The Internal Validation for Termination of Low Quantity DNA Samples and Male Female Mixture Samples utilizing Applied Biosystems® Quantifiler® Trio

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Abstract

DNA quantification is a mandated requirement for forensic samples as stated by the Federal Bureau of Investigation (FBI) Quality Assurance Standard 9.4 which states: the laboratory shall quantify the amount of human DNA in forensic samples prior to nuclear DNA amplification.[1] This is the process by which the relative amount of human DNA extracted from a sample is determined. Before implementation of a new quantification system can occur, an internal validation must be conducted as mandated by the FBI Quality Assurance Standard 8.3.1.1 which states: internal validation of all manual and robotic methods shall be conducted by each laboratory and reviewed and approved by the laboratory's technical leader prior to using a procedure for forensic applications.[1]

The Scientific Working Group of DNA Analysis Methods (SWGDAM) has provided guidelines for the validation of DNA analysis methods in validating procedures consistent with the FBI Director's Quality Assurance Standards (QAS). Within these guidelines, they recommend for the following studies to be conducted: sensitivity and stochastic, mixture, known and non-probative evidence samples or mock evidence samples, precision and accuracy, and contamination assessment.[7]

In concordance with the SWGDAM guidelines and FBI Quality Assurance Standards, each of these studies was conducted utilizing the Applied Biosystems® Quantifiler® Trio DNA Quantification Kit to help determine a reliable stopping point for low quantity DNA samples prior to amplification with the GlobalFiler™ Amplification Kit. The Quantifiler® Trio kit was designed to be a more predictive STR screen when working in coordination with the enhanced robustness of the newer-generation STR kits such as the GlobalFiler™ Amplification Kit by

utilizing three multi-copy targets: large autosomal, small autosomal, and Y in addition to providing indicators of inhibition and degradation.

The internal validation for termination of low quantity DNA samples and male female mixture samples utilizing Applied Biosystems® Quantifiler® Trio demonstrated that it was a robust and reliable quantification kit with the potential to be utilized as a screening tool by the SFPD crime lab for termination prior to amplification of low quantity DNA samples.

The sensitivity series study with support of all the other low quantity samples analyzed, demonstrated that termination of sample at small autosomal concentrations of 0.00024 ng/ µL and below and Y concentrations of 0.00025 ng/ µL and below as no more than 4 alleles were observed in such instances and results were not interpretable. It was further exemplified in the male female mixture study that no more than 3 alleles from the male profile (excluding alleles shared by the female profile) were able to be obtained when Quantifiler® Trio determined the male to female ratio to be at or below 1:100.

Section 1: Introduction

DNA quantification is a process by which the relative amount of human DNA extracted from a sample is determined. It is mandated for forensic samples as stated by the Federal Bureau of Investigation (FBI) Quality Assurance Standard 9.4- The laboratory shall quantify the amount of human DNA in forensic samples prior to nuclear DNA amplification.[1] Quantification for casework reference samples is not required for human DNA if the laboratory has a validated system that has been demonstrated to reproducibly and reliably yield successful DNA amplification and typing without prior quantitation.[2] A typical quantification assay yields a DNA concentration in ng/ µL and aids the analyst by indicating if normalization of the human DNA sample is necessary in order to optimize the results of downstream processes.

One such quantitation method utilizes the Applied Biosystems® Quantifiler® Trio DNA Quantification Kit. The Quantifiler® Trio kit was designed to better match the improved robustness of newer-generation STR kits as the multi-copy target utilized in the Quantifiler® Trio kit allows for ten-fold more available copies when compared with earlier quantification kits such as Quantifiler® Duo; this allows for the detection of less than a cell's worth of DNA enabling detection into the sub-pg/ µL range.[4] Although powerful, the short tandem repeat DNA process is also expensive, time consuming, and labor intense. Because of this, the use of Quantifiler® Trio as a Y-STR screen can be both time saving and cost effective. In order for a laboratory to determine termination of evidentiary sample processing after quantitation, the laboratory shall conduct a validation study in support of that determination.[6]

Quantifiler® Trio utilizes the Applied Biosystems® 7500 Real-Time PCR System with HID Real-Time PCR Analysis Software. The Applied Biosystems® 7500 Real-Time PCR System requires the use of a Tungsten-halogen lamp, 5 emission filters, a CCD Camera, 5 excitation filters, and a 96-well plate with a range of recording 500 to 700 nm. Each run takes approximately one hour allowing for faster processing and better efficiency within the casework workflow.2, 4

Quantifiler® Trio employs the use of Real-Time PCR. This allows for the PCR process to be monitored as it is occurring, and enables data to be collected throughout the process rather than at the end. It emphasizes the point at which amplification is first detected instead of target accumulation after a certain number of cycles as done with traditional PCR. This is achieved using Applied Biosystems® TaqMan MBG Probe-based chemistry which employs the use of a fluorogenic probe to enable the detection of a specific PCR product as it accumulates throughout the PCR cycles allowing for increased melting temperature without requiring increased probe

length. As the TaqMan MBG Probe binds the product, separation of the fluorophore and quencher occurs resulting in fluorescence. This is exemplified in Figure 1. Fluorescence intensity is then measured and is proportional to the amount of amplified product. Normalization of the fluorescence is conducted by the presence of a passive reference dye in every sample.[3]

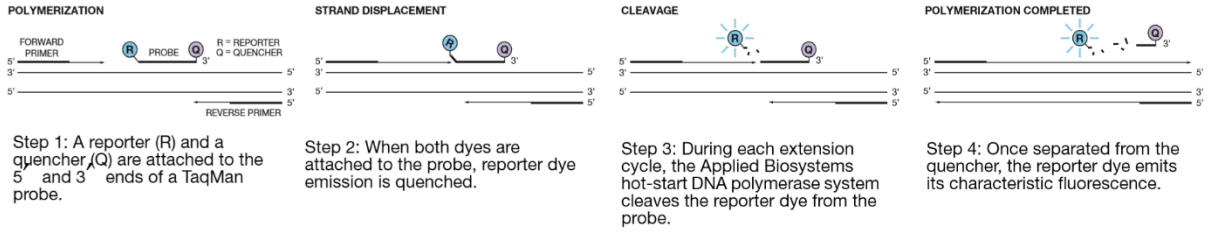

Figure 1. Representation of how the 5′ nuclease chemistry uses a fluorogenic probe to enable detection of a specific PCR product[3]

The Quantifiler® Trio DNA Quantification Kit combines four 5' nuclease assays, a target-specific large autosomal human DNA assay, a target-specific small autosomal human DNA assay, a target-specific human male DNA assay, and an internal PCR control as can be seen in Table 1. This allows for **s**imultaneous quantification of the total amount of amplifiable human (small and large autosomal) and human male DNA in a sample. A five standard point calibration curve is created ranging in concentrations of 50.00 to 0.005 ng/ µL and is ran in duplicate. The standards used are created by conducting serial ten-fold dilutions. Contamination of the Quantifiler® Trio reagents is monitored by a quantitation negative control.

The internal positive control is included in the reaction mix, and added to every sample at the same concentration to monitor for successful amplification and the presence of possible PCR inhibitors. The presence of degraded DNA can further be examined by the quantitation results yielded for the large and small autosomal human DNA. If the small autosomal human DNA yields a higher quantitation result than the large autosomal human DNA, it is indicated that degradation has occurred. Quantifiler® Trio calculates a degradation Index by dividing the small

autosomal result by the large autosomal result. Values of less than 1 are commonly generated and likely due to minor differences in the detection of the probes during PCR.[5]

Table 1. Targets, Reporter Dyes, Quenchers, and Amplicon Length

Target	Reporter Dye	Quencher	Amplicon Length
Large Autosomal	ABY®	QSY7	214 bp
Small Autosomal	VIC®	NFQ-MGB	80 bp
Human Male (Y)	FAM™	NFQ-MGB	75 bp
Internal PCR Control	JUN®	QSY7	130 bp

In order to implement to use of Applied Biosystems® Quantifiler® Trio DNA Quantification Kit for forensic casework, an internal validation is required to be conducted as mandated by the FBI Quality Assurance Standard 8.3.1.1which states internal validation of all manual and robotic methods shall be conducted by each laboratory and reviewed and approved by the laboratory's technical leader prior to using a procedure for forensic applications.[1] To aid in the validation process, the Scientific Working Group of DNA Analysis Methods (SWGDAM) has provided guidelines for the validation of DNA analysis methods in validating procedures consistent with the FBI Director's QAS. Within these guidelines, they recommend for the following studies to be conducted: sensitivity and stochastic, mixture, known and non-probative evidence samples or mock evidence samples, precision and accuracy, and contamination assessment.[7]

Each of these studies was conducted in concordance with the SWGDAM guidelines and FBI Quality Assurance Standards with the use of Applied Biosystems® Quantifiler® Trio DNA Quantification Kit to help determine a reliable stopping point for low quantity DNA samples and male female mixtures samples to avoid amplification with the GlobalFiler™ Amplification Kit.

Section 2: Methods

2.1 Sensitivity and Stochastic

A sensitivity study was conducted in which previously extracted male DNA with a small autosomal quantitation result of 2.9069 ng/μL and a Y result of 2.9287 ng/μL was used to target quantitation concentrations of 0.06 ng/μL, 0.05 ng/μL, 0.04 ng/μL, 0.03 ng/μL, 0.02 ng/μL 0.01 ng/μL 0.0075 ng/μL, 0.005 ng/μL, 0.0025 ng/μL, 0.00125 ng/μL, 0.0005 ng/μL, 0.00025 ng/μL, and 0.0001 ng/μL. A 120 μL solution of each concentration was created and then three 40 μL aliquots were created allowing for quantitation in triplicate using the Tecan Freedom EVO® for plate set up and the Quantifiler® Trio DNA Quantification Kit using the Applied Biosystems® 7500 Real-Time PCR System with HID Real-Time PCR Analysis Software. The Tecan Freedom EVO® was used for amplification plate set up using the GlobalFiler™ Amplification Kit and amplification was conducted on the GeneAmp™ PCR System 9700. Capillary electrophoresis was conducted using the Applied Biosystems® 3130xl genetic analyzer. The data was analyzed via the GeneMapper® ID-X Software.

A second sensitivity study was created in which seventeen replicates of each of the targeted 0.00125 ng/μL, 0.0005 ng/μL, 0.00025 ng/μL, and 0.0001 ng/μL concentrations were created resulting in twenty data points total at these concentrations between the two studies. These additional samples were analyzed using the same equipment and procedures as the previous samples.

2.2 Male Female Mixtures

Male-female mixtures were created in which previously extracted male and female DNA was utilized. The female concentration was held constant at 100 ng per 40 μL while the male concentration was varied at 100 ng, 50 ng, 25 ng, 15 ng, 10 ng, 7.5 ng, 5 ng, 2.5 ng, 1 ng, 0.5 ng, 0.25 ng, and 0.1 ng per 40 μL. The Tecan Freedom EVO® was used for plate set up, and the Quantifiler® Trio DNA Quantification Kit using the Applied Biosystems® 7500 Real-Time PCR

System with HID Real-Time PCR Analysis Software was utilized to perform three separate quantitations for each sample. Tecan Freedom EVO® was used for amplification plate set up using the GlobalFiler™ Amplification Kit and amplification was conducted on the GeneAmp™ PCR System 9700.Capillary electrophoresis was conducted using the Applied Biosystems® 3130xl genetic analyzer. The data was analyzed via the GeneMapper® ID-X Software.

Eleven additional replicates of the 2.5 ng, 1 ng, 0.5 ng, 0.25 ng, and 0.1 ng male DNA to 100 ng female DNA per 40 μL were analyzed. This resulted in fourteen total replicate points at these concentrations. These additional samples were analyzed using the same equipment and procedures as the previous samples.

2.3 Case Type Samples

Case type samples were simulated using female buccal swabs applied with 20 μL of varying semen dilutions. The dilutions used were 1/150, 1/200, 1/250, 1/300, 1/350, 1/400, 1/450, 1/500, 1/550, 1/600, 1/650, and 1/700. These samples were then taken through the differential separation process using the QIAGEN® QIAcube®, and extracted using the EZ1® Advanced XL and the EZ1® DNA Investigator kit. The Tecan Freedom EVO® was used for plate set up, and the Quantifiler® Trio DNA Quantification Kit using the Applied Biosystems® 7500 Real-Time PCR System with HID Real-Time PCR Analysis Software was utilized to obtain quantitation results for each sample. Tecan Freedom EVO® was used for amplification plate set up using the GlobalFiler™ Amplification Kit and amplification was conducted on the GeneAmp™ PCR System 9700.Capillary electrophoresis was conducted using the Applied Biosystems®3130xl genetic analyzer. The data was analyzed via the GeneMapper® ID-X Software.

This procedure was repeated on a second QIAcube®, in addition to case type samples ran on the QIAcube® which consisted of 20 µL of male saliva dilutions of 1/5, 1/10, 1/20, 1/30, 1/40, 1/50, 1/60, 1/70, 1/80, and 1/90 added to female buccal swabs.

2.4 Touch Samples

Touch samples were collected from various locations throughout the facility and extracted using the EZ1® Advanced XL and the EZ1® DNA Investigator kit. The Tecan Freedom EVO® was used for plate set up, and the Quantifiler® Trio DNA Quantification Kit using the Applied Biosystems® 7500 Real-Time PCR System with HID Real-Time PCR Analysis Software was utilized to perform three separate quantitations for each sample. Tecan Freedom EVO® was used for amplification plate set up using the GlobalFiler™ Amplification Kit and amplification was conducted on the GeneAmp™ PCR System 9700. Capillary electrophoresis was conducted using the Applied Biosystems®3130xl genetic analyzer. The data was analyzed via the GeneMapper® ID-X Software.

2.5 Repeatability, Accuracy, and Precision

Repeatability was demonstrated by performing at least three separate quantitations on each sample as was done with the male female mixtures and touch samples or running the sample in triplicate on a single quantitation plate as was done with the sensitivity series. Additional quantitations were performed on the samples at which approximately ½ or more of the desired profile began to drop out. The standard deviation as well as percent relative standard deviation was then calculated for each sample. Quantitation of the case type samples was only conducted once; however two trials were conducted for the female buccal swab and semen dilution samples (one on QIAcube 1 and one on QIAcube 2).

2.6 Contamination

Contamination was evaluated by monitoring reagent blanks, quantitation no template controls, and amplification negatives. Reagent blanks are used to ensure absence of contamination throughout the extraction, quantitation, amplification, and capillary electrophoresis process. This control contains all reagents used in the extraction, quantitation, amplification and capillary electrophoresis procedure but contains no DNA sample. The quantitation negative control monitors for contamination in the Quantifiler® Trio reagents. It consists of all reagents used in the quantitation procedure but contains no DNA sample. Quantifiler® THP DNA Dilution Buffer is added in place of a DNA sample. The amplification negative contains all the reagents used in the amplification and capillary electrophoresis procedure but contains TE buffer in place of the DNA sample. If no DNA was present in the results for the controls for quantitation and capillary electrophoresis, it could be determined that contamination was not present.

Section 3: Results

3.1 Sensitivity and Stochastic

Table 2. Sensitivity Series Large, Small, and Y Quant Results with CE results

Sample	Theoretical [SA]	Quant [SA]	Theoretical [Y]	Quant [Y]	Male Profile	Drop Out
S1_A	0.05886	0.07117	0.06049	0.08357	Full	0
S1_B	0.05886	0.07991	0.06049	0.08538	Full	0
S1_C	0.05886	0.07312	0.06049	0.07848	Full	0
S2_A	0.04893	0.06583	0.05028	0.06955	Full	0
S2_B	0.04893	0.06063	0.05028	0.06948	Full	0
S2_C	0.04893	0.05595	0.05028	0.06868	Full	0
S3_A	0.03924	0.05433	0.04032	0.06545	Full	0
S3_B	0.03924	0.04273	0.04032	0.04772	Full	0
S3_C	0.03924	0.04840	0.04032	0.05204	Full	0
S4_A	0.02931	0.03987	0.03012	0.04139	Full	0
S4_B	0.02931	0.03956	0.03012	0.03824	Full	0
S4_C	0.02931	0.03916	0.03012	0.04098	Full	0
S5_A	0.01962	0.02443	0.02016	0.02567	Full	0
S5_B	0.01962	0.02628	0.02016	0.02711	Full	0
S5_C	0.01962	0.01983	0.02016	0.01804	Full	0
S6_A	0.00969	0.01342	0.00996	0.01635	Full	0
S6_B	0.00969	0.01226	0.00996	0.01321	Full	0
S6_C	0.00969	0.01169	0.00996	0.01086	Full	0
S7_A	0.00727	0.01127	0.00747	0.01260	Full	0
S7_B	0.00727	0.00961	0.00747	0.00991	Full	0
S7_C	0.00727	0.00789	0.00747	0.00621	Full	0
S8_A	0.00484	0.00646	0.00498	0.00754	Full	0
S8_B	0.00484	0.00820	0.00498	0.00761	Full	0
S8_C	0.00484	0.00568	0.00498	0.00804	Partial	-1
S9_A	0.00245	0.00309	0.00251	0.00370	Partial	-8/39
S9_B	0.00245	0.00250	0.00251	0.00309	Partial	-10/39
S9_C	0.00245	0.00231	0.00251	0.00349	Partial	-9/39

*All Concentrations in ng/ µL

Table 3. Sensitivity Series Large, Small, and Y Quant Results with CE results

Sample	Theoretical [SA]	Quant [SA]	Theoretical [Y]	Quant [Y]	Male Profile	Drop Out
S10_A	0.00124	0.00127	0.00127	0.00222	Partial	-17/39
S10_B	0.00124	0.00101	0.00127	0.00231	Partial	-14/39
S10_C	0.00124	0.00101	0.00127	0.00122	Partial	-20/39
S10.1_A	0.00124	0.00113	0.00127	0.00098	Partial	-21/39
S10.1_B	0.00124	0.00172	0.00127	0.00175	Partial	-18/39
S10.1_C	0.00124	0.00113	0.00127	0.00159	Partial	-11/39
S10.1_D	0.00124	0.00070	0.00127	0.00078	Partial	-16/39
S10.1_E	0.00124	0.00092	0.00127	0.00139	Partial	-16/39
S10.1_F	0.00124	0.00092	0.00127	0.00171	Partial	-13/39
S10.1_G	0.00124	0.00111	0.00127	0.00062	Partial	-16/39
S10.1_H	0.00124	0.00121	0.00127	0.00066	Partial	-26/39
S10.1_I	0.00124	0.00100	0.00127	0.00171	Partial	-21/39
S10.1_J	0.00124	0.00104	0.00127	0.00138	Partial	-21/39
S10.1_K	0.00124	0.00125	0.00127	0.00129	Partial	-14/39
S10.1_L	0.00124	0.00154	0.00127	0.00281	Partial	-14/39
S10.1_M	0.00124	0.00097	0.00127	0.00099	Partial	-19/39
S10.1_N	0.00124	0.00076	0.00127	0.00081	Partial	-17/39
S10.1_O	0.00124	0.00107	0.00127	0.00065	Partial	-18/39
S10.1_P	0.00124	0.00123	0.00127	0.00068	Partial	-17/39
S10.1_Q	0.00124	0.00067	0.00127	0.00108	Partial	-13/39
						*Average of 17 alleles dropped out (~1/2)

*All Concentrations in ng/ µL

Table 4. Sensitivity Series Large, Small, and Y Quant Results with CE results

Sample	Theoretical [SA]	Quant [SA]	Theoretical [Y]	Quant [Y]	Male Profile	Drop Out
S11_A	0.00048	0.00055	0.00050	0.00218	7/39	-32/39
S11_B	0.00048	0.00016	0.00050	0.00108	No Profile	-39/39
S11_C	0.00048	0.00032	0.00050	0.00022	4/39	-34/39
S11.1_A	0.00048	0.00034	0.00050	0.00046	11/39	-28/39
S11.1_B	0.00048	0.00000	0.00050	0.00049	9/39	-30/39
S11.1_C	0.00048	0.00044	0.00050	0.00039	9/39	-30/39
S11.1_D	0.00048	0.00031	0.00050	0.00000	7/39	-32/39
S11.1_E	0.00048	0.00026	0.00050	0.00062	3/39	-36/39
S11.1_F	0.00048	0.00030	0.00050	0.00045	5/39	-34/39
S11.1_G	0.00048	0.00032	0.00050	0.00037	8/39	-31/39
S11.1_H	0.00048	0.00041	0.00050	0.00104	5/39	-34/39
S11.1_I	0.00048	0.00076	0.00050	0.00095	12/39	-27/39
S11.1_J	0.00048	0.00059	0.00050	0.00043	9/39	-30/39
S11.1_K	0.00048	0.00053	0.00050	0.00000	4/39	-35/39
S11.1_L	0.00048	0.00031	0.00050	0.00027	4/39	-35/39
S11.1_M	0.00048	0.00057	0.00050	0.00000	8/39	-31/39
S11.1_N	0.00048	0.00033	0.00050	0.00022	2/39	-37/39
S11.1_O	0.00048	0.00045	0.00050	0.00061	7/39	-32/39
S11.1_P	0.00048	0.00044	0.00050	0.00044	4/39	-35/39
S11.1_Q	0.00048	0.00040	0.00050	0.00019	13/39	-26/39
						*Average of 32 alleles dropped out

*All Concentrations in ng/ µL

*Quant value below determined cut off

Table 5. Sensitivity Series Large, Small, and Y Quant Results with CE results

Sample	Theoretical [SA]	Quant [SA]	Theoretical [Y]	Quant [Y]	Male Profile	Drop Out
S12_A	0.00024	0.00035	0.00025	0.00000	1/39	-38/39
S12_B	0.00024	0.00036	0.00025	0.00000	1/39	-38/39
S12_C	0.00024	0.00027	0.00025	0.00037	No Profile	-39/39
S12.1_A	0.00024	0.00026	0.00025	0.00013	5/39	-34/39
S12.1_B	0.00024	0.00015	0.00025	0.00048	1/39	-38/39
S12.1_C	0.00024	0.00000	0.00025	0.00026	1/39	-38/39
S12.1_D	0.00024	0.00019	0.00025	0.00031	2/39	-37/39
S12.1_E	0.00024	0.00019	0.00025	0.00014	1/39	-38/39
S12.1_F	0.00024	0.00023	0.00025	0.00027	3/39	-35/39
S12.1_G	0.00024	0.00027	0.00025	0.00000	6/39	-33/39
S12.1_H	0.00024	0.00000	0.00025	0.00000	3/39	-36/39
S12.1_I	0.00024	0.00000	0.00025	0.00041	5/39	-34/39
S12.1_J	0.00024	0.00014	0.00025	0.00066	No Profile	-39/39
S12.1_K	0.00024	0.00000	0.00025	0.00000	3/39	-36/39
S12.1_L	0.00024	0.00013	0.00025	0.00058	1/39	-38/39
S12.1_M	0.00024	0.00030	0.00025	0.00000	4/39	-35/39
S12.1_N	0.00024	0.00013	0.00025	0.00038	No Profile	-39/39
S12.1_O	0.00024	0.00022	0.00025	0.00000	2/39	-37/39
S12.1_P	0.00024	0.00047	0.00025	0.00037	3/39	-36/39
S12.1_Q	0.00024	0.00000	0.00025	0.00042	4/39	-35/39
						*Average of 37 alleles dropped out

*All Concentrations in ng/ µL

*Quant value below determined cut off

Table 6. Sensitivity Series Large, Small, and Y Quant Results with CE results

Sample	Theoretical [SA]	Quant [SA]	Theoretical [Y]	Quant [Y]	Male Profile	Dropped Out
S13_A	0.00010	0.00000	0.00010	0.00007	No Profile	-39/39
S13_B	0.00010	0.00024	0.00010	0.00000	1/39	-38/39
S13_C	0.00010	0.00000	0.00010	0.00036	No Profile	-39/39
S13.1_A	0.00010	0.00012	0.00010	0.00024	No Profile	-39/39
S13.1_B	0.00010	0.00000	0.00010	0.00005	No Profile	-39/39
S13.1_C	0.00010	0.00000	0.00010	0.00000	1/39	-38/39
S13.1_D	0.00010	0.00030	0.00010	0.00000	1/39	-38/39
S13.1_E	0.00010	0.00000	0.00010	0.00000	1/39	-38/39
S13.1_F	0.00010	0.00021	0.00010	0.00000	1/39	-38/39
S13.1_G	0.00010	0.00000	0.00010	0.00020	No Profile	-39/39
S13.1_H	0.00010	0.00000	0.00010	0.00013	No Profile	-39/39
S13.1_I	0.00010	0.00000	0.00010	0.00000	1/39	-38/39
S13.1_J	0.00010	0.00012	0.00010	0.00000	No Profile	-39/39
S13.1_K	0.00010	0.00012	0.00010	0.00000	1/39	-38/39
S13.1_L	0.00010	0.00000	0.00010	0.00000	No Profile	-39/39
S13.1_M	0.00010	0.00026	0.00010	0.00000	No Profile	-39/39
S13.1_N	0.00010	0.00017	0.00010	0.00014	2/39	-37/39
S13.1_O	0.00010	0.00011	0.00010	0.00000	1/39	-38/39
S13.1_P	0.00010	0.00000	0.00010	0.00000	No Profile	-39/39
S13.1_Q	0.00010	0.00000	0.00010	0.00000	No Profile	-39/39
						*Average of 39 alleles dropped out

*All Concentrations in ng/ µL

* Quant value below determined cut off

3.2 Male Female Mixtures

Table 7. Male Female Mixture Small Autosomal, Y, M:F Ratio, and CE Results

Sample	Name	[SA] Theoretical	[SA] Quant	[Y] Theoretical	[Y] Quant	Male Profile	Male Drop Out	Trio M:F Ratio
100 ng F:100 ng M	100M	4.9950	5.8726	2.4953	3.4115	Full	0	-
100 ng F:50 ng M	50M	3.7474	4.5225	1.2476	1.7395	Full	0	1:1.600
100 ng F:25ng M	25M	3.1263	3.8966	0.6266	0.9797	Full	0	1:2.981
100 ng F:15 ng M	15M	2.8768	3.3581	0.3771	0.5671	Full	0	1:4.923
100 ng F:10 ng M	10M	2.7493	3.079	0.2495	0.3877	Partial	-2	1:6.967
Reamped	10M					Full	0	
100 ng F:7.5 ng M	7.5M	2.6883	3.4875	0.1885	0.3108	Partial	-1	1:10.220
Reamped	7.5M					Partial	-5	
100 ng F:5 ng M	5M	2.6273	3.0338	0.1275	0.2120	Partial	-13	1:13.373
Reamped	5M					Partial	-6	

*All Concentrations in ng/ µL
*Full female profile was obtained for every sample
*NSH= allele not shared in the male and female profile
*Average of 3 replicates
*Male female ratios of 1:100 and below were determined to not yield a useable profile

Table 8. Male Female Mixture Small Autosomal, Y, M:F Ratio, and CE Results

Sample	Name	[SA] Theoretical	[SA] Quant	[Y] Theoretical	[Y] Quant	Male Profile	Male Drop Out	Trio M:F Ratio
100 ng F:2.5ng M	2.5M	2.5546	3.6535	0.0625	0.0962	Partial	-19	1:37.350
Reamped	2.5M					Partial	-13	
100 ng F:2.5ng M	2.5M_A	2.5613	1.8309	0.0625	0.0962	Partial	-12	1:18.036
100 ng F:2.5ng M	2.5M_B	2.5613	2.6291	0.0625	0.1215	Partial	-13	1:20.637
100 ng F:2.5ng M	2.5M_C	2.5613	2.1384	0.0625	0.0940	Partial	-13	1:21.749
100 ng F:2.5ng M	2.5M_D	2.5613	2.2297	0.0625	0.0971	Partial	-16	1:21.97
100 ng F:2.5ng M	2.5M_E	2.5613	2.1613	0.0625	0.1021	Partial	-13	1:20.17
100 ng F:2.5ng M	2.5M_F	2.5613	2.2278	0.0625	0.1074	Partial	-13	1:19.734
100 ng F:2.5ng M	2.5M_G	2.5613	2.066	0.0625	0.0934	Partial	-14	1:21.124
100 ng F:2.5ng M	2.5M_H	2.5613	1.948	0.0625	0.0919	Partial	-12	1:20.191
100 ng F:2.5ng M	2.5M_I	2.5613	2.1486	0.0625	0.1096	Partial	-14	1:18.596
100 ng F:2.5ng M	2.5M_J	2.5613	2.5296	0.0625	0.1147	Partial	-17	1:21.058
100 ng F:2.5ng M	2.5M_K	2.5613	2.259	0.0625	0.1079	Partial	-8	1:19.943
Average							-14	1:24.018

*All Concentrations in ng/ µL
*Full female profile was obtained for every sample
*NSH= allele not shared in the male and female profile
*Average of 3 replicates
*Male female ratios of 1:100 and below were determined to not yield a useable profile

Table 9. Male Female Mixture Small Autosomal, Y, M:F Ratio, and CE Results

Sample	Name	[SA] Theoretical	[SA] Quant	[Y] Theoretical	[Y] Quant	Male Profile	Male Drop Out	Trio M:F Ratio
100 ng F:1 ng M	1M	2.5171	4.0187	0.0250	0.0537	None	All	1:78.766
Reamped	1M					1 allele	-23	
100 ng F:1 ng M	1M_A	2.5515	2.3443	0.0250	0.0461	5 NSH	-19	1:49.87
100 ng F:1 ng M	1M_B	2.5515	1.9702	0.0250	0.0334	5 NSH	-19	1:57.977
100 ng F:1 ng M	1M_C	2.5515	2.186	0.0250	0.0557	3 NSH	-21	1:38.227
100 ng F:1 ng M	1M_D	2.5515	1.8751	0.0250	0.0372	3 NSH	-21	1:49.435
100 ng F:1 ng M	1M_E	2.5515	1.7243	0.0250	0.0293	8 NSH	-16	1:57.865
100 ng F:1 ng M	1M_F	2.5515	1.7722	0.0250	0.0401	6 NSH	-18	1:43.248
100 ng F:1 ng M	1M_G	2.5515	2.4555	0.0250	0.0484	4 NSH	-20	1:49.783
100 ng F:1 ng M	1M_H	2.5515	2.072	0.0250	0.0388	8 NSH	-16	1:52.392
100 ng F:1 ng M	1M_I	2.5515	2.1363	0.0250	0.0466	4 NSH	-20	1:44.852
100 ng F:1 ng M	1M_J	2.5515	2.237	0.0250	0.0358	6 NSH	-18	1:61.506
100 ng F:1 ng M	1M_K	2.5515	2.2405	0.0250	0.0575	4 NSH	-20	1:37.937
Average						5 NSH		1:55.710

*All Concentrations in ng/ µL
*Full female profile was obtained for every sample
*NSH= allele not shared in the male and female profile
*Average of 3 replicates
*Male female ratios of 1:100 and below were determined to not yield a useable profile

Table 10. Male Female Mixture Small Autosomal, Y, M:F Ratio, and CE Results

Sample	Name	[SA] Theoretical	[SA] Quant	[Y] Theoretical	[Y] Quant	Male Profile	Male Drop Out	Trio M:F Ratio
100 ng F:0.5 ng M	0.5M	2.5047	3.9136	0.0126	0.0226	None	All	1:175.555
Reamped	0.5M					2 alleles	-22	
100 ng F:0.5 ng M	0.5M_A	2.5132	2.4278	0.0125	0.0255	1 NSH	-23	1:94.158
100 ng F:0.5 ng M	0.5M_B	2.5132	2.4369	0.0125	0.0192	2 NSH	-22	1:126.092
100 ng F:0.5 ng M	0.5M_C	2.5132	2.0723	0.0125	0.0242	4 NSH	-20	1:84.561
100 ng F:0.5 ng M	0.5M_D	2.5132	2.6135	0.0125	0.0210	1 pu?	-23	1:123.428
100 ng F:0.5 ng M	0.5M_E	2.5132	2.4313	0.0125	0.0183	4 NSH	-20	1:131.931
100 ng F:0.5 ng M	0.5M_F	2.5132	2.4493	0.0125	0.0229	None	All	1:105.757
100 ng F:0.5 ng M	0.5M_G	2.5132	2.4501	0.0125	0.0267	1 pu?	-23	1:90.877
100 ng F:0.5 ng M	0.5M_H	2.5132	2.4301	0.0125	0.0162	1 NSH	-23	1:149.157
100 ng F:0.5 ng M	0.5M_I	2.5132	2.3412	0.0125	0.0185	None	All	1:125.513
100 ng F:0.5 ng M	0.5M_J	2.5132	2.2206	0.0125	0.0151	1 pu?	-23	1:146.469
100 ng F:0.5 ng M	0.5M_K	2.5132	2.031	0.0125	0.0176	2 NSH	-22	1:114.405
Average						1 NSH		1:129.963

*All Concentrations in ng/ µL
*Full female profile was obtained for every sample
*NSH= allele not shared in the male and female profile
*Average of 3 replicates
*Male female ratios of 1:100 and below were determined to not yield a useable profile

Table 11. Male Female Mixture Small Autosomal, Y, M:F Ratio, and CE Results

Sample	Name	[SA] Theoretical	[SA] Quant	[Y] Theoretical	[Y] Quant	Male Profile	Male Drop Out	Trio M:F Ratio
100 ng F:0.25 ng M	0.25M	2.4985	4.2473	0.0063	0.0114	None	All	1:376.266
Reamped	0.25M					None	All	
100 ng F:0.25 ng M	0.25M_A	2.5066	2.743	0.0063	0.0101	None	All	1:269.583
100 ng F:0.25 ng M	0.25M_B	2.5066	2.096	0.0063	0.0062	1 NSH	-23	1:335.508
100 ng F:0.25 ng M	0.25M_C	2.5066	2.1226	0.0063	0.0098	1 NSH	-23	1:216.65
100 ng F:0.25 ng M	0.25M_D	2.5066	2.2245	0.0063	0.0109	1 pu?	-23	1:203.932
100 ng F:0.25 ng M	0.25M_E	2.5066	2.4822	0.0063	0.0111	2 NSH	-22	1:222.982
100 ng F:0.25 ng M	0.25M_F	2.5066	2.1789	0.0063	0.0102	1 NSH	-23	1:212.958
100 ng F:0.25 ng M	0.25M_G	2.5066	2.066	0.0063	0.0122	3 NSH	-21	1:170.566
100 ng F:0.25 ng M	0.25M_H	2.5066	2.0939	0.0063	0.0126	None	All	1:175.91
100 ng F:0.25 ng M	0.25M_I	2.5066	2.5049	0.0063	0.0095	1 pu?	-23	1:261.601
100 ng F:0.25 ng M	0.25M_J	2.5066	2.2499	0.0063	0.0083	1 NSH	-23	1:268.686
100 ng F:0.25 n M	0.25M_K	2.5066	2.0689	0.0063	0.0108	None	All	1:189.701
Average						<1 NSH		1:261.250

*All Concentrations in ng/ µL
*Full female profile was obtained for every sample
*NSH= allele not shared in the male and female profile
*Average of 3 replicates
*Male female ratios of 1:100 and below were determined to not yield a useable profile

Table 12. Male Female Mixture Small Autosomal, Y, M:F Ratio, and CE Results

Sample	Name	[SA] Theoretical	[SA] Quant	[Y] Theoretical	[Y] Quant	Male Profile	Male Drop Out	Trio M:F Ratio
100 ng F:0.1 ng M	0.1M	2.4948	4.0977	0.0026	0.0068	None	All	1:603.947
Reamped	0.1M					None	All	
100 ng F:0.1 ng M	0.1M_A	2.5037	1.9983	0.0025	0.0020	None	All	1:991.232
100 ng F:0.1 ng M	0.1M_B	2.5037	2.2956	0.0025	0.0072	1 NSH	-23	1:316.044
100 ng F:0.1 ng M	0.1M_C	2.5037	1.973	0.0025	0.0060	None	All	1:326.207
100 ng F:0.1 ng M	0.1M_D	2.5037	1.9518	0.0025	0.0061	1 pu ?	-23	1:316.659
100 ng F:0.1 ng M	0.1M_E	2.5037	1.8449	0.0025	0.0037	None	All	1:500.897
100 ng F:0.1 ng M	0.1M_F	2.5037	2.3587	0.0025	0.0045	None	All	1:525.427
100 ng F:0.1 ng M	0.1M_G	2.5037	2.0479	0.0025	0.0058	2 pu?	-22	1:350.432
100 ng F:0.1 ng M	0.1M_H	2.5037	2.258	0.0025	0.0071	None	All	1:315.784
100 ng F:0.1 ng M	0.1M_I	2.5037	2.4719	0.0025	0.0029	None	All	1:862.626
100 ng F:0.1 ng M	0.1M_J	2.5037	2.3413	0.0025	0.0040	3 NSH	-21	1:590.536
100 ng F:0.1 ng M	0.1M_K	2.5037	2.1038	0.0025	0.0020	1 NSH	-23	1:1064.417
Average						~0 NSH		1:569.463

*All Concentrations in ng/ µL
*Full female profile was obtained for every sample
*NSH= allele not shared in the male and female profile
*Average of 3 replicates
*Male female ratios of 1:100 and below were determined to not yield a useable profile

3.3 Case Type Samples

Table 13. Semen Dilution QIAcube 1 Separation Samples with Large Autosomal, Small Autosomal, and Y Quant Results, M:F Ratio Results, and CE Results

QIAcube 1	Quant [SA]	Quant [Y]	M:F Ratio	Female Profile	Male Profile
MC150NS	1.1220	0.0033	1:344.217	Full profile	None
MC200NS	1.3555	0.0006	1:2380.68	Full profile	None
MC250NS	0.7820	0.0013	1:593.989	Full profile	None
MC300NS	0.9712	0.0002	1:6396.162	Full profile	None
MC350NS	2.3408	0.0006	1:3758.751	Full profile	None
MC400NS	2.5179	0.0001	1:48609.55	Full profile	None
MC450NS	0.9449	0.0006	1:1490.497	Full profile	None
MC500NS	0.5875	0.0001	1:4674.143	Full profile	None
MC550NS	0.5538	0.0000	-	Full profile	None
MC600NS	0.3567	0.0003	1:1369.954	Full profile	None
MC650NS	1.5633	0.0003	1:4504.495	Full profile	None
MCRB1NS	0.0000	0.0000	-	None	None
MC700NS	2.1454	0.0002	1:8864.881	Full profile	None
MCRB2NS					
MC150S	0.0423	0.0492	-	2-3 alleles	Full Profile
MC200S	0.0125	0.0155	-	1-2 allele	Full Profile
MC250S	0.0138	0.0153	-	3-4 alleles	Full Profile
MC300S	0.0137	0.0170	-	Partial profile	Full Profile
MC350S	0.0177	0.0162	-	Partial profile	Full Profile
MC400S	0.0049	0.0057	-	Partial profile	Partial profile (-1)
MC450S	0.0026	0.0041	-	Partial profile	Partial profile (-1)
MC500S	0.0027	0.0014	-	Partial profile	Partial profile (-5)
MC550S	0.0024	0.0020	-	Partial profile	Partial profile (-2)
MC600S	0.0024	0.0030	-	Partial profile	Full profile
MC650S	0.0039	0.0046	-	Partial profile	Full profile
MCRB1S	0.0000	0.0000	-	None	No Profile
MC700S	0.0129	0.0132	-	all but 1 allele	Full profile
MCRB2S	0.0000	0.0000	-	None	No Profile

*All Concentrations in ng/ µL

*Below determined cut off

Table 14. Semen Dilution QIAcube 2 Separation Samples with Large Autosomal, Small Autosomal, and Y Quant Results, M:F Ratio Results, and CE Results

QIAcube 2	Quant [SA]	Quant [Y]	M:F Ratio	Female Profile	Male Profile
2_MC150NS	0.8151	0.0007	1:1246.764	Full profile	1 allele
2_MC200NS	0.2485	0.0005	1:512.806	Full profile	None
2_MC250NS	0.3519	0.0005	1:755.638	Full profile	1 allele
2_MC300NS	0.1680	0.0001	1:1831.189	Full profile	None
2_MC350NS	0.3322	0.0001	1:3071.222	Full profile	1 allele
2_MC400NS	0.1276	0.0003	1:417.067	Full profile	None
2_MC450NS	0.1309	0.0000	-	Full profile	None
2_MC500NS	0.0407	0.0002	1:197.883	Full profile	2 alleles
2_MC550NS	0.0407	0.0002	1:804.911	Full profile	None
2_MC600NS	0.0429	0.0000	-	Full profile	1 allele
2_MC650NS	0.3598	0.0000	-	Full profile	None
RB9NS	0.0000	0.0000	-	None	None
2_MC700NS	0.1324	0.0000	-	Full profile	None
2_MC150S	0.0224	0.0227	-	12 alleles	Full profile
2_MC200S	0.0137	0.0137	-	2 alleles	Full profile
2_MC250S	0.0100	0.0108	-	2 alleles	Full profile
2_MC300S	0.0053	0.0058	-	2 alleles	Full profile
2_MC350S	0.0073	0.0099	-	2 alleles	Full profile
2_MC400S	0.0019	0.0023	-	1 allele	Partial profile (-7)
2_MC450S	0.0017	0.0018	-	3 alleles	Partial profile (-4)
2_MC500S	0.0027	0.0028	-	1 allele	Partial profile (-6)
2_MC550S	0.0015	0.0018	-	1 allele	Partial profile (-8)
2_MC600S	0.0010	0.0000	-	None	None
2_MC650S	0.0043	0.0036	-	15 alleles	Partial profile (-1)
RB9S	0.0000	0.0000	-	None	None
2_MC700S	0.0019	0.0026	-	3 alleles	Partial profile (-9)

*All Concentrations in ng/ µL

*Below determined cut off

Table 15. Saliva Dilution QIAcube 2 Separation Samples with Large Autosomal, Small Autosomal, and Y Quant Results, M:F Ratio Results, and CE Results

Sample	[SA] Quant	[Y] Quant	Female Profile	Male Profile	M:F Ratio
SAL5NS	1.0925	0.0413	Full female	Partial male (-10)	1:25.462
SAL10NS	2.8847	0.0988	Full female	Partial male (-7)	1:28.196
SAL20NS	0.9080	0.0342	Full female	Partial male (-8)	1:25.547
SAL30NS	0.6980	0.0895	Full female	Full male	1:6.802
SAL40NS	0.1095	0.0075	Full female	Partial male (-5)	1:13.626
SAL50NS	0.8083	0.0230	Full female	Partial male (-17) (8 NSH)	1:34.129
SAL60NS	0.2930	0.0084	Full female	Partial male (-18) (6 NSH) 1 pu?	1:34.006
SAL70NS	0.9333	0.0225	Full female	Partial male (-13) (11 NSH) 1 pu?	1:40.502
SAL80NS	0.6304	0.0146	Full female	Partial male (-17) (7 NSH) 1 pu?	1:42.195
SAL90NS	0.5635	0.0047	Full female	Partial male (-23) (1 NSH)	1:118.987
RB10NS	0.0000	0.0000	No Profile	No Profile	-
SAL5S	0.0010	0.0000	11 (5 NSH)	8 (2 NSH)	-
SAL10S	0.0065	0.0027	Full female	Partial male (-1)	1:1.372
SAL20S	0.0019	0.0000	Partial female(-11)	Partial male (-26) (3 NSH)	-
SAL30S	0.0025	0.0008	1 female allele	Partial male (-9)	1:2.151
SAL40S	0.0005	0.0001	10 (5 NSH)	8 (3 NSH)	1:3.306
SAL50S	0.0052	0.0006	Full female	(-24) 1 NSH	1:8.202
SAL60S	0.0010	0.0002	14 (6 NSH)	8 (1 NSH)	1:5.078
SAL70S	0.0011	0.0000	10 (5 NSH)	5?	-
SAL80S	0.0017	0.0000	Partial female (-13)	1 NSH	-
SAL90S	0.0012	0.0003	Partial female (-14)	No Profile	1:3.53
RB10S	0.0000	0.0000	No Profile	No Profile	-

*All Concentrations in ng/ µL

*Below Determined cut off

*NSH= allele not shared in the male and female profile

3.4 Touch Samples

Table 16. Touch Samples Collected from Various Locations throughout the Criminalistics' Building

Sample	Collected From	[SA] Quant Avg	Stdev	[Y] Quant Avg	Stdev	Profile	Degradation Index Avg
A	DNA lab outside door handle	0.0252	0.0038	0.0216	0.0009	Mix- ≥ 2	0.8303
B	Front lobby inside left door handle	0.0003	0.0001	0.0001	0.0001	None	1.9049
C	Front Desk	0.0290	0.0043	0.0002	0.0002	Full Female	0.6226
D	Green button on front desk copier	0.0005	0.0002	0.0003	0.0002	3 alleles	1.7556
E	Sign-in pen	0.0002	0.0002	0.0001	0.0001	3 alleles	1.0428
F	Front desk phone	0.0028	0.0005	0.0029	0.0001	Partial Male	0.8352
G	Front lobby right light switch	0.0001	0.0002	0.0001	0.0001	No Profile	0.0000
H	Space bar on front desk computer	0.0008	0.0003	0.0004	0.0001	7 alleles	2.0244
I	Hand rail	0.0043	0.0012	0.0042	0.0010	Partial Male	1.6530
J	Double Door handle to lab	0.0014	0.0003	0.0011	0.0004	6 alleles	1.1938
K	Green button on copier	0.0011	0.0003	0.0006	0.0002	6 alleles	3.0331
L	Water cooler handle	0.0004	0.0002	0.0001	0.0001	3 alleles	0.9150
M	Lab mop handle	0.0001	0.0001	0.0002	0.0001	2 alleles	0.1413
N	Broom handle	0.0000	0.0000	0.0000	0.0000	None	0.0000
O	File cabinet door handle	0.0010	0.0000	0.0005	0.0002	None	2.6244
P	Narcotic lab outside door handle	0.0005	0.0002	0.0005	0.0005	1 allele	1.0518
Q	Mouse to computer in main DNA lab	0.0000	0.0001	0.0000	0.0000	None	0.0000
R	Hole punch	0.0007	0.0002	0.0004	0.0006	4 alleles	1.5754
S	Phone earpiece in processing room	0.0131	0.0013	0.0119	0.0004	Mix- ≥ 2	1.0278
T	Door handle to outside of PCR lab	0.0006	0.0001	0.0003	0.0004	5 alleles	1.3138

*All Concentrations in ng/ µL
*C gave a M:F ratio of 1:131.135 for run 1.
*Below determined cut off

Table 17. Touch Samples Collected from Various Locations throughout the Criminalistics' Building

Sample	Collected From	Quant [SA]	Quant [Y]	Profile	Degradation Index	M:F Ratio
V	EC mug	0.1390	0.0000	Full Female	0.7674	-
W	Room 203 microwave	0.0324	0.0192	Mix- ≥ 3	1.8335	-
X	Guitar neck	0.0027	0.0021	Mix- ≥ 2	0.7877	-
Y	Codis workstation	0.0384	0.0334	Mix- ≥ 4	1.1069	-
Z	MTD phone	0.0690	0.0449	Mix- ≥ 4	0.9106	-
AA	MC computer space bar	0.0018	0.0003	Partial Female	1.3264	1:5.438
BB	Pen in main DNA office	0.0020	0.0020	Partial	0.9845	-
CC	Door knob to main DNA office	0.0023	0.0012	5 alleles	1.3762	1:1.008
DD	Stapler in main DNA office	0.0070	0.0063	Mix- ≥ 2	0.9729	-
EE	Start button on DNA office copier	0.0018	0.0010	8 alleles	0.7917	-
FF	Microwave button in main DNA section	0.0069	0.0014	Mix- ≥ 2	1.1305	1:3.806
GG	Toaster in main DNA office	0.0064	0.0050	Mix- ≥ 2	0.7556	-
HH	Sharpie from huddle board	0.0048	0.0036	Mix- ≥ 2	1.2502	-
II	Hand sanitizer button on wall in main DNA office	0.0012	0.0010	5 alleles (X)	2.2545	-
JJ	Door handle exiting DNA offices	0.0129	0.0107	Mix- ≥ 3	1.3617	-
KK	Room 203 water cooler	0.0069	0.0064	Mix- ≥ 2	1.4972	-
LL	Room 203 fridge handle	0.0186	0.0138	Mix- ≥ 3	2.1230	-
MM	Room 203 toaster handle	0.0027	0.0000	Partial Female	0.9421	-
NN	Room 203 coffee pot handle	0.0022	0.0001	Partial Female	1.2767	1:17.969
OO	Room 203 light switch	0.0011	0.0007	4 alleles	1.1879	-
PP	Room 203 keypad	0.0118	0.0089	Mix- ≥ 2	0.8492	-
QQ	DJ phone	0.0598	0.0608	Mix- ≥ 3	0.8003	-
RR	MC phone	0.0225	0.0006	Full Female	0.7337	1:34.843
SS	AB mouse	0.0349	0.0115	Mix- ≥ 3	2.1344	1:2.034
TT	Erin coffee cup	0.5249	0.0002	Full Female	0.6930	1:2415.21
UU	MC mug	2.0397	0.0002	Full Female	0.7650	1:12265.522
VV	SB pen	0.1255	0.0095	Mix- ≥ 3	0.7986	1:12.159
WW	Tech/Admin Review drawer	0.1661	0.1153	Mix- ≥ 4	0.9328	-
XX	TA arm rest	0.0553	0.0200	Mix- ≥ 4	1.5491	1:1.759
YY	Main DNA office water cooler	0.1537	0.1279	Mix- ≥ 4	0.7160	-
ZZ	EC water bottle	0.0180	0.0005	Mix- ≥ 2	0.7337	1:32.025

*All Concentrations in ng/ µL

*Below determined cut off

3.5 Repeatability, Accuracy, and Precision

Table 18. Sensitivity Series Quantifiler® Trio Small Autosomal Quant Results, Average, Standard Deviation, and %RSD

Sample	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**
Theoretical Conc.	**0.05886**	**0.04893**	**0.03924**	**0.02931**	**0.01962**	**0.00969**	**0.00727**
Quant SA A Results	0.07117	0.06583	0.05433	0.03987	0.02443	0.01342	0.01127
Quant SA B Results	0.07991	0.06063	0.04273	0.03956	0.02628	0.01226	0.00961
Quant SA C Results	0.07312	0.05595	0.04840	0.03916	0.01983	0.01169	0.00789
Average	0.07474	0.06080	0.04849	0.03953	0.02351	0.01246	0.00959
Std. Dev.	0.00458	0.00494	0.00580	0.00036	0.00332	0.00088	0.00169
%RSD	6.13	8.13	11.96	0.90	14.12	7.08	17.61

*All Concentrations in ng/ µL

Table 19. Sensitivity Series Quantifiler® Trio Small Autosomal Quant Results, Average, Standard Deviation, and %RSD

Sample	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**	**RB6**
Theoretical Conc.	**0.00484**	**0.00245**	**0.00124**	**0.00048**	**0.00024**	**0.00010**	**0.00000**
Quant SA A Results	0.00646	0.00309	0.00127	0.00055	0.00035	0.00000	0.00000
Quant SA B Results	0.00820	0.00250	0.00101	0.00016	0.00036	0.00024	0.00000
Quant SA C Results	0.00568	0.00231	0.00101	0.00032	0.00027	0.00000	0.00000
Quant SA .1_A Results			0.00113	0.00034	0.00026	0.00012	0.00000
Quant SA .1_B Results			0.00172	0.00000	0.00015	0.00000	
Quant SA .1_C Results			0.00113	0.00044	0.00000	0.00000	
Quant SA .1_D Results			0.00070	0.00031	0.00019	0.00030	
Quant SA .1_E Results			0.00092	0.00026	0.00019	0.00000	
Quant SA .1_F Results			0.00092	0.00030	0.00023	0.00021	
Quant SA .1_G Results			0.00111	0.00032	0.00027	0.00000	
Quant SA .1_H Results			0.00121	0.00041	0.00000	0.00000	
Quant SA .1_I Results			0.00100	0.00076	0.00000	0.00000	
Quant SA .1_J Results			0.00104	0.00059	0.00014	0.00012	
Quant SA .1_K Results			0.00125	0.00053	0.00000	0.00012	
Quant SA .1_L Results			0.00154	0.00031	0.00013	0.00000	
Quant SA .1_M Results			0.00097	0.00057	0.00030	0.00026	
Quant SA .1_N Results			0.00076	0.00033	0.00013	0.00017	
Quant SA .1_O Results			0.00107	0.00045	0.00022	0.00011	
Quant SA .1_P Results			0.00123	0.00044	0.00047	0.00000	
Quant SA .1_Q Results			0.00067	0.00040	0.00000	0.00000	
Average	0.00678	0.00263	0.00108	0.00039	0.00018	0.00008	0.00000
Std. Dev.	0.00129	0.00040	0.00026	0.00017	0.00014	0.00010	0.00000
%RSD	19.04	15.37	23.56	42.91	74.80	125.89	0.00

*All Concentrations in ng/ µL

Table 20. Sensitivity Series Quantifiler® Trio Y Quant Results, Average, Standard Deviation, and %RSD

Sample	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**
Theoretical Conc.	**0.06049**	**0.05028**	**0.04032**	**0.03012**	**0.02016**	**0.00996**	**0.00747**
Quant Y A Results	0.08357	0.06955	0.06545	0.04139	0.02567	0.01635	0.01260
Quant Y B Results	0.08538	0.06948	0.04772	0.03824	0.02711	0.01321	0.00991
Quant Y C Results	0.07848	0.06868	0.05204	0.04098	0.01804	0.01086	0.00621
Average	0.08248	0.06924	0.05507	0.04020	0.02361	0.01347	0.00957
Std. Dev.	0.00358	0.00048	0.00925	0.00171	0.00487	0.00276	0.00321
%RSD	4.33	0.70	16.79	4.26	20.64	20.47	33.53

*All Concentrations in ng/ µL

Table 21. Sensitivity Series Quantifiler® Trio Y Quant Results, Average, Standard Deviation, and %RSD

Sample	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**	**RB6**
Theoretical Conc.	**0.00498**	**0.00251**	**0.00127**	**0.00050**	**0.00025**	**0.00010**	**0.00000**
Quant Y A Results	0.00754	0.00370	0.00222	0.00218	0.00000	0.00007	0.00000
Quant Y B Results	0.00761	0.00309	0.00231	0.00108	0.00000	0.00000	0.00000
Quant Y C Results	0.00804	0.00349	0.00122	0.00022	0.00037	0.00036	0.00000
Quant Y .1_A Results			0.00098	0.00046	0.00013	0.00024	0.00000
Quant Y .1_B Results			0.00175	0.00049	0.00048	0.00005	
Quant Y .1_C Results			0.00159	0.00039	0.00026	0.00000	
Quant Y .1_D Results			0.00078	0.00000	0.00031	0.00000	
Quant Y .1_E Results			0.00139	0.00062	0.00014	0.00000	
Quant Y .1_F Results			0.00171	0.00045	0.00027	0.00000	
Quant Y .1_G Results			0.00062	0.00037	0.00000	0.00020	
Quant Y .1_H Results			0.00066	0.00104	0.00000	0.00013	
Quant Y .1_I Results			0.00171	0.00095	0.00041	0.00000	
Quant Y .1_J Results			0.00138	0.00043	0.00066	0.00000	
Quant Y .1_K Results			0.00129	0.00000	0.00000	0.00000	
Quant Y .1_L Results			0.00281	0.00027	0.00058	0.00000	
Quant Y .1_M Results			0.00099	0.00000	0.00000	0.00000	
Quant Y .1_N Results			0.00081	0.00022	0.00038	0.00014	
Quant Y .1_O Results			0.00065	0.00061	0.00000	0.00000	
Quant Y .1_P Results			0.00068	0.00044	0.00037	0.00000	
Quant Y .1_Q Results			0.00108	0.00019	0.00042	0.00000	
Average	0.00773	0.00343	0.00133	0.00052	0.00024	0.00006	0.00000
Std. Dev.	0.00027	0.00031	0.00062	0.00050	0.00022	0.00010	0.00000
%RSD	3.49	9.14	46.33	96.23	90.77	171.92	0.00

*All Concentrations in ng/ µL

Table 22. Touch Samples 1 Quantifiler® Trio Small Autosomal and Y Quant Results, Average, Standard Deviation, and %RSD

Sample	Collected From	[SA] Quant Avg	Stdev	%RSD	[Y] Quant Avg	Stdev	%RSD
A	DNA lab outside door handle	0.0252	0.0038	15.11	0.0216	0.0009	4.11
B	Front lobby inside left door handle	0.0003	0.0001	17.95	0.0001	0.0001	173.21
C	Front Desk	0.0290	0.0043	14.84	0.0002	0.0002	96.11
D	Green button on front desk copier	0.0005	0.0002	41.64	0.0003	0.0002	56.53
E	Sign-in pen	0.0002	0.0002	66.61	0.0001	0.0001	124.51
F	Front desk phone	0.0028	0.0005	15.93	0.0029	0.0001	4.57
G	Front lobby right light switch	0.0001	0.0002	173.21	0.0001	0.0001	95.18
H	Space bar on front desk computer	0.0008	0.0003	34.55	0.0004	0.0001	23.91
I	Hand rail	0.0043	0.0012	27.34	0.0042	0.0010	23.21
J	Double Door handle to lab	0.0014	0.0003	22.17	0.0011	0.0004	37.44
RB3	Sterile water	0.0000	0.0000	0.00	0.0000	0.0000	0.00
K	Green button on copier	0.0011	0.0003	29.20	0.0006	0.0002	32.70
L	Water cooler handle	0.0004	0.0002	43.58	0.0001	0.0001	173.21
M	Lab mop handle	0.0001	0.0001	173.21	0.0002	0.0001	86.83
N	Broom handle	0.0000	0.0000	0.00	0.0000	0.0000	0.00
O	File cabinet door handle	0.0010	0.0000	1.91	0.0005	0.0002	36.88
P	Narcotic lab outside door handle	0.0005	0.0002	33.21	0.0005	0.0005	91.16
Q	Mouse to computer in main DNA lab	0.0000	0.0001	173.21	0.0000	0.0000	173.21
R	Hole punch	0.0007	0.0002	28.67	0.0004	0.0006	146.88
S	Phone earpiece in processing room	0.0131	0.0013	9.83	0.0119	0.0004	3.48
T	Door handle to outside of PCR lab	0.0006	0.0001	15.90	0.0003	0.0004	129.32
RB4	Sterile water	0.0000	0.0000	0.00	0.0000	0.0000	0.00

*All Concentrations in ng/ µL

Table 23. Male Female Mixtures 1 Quantifiler® Trio Y Quant Results, Average, Standard Deviation, and %RSD

	Sample	[SA] Theoretical	[SA] Quant Avg.	Stdev	%RSD	[Y] Theoretical	[Y] Quant Avg.	Stdev	%RSD
100 ng F:100 ng M	100M	4.9950	5.8726	0.5965	10.14	2.4953	3.4115	0.0234	6.87
100 ng F:50 ng M	50M	3.7474	4.5225	0.2488	5.50	1.2476	1.7395	0.0036	0.20
100 ng F:25ng M	25M	3.1263	3.8966	0.3631	9.32	0.6266	0.9797	0.1015	10.37
100 ng F:15 ng M	15M	2.8768	3.3581	0.0757	2.25	0.3771	0.5671	0.0069	1.21
100 ng F:10 ng M	10M	2.7493	3.079	0.1098	3.57	0.2495	0.3877	0.0320	8.26
100 ng F:7.5 ng M	7.5M	2.6883	3.4875	0.2231	6.40	0.1885	0.3108	0.0136	4.39
100 ng F:5 ng M	5M	2.6273	3.0338	0.1175	3.87	0.1275	0.2120	0.0200	9.45
100 ng F:2.5ng M	2.5M	2.5546	3.6535	0.3135	8.58	0.0625	0.0962	0.0116	12.10
100 ng F:1 ng M	1M	2.5171	4.0187	0.1929	4.70	0.0250	0.0537	0.0118	21.94
100 ng F:0.5 ng M	0.5M	2.5047	3.9136	0.1510	3.86	0.0126	0.0226	0.0033	14.81
100 ng F:0.25 ng M	0.25M	2.4985	4.2473	0.1506	3.55	0.0063	0.0114	0.0016	14.03
100 ng F:0.1 ng M	0.1M	2.4948	4.0977	0.2429	5.93	0.0026	0.0068	0.0004	6.16

*All Concentrations in ng/ µL

Table 24. Male Female Mixtures 2 Quantifiler® Trio Small Autosomal Quant Results, Average, Standard Deviation, and %RSD

Sample name	**2.5M_**	**1M_**	**0.5M_**	**0.25M_**	**0.1M_**	**RB8**
Theoretical Conc.	**2.5613**	**2.5515**	**2.5132**	**2.5066**	**2.5037**	**0.0000**
Quant SA A Results	1.8309	2.3443	2.4278	2.7430	1.9983	0.0000
Quant SA B Results	2.6291	1.9702	2.4369	2.0960	2.2956	
Quant SA C Results	2.1384	2.1860	2.0723	2.1226	1.9730	
Quant SA D Results	2.2297	1.8751	2.6135	2.2245	1.9518	
Quant SA E Results	2.1613	1.7243	2.4313	2.4822	1.8449	
Quant SA F Results	2.2278	1.7722	2.4493	2.1789	2.3587	
Quant SA G Results	2.0660	2.4555	2.4501	2.0660	2.0479	
Quant SA H Results	1.9480	2.0720	2.4301	2.0939	2.2580	
Quant SA I Results	2.1486	2.1363	2.3412	2.5049	2.4719	
Quant SA J Results	2.5296	2.2370	2.2206	2.2499	2.3413	
Quant SA K Results	2.2590	2.2405	2.0310	2.0689	2.1038	
Average	2.1971	2.0921	2.3549	2.2573	2.1496	0.0000
Std. Dev.	0.2288	0.2344	0.1765	0.2232	0.2039	0.0000
%RSD	10.41	11.20	7.49	9.89	9.49	0.00

*All Concentrations in ng/ µL

Table 25. Male Female Mixtures 2 Quantifiler® Trio Y Quant Results, Average, Standard Deviation, and %RSD

Sample name	**2.5M_**	**1M_**	**0.5M_**	**0.25M_**	**0.1M_**	**RB8**
Theoretical Conc.	**0.0625**	**0.0250**	**0.0125**	**0.0063**	**0.0025**	**0**
Quant Y A Results	0.0962	0.0461	0.0255	0.0101	0.0020	0.0000
Quant Y B Results	0.1215	0.0334	0.0192	0.0062	0.0072	
Quant Y C Results	0.0940	0.0557	0.0242	0.0098	0.0060	
Quant Y D Results	0.0971	0.0372	0.0210	0.0109	0.0061	
Quant Y E Results	0.1021	0.0293	0.0183	0.0111	0.0037	
Quant Y F Results	0.1074	0.0401	0.0229	0.0102	0.0045	
Quant Y G Results	0.0934	0.0484	0.0267	0.0122	0.0058	
Quant Y H Results	0.0919	0.0388	0.0162	0.0126	0.0071	
Quant Y I Results	0.1096	0.0466	0.0185	0.0095	0.0029	
Quant Y J Results	0.1147	0.0358	0.0151	0.0083	0.0040	
Quant Y K Results	0.1079	0.0575	0.0176	0.0108	0.0020	
Average	0.1033	0.0426	0.0205	0.0102	0.0047	0.0000
Std. Dev.	0.0097	0.0090	0.0039	0.0018	0.0019	0.0000
%RSD	9.41	21.18	18.95	17.45	41.29	0.00

*All Concentrations in ng/ µL

3.6 Contamination

All reagent blanks extracted or created during sample preparation were found to contain 0.0000 ng/ µL DNA. The capillary electrophoresis results obtained further indicated lack of DNA present as did the capillary electrophoresis results obtained for the amplification negative controls. All quantitation negative controls were also found to contain 0.0000 ng/ µL DNA excluding the NTC from the first set of touch samples which gave a result of 0.0002 ng/ µL.

Section 4: Discussion

4.1 Sensitivity and Stochastic

Full profiles were obtained for all three replicates of sensitivity samples S1 through S7 which correspond to theoretical small autosomal concentrations of 0.05886, 0.04893, 0.03924, 0.02931, 0.01962, 0.00969, and 0.00727 ng/ µL DNA and theoretical Y concentrations of 0.06049, 0.05028, 0.04032, 0.03012, 0.02016, 0.00996, 0.00747 ng/ µL DNA.

Sample S8 which corresponds to a theoretical small autosomal concentration of 0.00484 ng/ µL DNA and a theoretical Y concentration of 0.00498 ng/ µL resulted in full profiles for two of the three replicates while only 1 allele dropped out on the third replicate. Sample S9 which corresponds to a theoretical small autosomal concentration of 0.00245 ng/ µL and a theoretical Y concentration of 0.00251 ng/ µL had an average of 9 alleles dropped out between the three replicates which corresponds to 23% drop out of the profile.

S10 (theoretical small autosomal 0.00124 ng/ µL and theoretical Y 0.00127 ng/ µL) is where approximately half of the profile began to drop out. Because of this, additional replicates at this concentration and below were added. S11 (theoretical small autosomal 0.00048 ng/ µL and theoretical Y 0.00050 ng/ µL) resulted in an average of 32 alleles dropped out resulting in an average of 7 alleles obtained. At S12 (theoretical small autosomal 0.00024 ng/ µL and theoretical Y 0.00025 ng/ µL) an average of 37 alleles out of 39 dropped out resulting in the conclusion that termination of sample is possible at small autosomal concentrations of 0.00024 ng/ µL and below and Y concentrations of 0.00025 ng/ µL and below as no more than 4 alleles were observed in such instances and results were not interpretable. At a theoretical small autosomal and Y concentration of 0.00010 ng/ µL (sample S13) on average, full profile drop out occurred with the maximum of 2 alleles being recovered for one replicate of S13.

There were two instances in which a small autosomal quant result of 0.00000 ng/ µL was obtained with 9 alleles and 5 alleles being obtained. However, the Y quant results yielded concentrations of 0.00049 ng/ µL and 0.00041 ng/ µL. There were also 5 samples in which a 0.00000 ng/ µL Y quant result was obtained but small autosomal quant results of 0.00031 ng/ µL, 0.00057 ng/ µL, 0.00040 ng/ µL, 0.00026 ng/ µL, and 0.00027 ng/ µL with 7, 8, 13, 5, and 6

alleles being recovered respectively. Profiles in which 4 or less alleles were obtained were determined to not be useful.

4.2 Male Female Mixtures

The male female mixtures of 100 ng female DNA to 100 ng, 50 ng, 25 ng, 15 ng, and 10 ng male DNA all resulted in full male profiles. The 100 ng female DNA to 100ng male DNA sample did not generate a male to female ratio in Trio. The male to female ratios generated by Trio for 3 replicates of each of the remaining mixtures were averaged and calculated to be 1:1.600, 1:2.981, 1:4.923, and 1:6.967 respectively. The 100 ng female DNA to 7.5 ng male DNA sample resulted in an average of 3 male alleles dropped out and an averaged male to female ratio of 1:10.220. An average of 10 alleles dropped out at 100 ng female DNA to 5ng male DNA with an averaged ratio of 1:13.373.

At 100 ng female DNA to 2.5 ng male DNA, the male to female ratios generated by Trio for 14 replicates were averaged and calculated to be 1:24.018. An average of 14 alleles from the male profile dropped out. At the 100 ng female DNA to 1 ng male DNA, the male to female ratios generated by Trio for 14 replicates were averaged and calculated to be 1:55.710. An average of 5 alleles from the male profile not shared in the female profile were obtained.

At the 100 ng female DNA to 0.5 ng male DNA, the male to female ratios generated by Trio for 14 replicates were averaged and calculated to be 1:129.936 with an average of 1 allele from the male profile not shared in the female profile obtained. Three of the profiles at this concentration had one allele present from the male profile. However, it was unable to be determined if it was pull up from another dye channel or actually the allele present. Therefore, they were excluded in the average. At the 100 ng female DNA to 0.25 ng and 0.1 ng male DNA, the male to female ratios generated by Trio for 14 replicates were averaged and calculated to be

1:261.205 and 1:569.436 respectively. No more than 3 alleles from the male profile not shared in the female profile were obtained at male to female ratios generated by Trio at 1:100 and below.

4.3 Case Type Samples

Two types of case type samples were examined one being female buccal swabs with the addition of 20 µL of semen dilution and the other being female buccal swabs with 20 µL of male saliva dilutions. Both types of samples were ran through a differential separation on the QIAcube. The quant results for each sample were compared to the previously determined cut off value.

For the semen dilution samples, all of the samples in the non-sperm fraction resulted in extremely low male to female ratios with at most 1 male allele (excluding alleles shared in both the male and female profile) being present; this supports the possible 1:100 M:F cut off ratio for male DNA previously determined. The saliva dilution samples, additionally support the potential cut offs determined as none of the samples below these values were found to provide a significant profile.

4.4 Touch Samples

The quant results for the touch samples were compared to potential male female ratio and sensitivity cut off values determined. It was found that none of the samples yielded more than 3 alleles when below the cut off. The degradation index of each sample was also examined and it was determined that none of the samples exemplified significant degradation.

4.5 Repeatability, Accuracy, and Precision

The repeatability, accuracy, and precision of the study was demonstrated by conducting multiple replicates of the same samples. This was demonstrated by calculating the standard

deviation and percent relative standard deviation of each replicate. As can be seen in the sensitivity series Tables 18, 19, 20, and 21 the %RSD becomes more significant at the low concentrations. This is further exemplified by the high %RSDs developed from the first set of touch samples.

The repeatability, accuracy, and precision of higher concentration samples were determined by examining the male female mixture samples as they had much higher expected concentrations. As can be seen in Table 23 and Table 24, there is much less significant fluctuations in determined concentration at higher concentrations as signified by the lower %RSDs.

4.6 Contamination

The Quantifiler® Trio small autosomal and Y quantitaion results of 0.00000 ng/ µL DNA for each reagent blank in addition to the lack of alleles present in the electropherograms indicates contamination of samples did not occur during the extraction process. All quantitation negative controls were also found to contain 0.00000 ng/ µL DNA excluding the NTC for one replicate of the first set of touch samples which gave a small autosomal result of 0.0002 ng/ µL. The fact that this concentration is below the level of cut off as determined by the sensitivity series and the lack of DNA detected in the reagent blanks lead to the conclusion that all reagents used are free from contamination.

Section 5: Conclusion

The internal validation for termination of low quantity DNA samples and male female mixture samples utilizing Applied Biosystems® Quantifiler® Trio demonstrated that it was a robust and reliable quantification kit with the potential to be utilized as a screening tool for amplification by the SFPD crime lab. Profiles in which 4 or less alleles were obtained were

determined to not be useful. It was determined that termination of sample is possible at small autosomal concentrations of 0.00024 ng/ µL and below and Y concentrations of 0.00025 ng/ µL and below as no more than 4 alleles were observed in such instances.

It was further exemplified in the male female mixture study that no more than 3 alleles from the male profile not shared in the female profile was able to be obtained when Quantifiler® Trio generated a male to female ratio at or below 1:100.

Acknowledgements

I thank Mark Powell and Eleanor Salmon for allowing me to intern with the San Francisco Police Department Crime Lab and being reviewers for my project as well as Maria Cownan and the rest of the SFPD crime lab employees for assisting me throughout the internship. I also thank Dr. Pamela Staton for being a reviewer for my project and Laura Kuyper for training me as well as Randy Price for assisting me with obtaining articles.

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