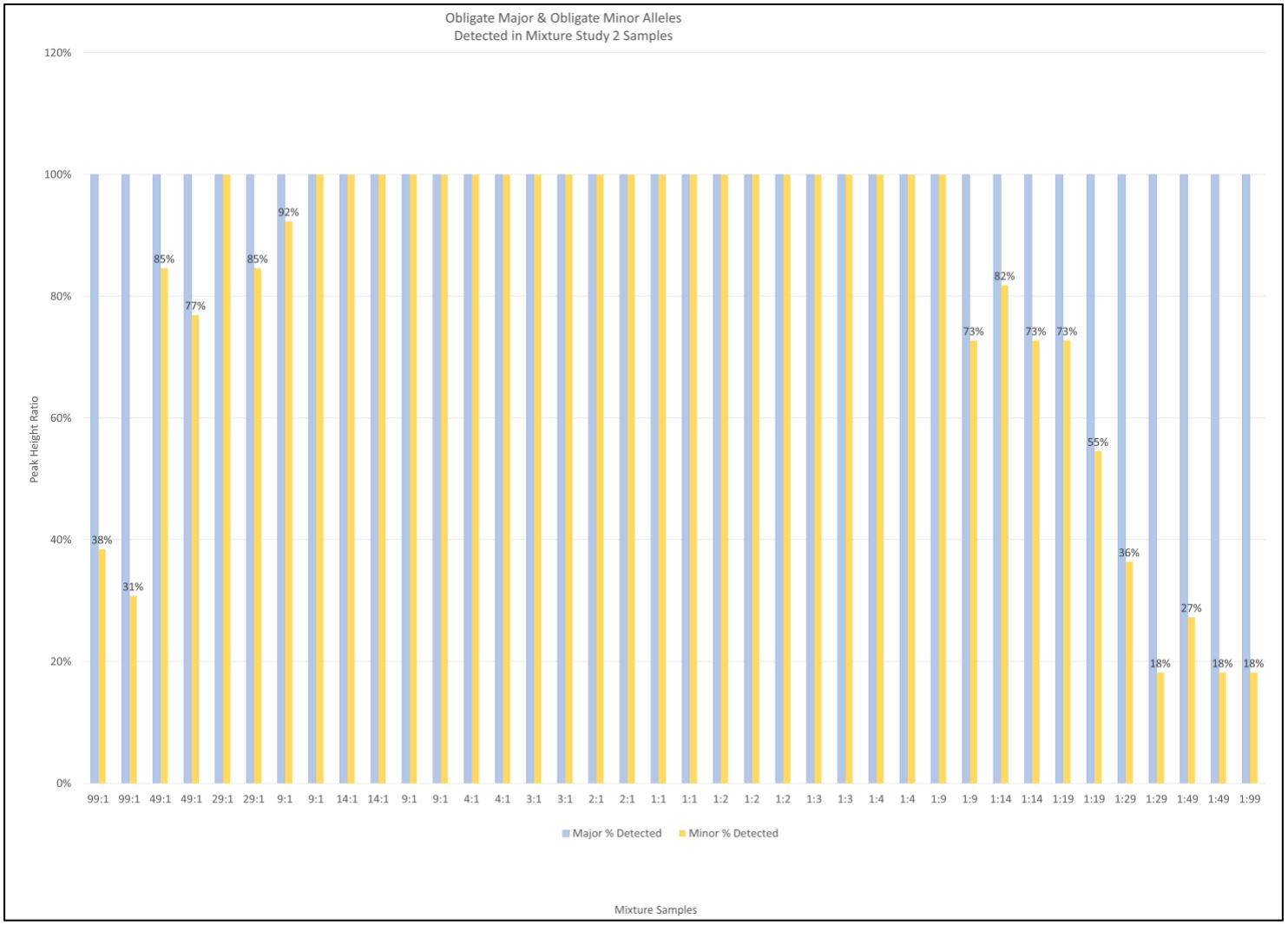

Figure 11 depicts the peak height ratio of the obligate major versus obligate minor alleles for the Mixture study 2 male donors. The average of the duplicates were used for this graph. The haplotype of the major DNA contrributor was detected in every sample. The minor DNA contributor varied from 18% alleles detected to 100 % alleles detected and varied according to template amounts for the minor contributor.

The haplotype of the major DNA contributor in the mixture samples was detected in every sample. The quantity of major male donor in the amplification reactions ranged from 0.99 ng to 0.50 ng, showing that 500 ng is sufficient to detect a full Y-STR haplotype.

Mixture Studies QAS 8.3.1(4)- Study 3

3 persons (female 1: male 1: male 2)

The DNA template per contributor into the mixture samples prepared in Mixture Study 3 may be referenced in the table below. The mixture samples for study 3 were prepared from two different male donors to allow for the respective ratios of DNA as defined in the Table below. The total template of male DNA for each reaction was 1.0ng. A background of female contributor DNA was added to each mixture sample and was held constant at 25ng. Each mixture sample was prepared with 10 uL sample volume into each PCR reaction. Each sample was amplified in duplicate and analyzed at 100 RFU.

Table 20

Sample	Ratio (M1:M2)	Female Donor (ng)	Male Donor 1 (ng)	Male Donor 2 (ng)
Mixture 1	99:1	25	0.99	0.01
Mixture 2	49:1	25	0.98	0.02
Mixture 3	29:1	25	0.97	0.03
Mixture 4	19:1	25	0.95	0.05
Mixture 5	14:1	25	0.93	0.07
Mixture 6	9:1	25	0.90	0.10
Mixture 7	4:1	25	0.80	0.20
Mixture 8	3:1	25	0.75	0.25
Mixture 9	2:1	25	0.67	0.33
Mixture 10	1:1	25	0.50	0.50
Mixture 11	1:2	25	0.33	0.67
Mixture 12	1:3	25	0.25	0.75
Mixture 13	1:4	25	0.25	0.75
Mixture 14	1:9	25	0.10	0.90
Mixture 15	1:14	25	0.07	0.93
Mixture 16	1:19	25	0.05	0.95
Mixture 17	1:29	25	0.03	0.97
Mixture 18	1:49	25	0.02	0.98
Mixture 19	1:99	25	0.01	0.99

Table 20 defines the template DNA per ratio of female: male 1: male 2 for samples prepared in Mixture study 3. The total male donor template was held constant at 1.0ng and are identical samples as prepared in Mixture study 2, except the background female DNA was held constant at 25ng per sample. Each sample was amplified in duplicate.

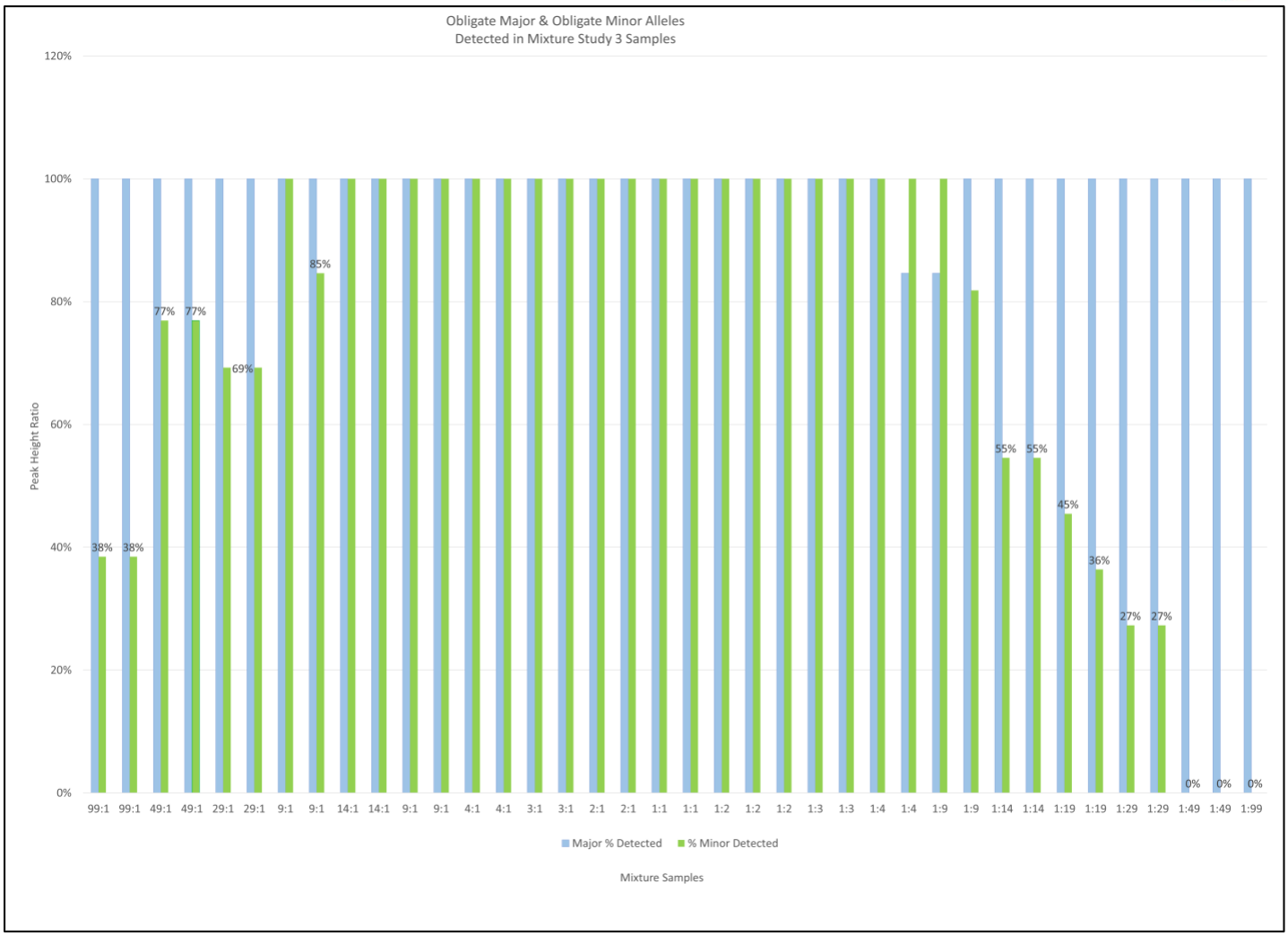

Figure 12 depicts the peak height ratio of the obligate major versus obligate minor alleles for the Mixture study 3 male donors. The average of the duplicates were used for this graph. The haplotype of the major DNA contrributor was detected in every sample. The minor DNA contributor varied from 0% alleles detected to 100 % alleles detected and varied according to template amounts for the minor contributor. The Mixture study 3 samples are identifical in preparation to the Mixture study 2 samples, except Mixture study 3 contained a constant backgroung of female DNA (25ng) for all samples. Overall the obligate minor alleles were less detected in Mixture study 3 than in the Mixture study 2 results, indicating an inhibitory effect from the background female component in the samples.

Two male contributors and one female contributor DNA were used in preparation of the mixture samples used in this study. The female DNA was held constant at 25ng in each amplification reaction. The obligate alleles within the two male haplotypes were determined for each male contributor. There were 11 obligate alleles for male contributor, M01; there were 13 obligate alleles for the other male contributor, M02. Presence or absence of the obligate alleles were determined and a percentage of the total was plotted in the table above. The haplotype of the major DNA contributor in the mixture samples were detected in every sample. The quantity of major male donor ranged from 0.99 ng to 0.50 ng.

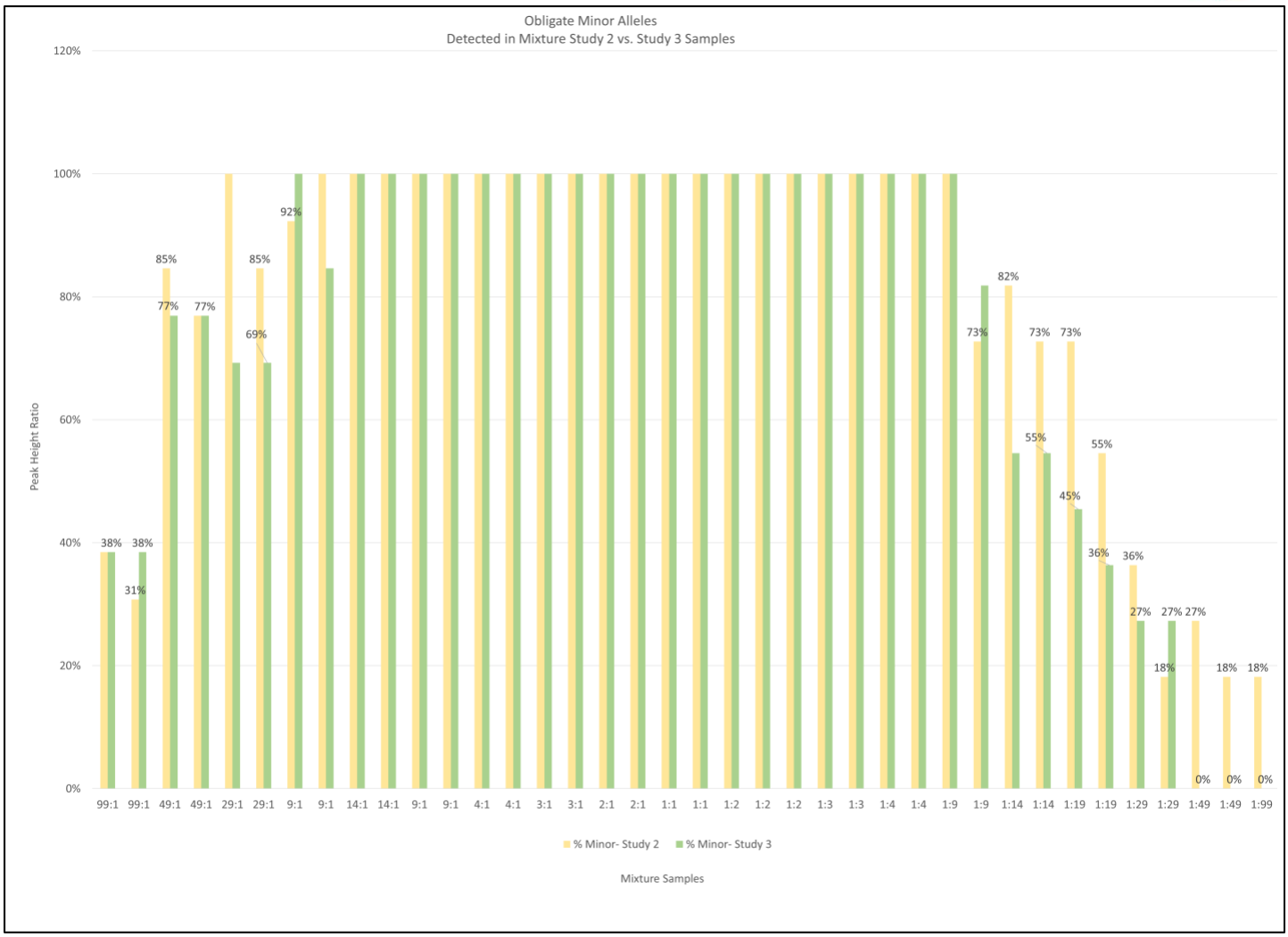

Figure 13 depicts the obligate minor alleles detcected for the male donors in Mixture study 2 versus Mixture study 3 male donors. The average of the duplicates were used for this graph. The Mixture study 3 samples are identicical in preparation to the Mixture study 2 samples, except Mixture study 3 contained a constant backgroung of female DNA (25ng) for all samples. Overall the obligate minor alleles were less detected in Mixture study 3 than in the Mixture study 2 results, indicating an inhibitory effect from the background female component in the samples.

Mixture study 2 and mixture study 3 samples containing male:male DNA were identically prepared. The difference between these two sample sets is that the mixture study 3 samples had an additional 25ng female DNA added to represent background for observation of suppressed amplification. To determine the effects of the background female DNA being added to the reactions, the obligate minor alleles from the male contributors were determined. Indeed the chart above shows that a larger number of obligate minor alleles were detected when female DNA was not present in the sample.

Known & Mock Evidence QAS 8.3.1(1)

Fourteen non-probative DNA samples were quantified using Quantiplex PRO and Quantifiler Trio. For each Y-STR amplification reaction setup, either 1.0ng or maximum volume (10 uL) was added to each reaction. Given the type of samples extracted, all results were as expected and concordant with those on file at the MSFL. Resultant quantification values from both Quantiplex PRO and Quantifiler Trio may be referenced in the table below, as well as whether a Y-Filer haplotype was detected. Electropherograms may be referenced in the Appendix.

Table 21

		Quantification Results (ng/uL)					STR Results
		QUANTIPLEX PRO		QUANTIFILER TRIO			Y-Filer Haplotype?
		HUMAN	MALE	LARGE	SMALL	Y	
Sample Identifiers	Source	2 uL	2 uL	2 uL	2 uL	2 uL	(YES, NO, Partial)
NP1	Blank Control	0.0000	0.0000	0.0000	0.0000	0.0000	**NO**
NP2	dried blood; unknown	0.7315	0.5413	0.6700	0.6700	0.6200	**YES**
NP3	dried blood; RLD; 9.4.2020	3.3789	3.6604	3.2000	3.5600	3.0400	**YES**
NP4	CTS 13-572 #2-4x 1.2 Harris Punch	0.1832	0.1229	0.0540	0.0910	0.0750	**YES**
NP5	QIAGEN R&D - Miro mix 1	55.5815	0.0233	63.2400	50.7400	0.0037	**Partial**
NP6	QIAGEN R&D - Miro mix 2	58.7299	0.0026	18.6700	20.8200	38.1100	**NO**
NP7	QIAGEN R&D - Miro mix 3	47.9612	0.0010	60.2700	52.0500	0.0000	**NO**
NP8	BLANK CONTROL	0.0002	0.0000	0.0000	0.0000	0.0000	**NO**
NP9	buccal swab; Jonas Guilliano	0.9754	0.6592	0.7500	0.9200	0.9600	**YES**
NP10	3x, 1.2 punch- indicating micro R.Roy	0.0989	0.0000	0.0800	0.1000	0.0000	**YES**
NP11	old proficiency; blue fabric; sperm +	0.0237	0.0030	0.0110	0.0210	0.0220	**NO**
NP12	baby tooth; WSFD	8.2226	7.1570	8.1200	7.3100	8.2100	**YES**
NP13	blood on swab; WSFD; Kendall swab	0.0875	0.1046	0.0400	0.0600	0.0600	**YES**
NP14	USACIL; flock swab "A"	0.0000	0.0000	0.0001	0.0000	0.0000	**NO**
M01	Male DNA- semen stock	13.6097	12.2410	20.7900	22.8300	22.8300	**YES**
M02	Joe saliva	18.6500	0.0000	16.8200	18.4400	20.2200	**YES**
F01	Female DNA- saliva	14.6700	0.0001	19.0500	15.3000	0.0000	**NO**
F02	Jen saliva	17.4000	0.0000	19.4400	21.3500	0.0000	**NO**

Table 21 shows the sample identifiers, source of sample extracted, and the quantification results (ng/uL) for the non-probative samples. Both the Quantiplex PRO and the Quantifiler Trio quantification kits were employed. Additionally, Y-Filer haplotype results for each sample are defined as YES, NO, or Partial. The STR results correspond the sample source and the quantification results for each sample used in this study.

Accuracy: NIST 2391d (QAS 8.4) & Concordance/Comparison to Original Procedures (QAS 8.5)

Y-STR haplotypes were obtained from the Standard Reference Material® 2391d PCR Based DNA Profiling Standard from components B, C, D. The resultant haplotypes were compared and found to be consistent with the published results in the Reference Material® 2391d Certified Haplotypes, 28 Y-STR Loci publication, located in Table 5.

Table 22

YFP_NIST2391-B		YFP_NIST2391-C		YFP_NIST2391-D	
DYS576	15	DYS576	17	DYS576	17
DYS389I	12	DYS389I	12	DYS389I	12
DYS635	21	DYS635	21	DYS635	21
DYS389II	30	DYS389II	31	DYS389II	31
DYS627	18	DYS627	20	DYS627	20
DYS460	10	DYS460	10	DYS460	10
DYS458	17	DYS458	18	DYS458	18
DYS19	15	DYS19	16	DYS19	16
YGATAH4	13	YGATAH4	12	YGATAH4	12
DYS448	21	DYS448	22	DYS448	22
DYS391	11	DYS391	10	DYS391	10
DYS456	15	DYS456	15	DYS456	15
DYS390	21	DYS390	21	DYS390	21
DYS438	11	DYS438	11	DYS438	11
DYS392	11	DYS392	11	DYS392	11
DYS518	37	DYS518	38	DYS518	38
DYS570	20	DYS570	18	DYS570	18
DYS437	14	DYS437	14	DYS437	14
DYS385	15,16	DYS385	16,17	DYS385	16,17
DYS449	32	DYS449	28	DYS449	28
DYS393	13	DYS393	13	DYS393	13
DYS439	13	DYS439	12	DYS439	12
DYS481	26	DYS481	28	DYS481	28
DYF387S1	36,38	DYF387S1	36,39	DYF387S1	36,39
DYS533	11	DYS533	11	DYS533	11

Table 22 defines the Y-STR haplotypes obtained from the Standard Reference Material® 2391d PCR Based DNA Profiling Standard from components B, C, D. Green cells indicate results corresponding to the published NIST results. The data in the white cells were not available for comparison given they were not published by NIST.

Ladder Precision

Table 23

Size (bp) %CV	DYS576 1	DYS389	DYS635	DYS389II	DYS627	
MIN	0.11%	0.03%	0.02%	0.01%	0.04%	
MAX	0.21%	0.04%	0.04%	0.03%	0.05%	
Size (bp) %CV	DYS460	DYS458	DYS19	YGATAH	DYS448	DYS391
MIN	0.08%	0.07%	0.01%	0.01%	0.03%	0.02%
MAX	0.15%	0.10%	0.03%	0.03%	0.04%	0.03%
Size (bp) %CV	DYS456 1	DYS390 1	DYS438	DYS392	DYS518	
MIN	0.05%	0.02%	0.02%	0.02%	0.01%	
MAX	0.13%	0.04%	0.03%	0.03%	0.02%	
Size (bp) %CV	DYS570	DYS437	DYS385	DYS449		
MIN	0.02%	0.02%	0.02%	0.01%		
MAX	0.05%	0.03%	0.03%	0.02%		
Size (bp) %CV	DYS393	DYS439	DYS481	DYF387S1	DYS533	
MIN	0.04%	0.02%	0.01%	0.04%	0.01%	
MAX	0.08%	0.05%	0.03%	0.05%	0.02%	

Table 23 defines the minimum and maximum coefficient of variance (CV) for all loci in all ladders run throughout the course of the validation (N=10).

Summary

Based upon the results obtained from the studies on sensitivity/linearity, reproducibility and precision, mixtures, concordance, cross-contamination, NIST-traceability, and known and non-probative, the following should be noted:

- The YFiler Plus PCR Amplification kit reliably and reproducibly generated full Y-STR haplotypes at a target concentration of 1.0 ng of input DNA.
- The YFiler Plus PCR Amplification kit reliably and reproducibly generated full Y-STR haplotypes from both buccal swabs and blood stains on paper.
- The YFiler Plus PCR Amplification kit produced concordant and reproducible results for extracted DNA at concentrations of 0.5ng, 0.25ng, and 0.125ng.
- YFiler Plus PCR Amplification kit produced Y-STR haplotypes results concordant with NIST 2391d, components B, C, and D.
- The YFiler Plus PCR Amplification kits produced allelic ladders with low standard deviations and low %CV.
- YFiler Plus PCR Amplification kit satisfactorily detected the presence of multiple contributors in mock mixture samples.

The validation studies performed on the YFiler Plus PCR Amplification kits support the chemistry as being robust and reliable tools for obtaining Y-STR results for forensic DNA casework operations at the Mississippi Forensics Laboratory.

Appendices

A. User Manuals

1) 01_User Manuals\4485610_yfilerplus_ug.pdf

B. Validation Guides

02_Validation Guides\MSFL_YF+_ValidationPlan2.pdf

02_Validation Guides\MSFL_YFiler+ Validation111623.xlsx

C. Quant Files

1) 03_YFP_Quant Files\QuantiplexPRO_mjd100822.eds
2) 03_YFP_Quant Files\QuantiplexPRO_mjd101622.eds
3) 03_YFP_Quant Files\QuantiplexPRO_mjd111622.eds
4) 03_YFP_Quant Files\QuantiplexPROLYO_FT2-mjd041823.eds
5) 03_YFP_Quant Files\041823jh.eds

D. Raw CE Data

1) 04_Raw Data\YFP_Validation_plate1_mjd101322
2) 04_Raw Data\YFP_validation_plate2_MJD10.21.2022
3) 04_Raw Data\YFP_valid_CEplate 3_Sens 4_mjd102722
4) 04_Raw Data\YFP_Validation_MixtureStudy_MJD120722
5) 04_Raw Data\YFP_VALID_mix2.3_MJD
6) 04_Raw Data\YFP_validation_NPs_022223
7) 04_Raw Data\YFP_All Ladders

E. GMIDX Exports

1) 05_YFP_GMIDX exports\YFP_Validation_Plate1_MJD.txt
2) 05_YFP_GMIDX exports\YFP_Validation Plate 2.txt
3) 05_YFP_GMIDX exports\YFP_Validation Plate 2_Allele Table.txt
4) 05_YFP_GMIDX exports\YFP_Validation Plate 2_VALIDATION.txt
5) 05_YFP_GMIDX exports\YFP_Validation Plate 2_VALIDATION_110922_Yfiler_Plus_Panel_v4.txt
6) 05_YFP_GMIDX exports\YFP_Validation Plate 2_VALIDATION_all.txt
7) 05_YFP_GMIDX exports\YFP_Validation Plate 2_Validation2.txt
8) 05_YFP_GMIDX exports\YFP_Validation Plate 2_Validation2_RFU.txt
9) 05_YFP_GMIDX exports\YFP_Validation Plate 2_Yfiler_Plus_Panel_v4.txt
10) 05_YFP_GMIDX exports\YFP_Validation Plate 2_VALIDATION_all.txt
11) 05_YFP_GMIDX exports\YFP_validation_plate3_mjd102822_Allele Table.txt

12) 05_YFP_GMIDX exports\YFP_validation_plate3_mjd102822_VALIDATION.txt
13) 05_YFP_GMIDX exports\YFP_validation_plate3_mjd102822_VALIDATION_ALL.txt
14) 05_YFP_GMIDX exports\YFP_validation_plate3_mjd102822_VALIDATION_Yfiler_Plus_Panel_v4.txt
15) 05_YFP_GMIDX exports\YFP_validation_plate3_mjd102822_Validation2.txt
16) 05_YFP_GMIDX exports\YFP_validation_plate3_mjd102822_Validation2_RFU.txt
17) 05_YFP_GMIDX exports\YFP_validation_plate3_mjd102822_Yfiler_Plus_Panel_v4.txt
18) 05_YFP_GMIDX exports\YFP validation_Mix2.3_VALIDATION_Yfiler_Plus_Panel_v4.txt
19) 05_YFP_GMIDX exports\YFP validation_Mix2.3_Validation2_Yfiler_Plus_Panel_v4.txt
20) 05_YFP_GMIDX exports\YFP_Validation_Mixtures_MJD_VALIDATION_Yfiler_Plus_Panel_v4.txt
21) 05_YFP_GMIDX exports\YFP_Validation_Mixtures_MJD_Validation2_Yfiler_Plus_Panel_v4.txt
22) 05_YFP_GMIDX exports\YFP_Validation_NPs_022623_VALIDATION.2_Yfiler_Plus_Panel_v4.xlsx
23) 05_YFP_GMIDX exports\YFP_Validation_NPs_022623_VALIDATION.txt
24) 06_YFP Analyzed Data\YFP_LaddersOnly_validation_Yfiler_Plus_Panel_060623.xlsx

F. Analyzed Data

1) 06_YFP Analyzed Data\YFP_Validation RESULTS_SENSITIVITY111622.xlsm
2) 06_YFP Analyzed Data\MixtureStudy2_Obligate Alleles.pptx
3) 06_YFP Analyzed Data\MSFL_Q.ProLyo_FieldTest_092622.xlsx
4) 06_YFP Analyzed Data\Reference Y-STR Profiles.pdf
5) 06_YFP Analyzed Data\YFP validation_Mixtures_2.3_032923.xlsx
6) 06_YFP Analyzed Data\YFP validation_Mixtures_2charted_032923.xlsx
7) 06_YFP Analyzed Data\YFP validation_Mixtures_3charted_032923.xlsx
8) 06_YFP Analyzed Data\YFP_LaddersOnly_validation_Yfiler_Plus_Panel_060623.xlsx
9) 06_YFP Analyzed Data\YFP_Validation_Mixtures_Mixtures_060923.xlsx
10) 06_YFP Analyzed Data\YFP_Validation_NIST results_VALIDATION.xlsx

G. Electropherograms

1) 07_Electropherograms\Mix2_13,14_DYS576.PNG
2) 07_Electropherograms\YFP_M01.PNG
3) 07_Electropherograms\YFP_NIST 2391-C.PNG
4) 07_Electropherograms\YFP_NIST 2391-B.PNG
5) 07_Electropherograms\YFP_NIST 2391-D.PNG
6) 07_Electropherograms\YFP_NP1.PNG
7) 07_Electropherograms\YFP_NP10.PNG
8) 07_Electropherograms\YFP_NP11.PNG
9) 07_Electropherograms\YFP_NP12.PNG
10) 07_Electropherograms\YFP_NP13.PNG
11) 07_Electropherograms\YFP_NP14.PNG
12) 07_Electropherograms\YFP_NP2.PNG
13) 07_Electropherograms\YFP_NP3.PNG
14) 07_Electropherograms\YFP_NP4.PNG
15) 07_Electropherograms\YFP_NP5.PNG

16) 07_Electropherograms\YFP_NP6.PNG
17) 07_Electropherograms\YFP_NP7.PNG
18) 07_Electropherograms\YFP_NP8.PNG
19) 07_Electropherograms\YFP_NP9.PNG
20) 07_Electropherograms\YFP_NP9.2.PNG
21) 07_Electropherograms\YFP_PC022223.PNG

H. Publications

1) 07_Publications\2391d.pdf
2) 07_Publications\FSIG_24 2016 p164-175 Developmental valid YFP.pdf

Document revision history:

12.29.2023

- MJD updated Methods to reflect assisted pipetting and the QIAgility instrument
- Added Sensitivity study 3 methods & results
- Added document revision history/ updated legend

12.18.2023

- MJD updated QAS numbers
- Changed color/ sizing/ font where needed
- Added statement to known & NPs regarding concordance
- Added document revision history/ updated legend

11.16.2023-

- MJD added DNA "Quantification using Quantifler Trio" to Methods
- Updated hyperlink to Validation Guides, 111623
- Updated legend
- Added revision history