Abraham, Lena, Internal Validation of the AmpFlSTR® Yfiler™ PCR Amplification Kit. Master of Science (Forensic Genetics), August, 2009, 93 pp., 17 tables, 28 illustrations, references, 15 titles.

The project conducted at the Forensic Testing Laboratories (FTL) in Las Cruces, NM involved an internal validation of the AmpFlSTR® Yfiler™ PCR Amplification kit. FTL currently implements the PowerPlex® Y kit which amplifies eleven Y-STR markers. The Yfiler™ kit provides more discriminatory power as it contains sixteen Y-STR markers. The validation included sensitivity, mixture, case type samples, contamination, precision and injection time change studies as required by the National Standards. Based on the data obtained, the Yfiler™ kit successfully detects male DNA in the presence of female DNA, and the correct number of male contributors in male/male mixtures is distinguishable. The additional loci included in the Yfiler™ kit can provide more insight than what is achieved through the PowerPlex® Y kit.

INTERNAL VALIDATION OF THE AMPFlSTR® YFILER™ PCR AMPLIFICATION KIT

INTERNSHIP PRACTICUM REPORT

Presented to the Graduate Council of the

Graduate School of Biomedical Sciences

University of North Texas

Health Science Center at Fort Worth

In Partial Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

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CHAPTER I

INTRODUCTION

Y-STR analysis has proven a useful tool in both forensic and paternity testing primarily due to the fact that these STRs are male-specific. Such analysis can be particularly helpful in male/female mixtures where the male DNA component is present at minute quantities. When autosomal analysis is unable to detect a male component due to overwhelming amounts of female DNA, Y-STR analysis may help overcome this situation by resolving the male component exclusively.

The Y chromosome is inherited from father to son thus making it male specific. There are about 60 million base pairs comprising the Y chromosome, with more than 400 identified short tandem repeat (STR) loci and more than 300 STRs that have been characterized (Li, 2008). The chromosome can be divided into two major regions, the non-recombining region (NRY), also known as the male-specific Y region (MSY), and the pseudo-autosomal region (PAR). The non-recombining region accounts for 95% of the entire Y-chromosome while the pseudo-autosomal region encompasses the telomeric regions, or the ends of the chromosome.

Unlike the PAR, where recombination takes place, no recombination is seen in the non-recombining region; limiting the amount of genetic variation. Since the alleles observed at the STR loci on the Y-chromosome are inherited together, they as a collection are referred to as a haplotype (Li, 2008). As a direct result of the absence of recombination, the power of

discrimination achieved through Y-STR analysis is significantly less than that of autosomal STR analysis. If DNA analysis is limited to only Y-STR typing, and if a match is made between a suspect and the evidence then, the suspect cannot be excluded as a potential contributor. However, the same could be said for any paternal male relatives of the suspect (Butler, 2005). Nevertheless, the information provided by the Y-chromosome analysis can still be valuable to help establish the innocence or guilt of an individual.

There are many commercially available kits that allow Y-STR analysis for forensic casework. The first multiplex kit, Y-PLEX™ 6 by ReliaGene Technologies, was made available in January 2001. Included in this kit are the loci DYS393, DYS19, DYS389II, DYS390, DYS391 and DYS385a/b. Since then another 7 kits have been introduced for Y-STR analysis which contain additional loci (Y-PLEX™ 5, genRES® DYSplex-1, genRES® DYSplex-2, Y-PLEX™ 12, PowerPlex® Y, MenPlex® Argus Y-MH, and Yfiler™).

The Powerplex® Y System (Promega) amplifies the nine European Minimal Haplotype (EMH) loci (DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392 and DYS393), two additional loci (DYS438 and DYS439) recommended by the Scientific Working Group on DNA Analysis Methods, and DYS437 (Krenke et al, 2005). The AmpFlSTR® Yfiler™ PCR Amplification Kit includes the EMH, the two SWGDAM recommendations as well as six additional highly polymorphic loci (DYS437, DYS448, DYS456, DYS458, DYS635(Y GATA C4) and Y GATA H4) (Applied Biosystems, 2004). The "a/b" nomenclature of locus DYS385 is due to its duplication on the Y-chromosome. As a result two PCR products are produced. When both products are the same size one peak is generated on the electropherogram. It is also possible to obtain two different alleles, where the shorter allele is designated as "a" and the larger allele is designated as "b". Similarly the locus DYS389 produces two PCR products. In

this case the DYS389II product contains the DYS389I product and they both differ in size by about 120 base pairs (Butler, 2005). With the exception of DYS392, DYS438 and DYS448 all other loci included in the Yfiler kit are tetranucleotide repeats. The three loci that are the exception are a trinucleotide repeat, a pentanucleotide repeat, and a hexanucleotide repeat respectively. Figure one depicts the repeat motifs and PCR product sizes for the European Minimal Haplotype and the two SWGDAM recommendations.

Figure 1 – Data on the European Minimal Haplotype and the two SWGDAM recommendations (Butler, 2005)

STR Marker	Position (Mb)	Repeat Motif	Allele Range	PCR Product Size	STR Diversity
DYS393	3.04	AGAT	8–16	104–136 bp	0.363
DYS19	9.44	TAGA	10–19	232–268 bp	0.498
DYS391	13.41	TCTA	6–13	90–118 bp	0.552
DYS437	13.78	TCTA	13–17	183–199 bp	0.583
DYS439	13.83	AGAT	8–15	203–231 bp	0.639
DYS389I/II	13.92	TCTG TCTA	10–15/24–34	148–168 bp/256–296 bp	0.538 /0.675
DYS438	14.25	TTTTC	8–12	101–121 bp	0.594
DYS390	16.52	TCTA TCTG	18–27	191–227 bp	0.701
DYS385 a/b	20.00, 20.04	GAAA	7–25	243–315 bp	0.838
DYS392	21.78	TAT	7–18	294–327 bp	0.596

Implementation of the Yfiler™ kit will increase the discrimination power in comparison studies of male DNA samples. The need for additional loci was evidenced in a study where two male individuals were found to have the same haplotype after using Powerplex® Y (Cainé et al, 2008) (Figure 2). Although these suspects had the same PowerPlex® Y haplotype they were not related. To help resolve the issue the Yfiler™ kit was also used with samples from the two men. The results demonstrated that Yfiler™ aided in distinguishing the suspects since they were found to have different alleles at four loci amplified by the Yfiler™ kit (Figure 3).

Figure 2 – Results Obtained from PowerPlex® Y (Cainé et al, 2008)

PowerPlex Y	Suspect 1	Suspect 2
DYS391	11	11
DYS389I	13	13
DYS439	11	11
DYS389II	29	29
DYS438	12	12
DYS437	15	15
DYS19	14	14
DYS392	13	13
DYS393	13	13
DYS390	24	24
DYS385	11,15	11,15

Figure 3 – Results Obtained from Yfiler™ with Emphasis on Additional Information (Cainé et al, 2008)

Y-filer	Suspect 1	Suspect 2
DYS456	**16**	**15**
DYS389I	13	13
DYS390	24	24
DYS389II	29	29
DYS458	**17**	**19**
DYS19	14	14
DYS385	11,15	11,15
DYS393	13	13
DYS391	11	11
DYS439	11	11
DYS635	**24**	**23**
DYS392	13	13
GATA H4	12	12
DYS437	15	15
DYS438	12	12
DYS448	**18**	**19**

The Yfiler™ kit has been shown to yield male profiles from male/female mixtures with overwhelming amounts of female DNA (up to 500 ng of female DNA) (Mulero et al, 2006). The addition of the six highly polymorphic loci, improves the discriminatory power of YSTRs (Mulero et al, 2006).

Internship Site and Intentions

The Forensic Testing Laboratory (FTL) of the Genesis Center is located on the New Mexico State University campus in Las Cruces, NM. FTL provides various DNA testing

services including: autosomal analysis, Y-chromosome analysis, Mini STR analysis and mitochondrial analysis. The lab recently acquired accreditation from Forensic Quality Services – International Division earlier this year. Y-chromosome analysis is currently performed at the lab using the PowerPlex® Y kit. The focus of the internship at FTL will be an internal validation of the AmpFlSTR® Yfiler™ PCR Amplification Kit.

There are two types of validation studies routinely performed, developmental and internal. Both types of validation are required by the National Standards issued in 1998 by the DNA Advisory Board. Since then the guidelines for validations have been revised by the Scientific Working Group on DNA Analysis Methods. Ultimately the purpose of any validation is to determine if reliable results can be obtained, under what conditions those results can be obtained, and any limitations of the procedure (SWGDAM, 2004). The developmental validation of the Yfiler™ kit has already been performed by the manufacturer Applied Biosystems to ensure accuracy, precision, and reproducibility of the procedure (SWGDAM, 2004). Internal validations also aim to achieve these three goals, as it is necessary to demonstrate that the procedure is reliable within the testing lab itself before it can be used on actual forensic casework.

According to the National Standards, internal validations should include the following studies: known and nonprobative evidence samples, reproducibility and precision, sensitivity and stochastic studies, mixture, and contamination (SWGDAM 2004). The first study involves using known samples, adjudicated case samples or simulated case samples. Comparisons of profiles obtained from known samples must be performed against profiles obtained from either the actual or simulated case samples: whichever is used in the study. Reproducibility and precision studies must be performed using controls. Likewise contamination studies require the evaluation of

contamination through the use of appropriate controls. The goal of the contamination study should show that as the procedure is being performed by the lab it is done so as to minimize contamination. Sensitivity studies are performed to show the reliability of the results obtained as well as to indicate the sensitivity of the procedure. Finally, mixture studies should aim to encompass the range of detectable mixture ratios, where major and minor contributors can also be detected. Included in the internal validation at FTL will be sensitivity, mixture, reproducibility and precision, and contamination studies as well as the application of case type samples.

AmpFlSTR® Yfiler™ PCR Amplification Kit

The Yfiler™ kit enables 100 reactions to be performed at a 25 µl final reaction volume (Applied Biosystems, 2004). The kit implements five fluorescent dyes: 6-FAM™ (blue), VIC® (green), NED™ (yellow), PET® (red) which label the samples and LIZ® (orange) which labels the GeneScan™ - 500 Size Standard (Applied Biosystems, 2004).

Figure 4 - Fluorescent Dyes And PCR product sizes (Short Tandem Repeat DNA Internet Database)

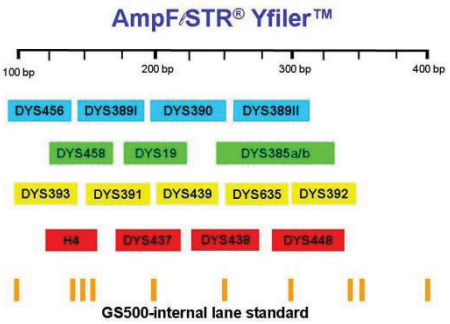
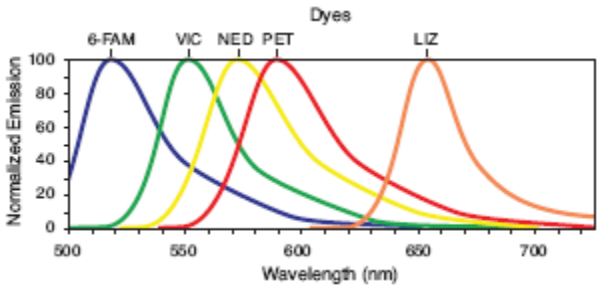


Figure 5 - Emission spectra (Applied Biosystems, 2004)



Although there is overlap seen in the emission spectra between the dyes, this is corrected during the data collection process of the capillary electrophoresis step through multicomponent analysis, which will separate the dyes (Applied Biosystems, 2004).

CHAPTER II

MATERIALS AND METHODS

At FTL extraction is performed using the BioRobot® EZ1™ Workstation which allows extraction of six samples in a single run. The EZ1™ Investigator kit is used for the purification of the DNA. The purification is made possible through a magnetic separation process by which silica particles bound to the DNA are separated by a magnet (Qiagen Inc., 2009). A Chaotropic salt facilitates the binding of the DNA to the silica particles by removing water from hydrated molecules (Qiagen Purification Technologies). Following magnetic separation the DNA is subsequently washed resulting in highly purified DNA (Qiagen Inc., 2009). Prior to proceeding with the extraction instrument it is necessary to first begin by pre-treating the sample to allow for protein digestion with Proteinase K (Pro K). FTL uses the trace protocol option of the instrument with TE as the elution buffer, and an elution volume set at 50 ul for samples containing blood, saliva, epithelial material or semen.

Samples which contain blood, saliva, or epithelial material will be pre-treated differently from those samples containing semen. They will either be collected using a swab or by taking an appropriate portion of the actual substrate itself. The aforementioned samples will be incubated in diluted G2 Buffer supplemented with Pro K and Dithiothreitol (DTT) for an hour on a 56° C heat block followed by 5 minute incubation on a 95° C heat block. After a centrifugation step with the swab portion or section of substrate plus a spin basket, 200 ul of

sample is transferred to extraction tubes, and the sample is then ready to be purified using the EZ1™.

Samples containing semen will undergo a similar pre-treatment step however DTT is not added until later in the process and the 5 minute incubation at 95° C is not required. The DTT is only added after two wash steps are performed so that sperm cells are only lysed after the sperm pellet is washed free of any female DNA. Following the hour incubation at 56° C and a centrifugation step using spin baskets, all but about 50 ul of the sample is transferred to an extraction tube which is ready for purification on the EZ1™: this is the non-sperm fraction.

The sperm pellet will then be treated with Differex™ Separation Solution (Promega) and Differex™ Digestion Buffer (Promega). After centrifugation three layers will be produced. The top yellow layer containing the digestion solution is discarded. This top layer consists of the non-sperm cell DNA fraction. The middle clear layer is the separation solution and the bottom layer is also clear and comprises the sperm cell pellet. Subsequently, two wash steps are performed using nuclease-free water. All of the water and a small fraction of the separation solution are removed after each wash. The sperm pellet is then treated with DNA IQ™ Lysis Buffer (Promega) supplemented with DTT to lyse the sperm cells. After incubation at room temperature for 15 to 30 minutes, 200 ul of the sample is transferred to an extraction tube, and the process is completed on the EZ1™.

Quantitation is performed using the Plexor® HY System, a real-time PCR system, which quantitates both autosomal and Y DNA. A decrease in fluorescence, which occurs when the incorporation of dabcyl-iso-dGTP in the primer of one strand quenches the fluorescent dye coupled to iso-dC in the primer on the complimentary strand, is seen with the accumulation of

product (Promega Corp., 2007). This is the opposite of what is seen with Plexor® HY's counterpart, the Quantifiler® Duo Kit (Applied Biosystems) in which an increase in fluorescence is observed with product accumulation (Applied Biosystems, 2008). Autosomal DNA is detected with the fluorescein dye while the Y chromosomal DNA is detected with the CAL® Fluor Orange 560 dye. The autosomal primers target a 99 base pair sequence of the human RNU2 locus which plays a role in pre-mRNA processing, found on chromosome 17 (Krenke et al, 2007). Y-chromosome primers amplify a 133 base pair sequence from the testis-specific protein, Y-encoded locus associated with spermatogenesis (Krenke et al, 2007).

The Plexor® HY Male Genomic DNA standard (50 ng/µl) is serial diluted to ultimately generate standard curves for both autosomal and Y DNA, which are used to determine the concentrations of unknown samples (Promega Corp., 2007). The concentrations of the standards range from 50 ng/µl to 0.0032 ng/µl. Samples are prepared first by creating a master mix with the following components and their appropriate volume per sample:

- Plexor® HY 2X Master Mix 10 ul
- Water, Amplification Grade 7 ul
- Plexor® HY 20X Primer/IPC Mix 1 ul

The master mix is added as a volume of 18 ul into the wells of an Optical 96 well plate, while samples/standards/no-template control are added as a volume of 2 ul into their appropriate wells. An Optical Adhesive Plate Cover is used to seal the plate. The quantitation process is carried out using the ABI 7500 Real-Time PCR System under the following parameters:

- Initial Denaturation 95° C, 2 minutes 1 Cycle

- Denaturation 95° C, 5 seconds
- Annealing and Extension 60° C, 35 seconds

} 38 Cycles

The expected values for the standard curve: Autosomal

- Slope -3.1 to -3.7
- R^2 ≥ 0.990

The expected values for the standard curve: Y

- Slope -3.0 to -3.6
- R^2 ≥ 0.990

The R^2 value indicates how well the data points fit the standard curve while the slope indicates the efficiency of PCR. The expected values for both the autosomal and Y standard curves are based on the average values obtained with the autosomal target (fluorescein) and the Y-chromosomal target (CAL® Fluor Orange 560 Dye) (Promega Corp., 2007).

The AmpFlSTR® Yfiler™ PCR Amplification Kit has been developed for the amplification of the following 17 Y-STR loci: DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS438, DYS439, DYS437, DYS448, DYS456, DYS458, DYS635 (Y GATA C4) and Y GATA H4. The amplification reactions are prepared in the pre-amplification room by first creating a master mix containing the following components and their appropriate volume per sample:

- AmpFlSTR® Yfiler™ Kit PCR Reaction Mix 9.2 ul
- AmpFlSTR® Yfiler™ Kit Primer Set 5.0 ul
- AmpliTaq Gold® DNA Polymerase 0.8 ul

A final reaction volume of 25 ul is achieved by adding 15 ul of master mix and 10ul of DNA extract/ positive control (AmpFlSTR® Control DNA 007 which has a concentration of 0.10 ng/µl))/ negative control (AmpFlSTR® Control DNA 9947A which has a concentration of 10 ng/µl). Once samples have been plated and the MicroAmp® tray has been capped, the plate is taken to the post-amplification room and centrifuged on the Eppendorf Centrifuge 5804. The amplification procedure is carried out on either of the Eppendorf Mastercycler instruments identified as Colonel Mustard and Professor Plumb. The thermal cycling parameters are as follows:

- Initial Incubation Step — Hold, 95° C, 11 minutes
- Denature — 94° C, 1 minute
- Anneal — 61° C, 1 minute
- Extend — 72° C, 1 minute

(30 Cycles)

- Final Extension — Hold, 60° C, 60 minutes
- Final Hold — Hold, 4° C, ∞

Fragment analysis is performed in the post-amplification room using the ABI 3130*xl* Genetic Analyzer with subsequent analysis of genotyping results from GeneMapper® *ID* Software version 3.2. Samples are prepared first by creating a master mix with the following components and their appropriate volume per sample:

- GeneScan™ 500 LIZ® Size Standard — 0.3 ul
- Hi-Di™ Formamide — 8.7 ul

The master mix is added as a volume of 9 ul into each well of a MicroAmp® Optical 96-Well reaction plate. Additionally, 1ul of PCR product or the AmpFlSTR® Yfiler™ allelic ladder is

added to the appropriate wells. In order to keep the capillary wet, wells that don't contain sample should have 10ul of Hi-Di™ formamide in them. Once the samples are plated a 96 well plate septa is placed on top of the wells and the plate is centrifuged in the Eppendorf Centrifuge 5804 briefly at full speed. The plate is then placed on a 95° C heat block for four minutes, followed by another four minutes on an ice block. The plate is secured in a 96 well plate base with the associated plate retainer. Once the instrument has been setup the plate is ready to go into the instrument. The normal run parameters are a 5 second injection time at 3kV injection voltage. FTL uses POP-6 polymer for fragment analysis.

Sensitivity Study

The sensitivity study will be performed using the 9948 male DNA standard (10 ng/µl concentration). The range of input DNA to be tested is 0.0234 ng to 3.0 ng. A series of serial dilutions (A – H) will be prepared along with two separate dilutions (I – J). The dilutions will be prepared as follows:

Table 1 – Sensitivity Study Dilution Series

	Amount of DNA to Add	Amount of TE-4 to Add	Final Concentration ng/µl
A	7.5 µl of 9948	42.5 µl	1.5
B	25 µl of A	25 µl	0.75
C	25 µl of B	25 µl	0.375
D	25 µl of C	25 µl	0.188
E	25 µl of D	25 µl	0.0938
F	25 µl of E	25 µl	0.0469
G	25 µl of F	25 µl	0.0234
H	25 µl of G	25 µl	0.0117
I	1.25 µl of 9948	48.75 µl	0.25
J	2.5 µl of 9948	47.5 µl	0.5

In order to ensure that the dilutions are made properly the samples will undergo quantitation using the Plexor® HY System Kit. Assuming that the dilutions are accurate, the next step will be to amplify each sample in duplicate along with two positive controls (AmpFlSTR® Control DNA 007), and four negative controls, one of which will be the AmpFlSTR® Control DNA 9947A and the rest nuclease-free water. The samples will be added as a volume of 2 ul with 8 ul of nuclease-free water. Samples will then be loaded on the 3130*xl* such that the entire amplification plate will be plated with the recommended load volume as well as an increased load volume which calls for 20 ul of master mix (19.5 ul Hi-Di™ Formamide and 0.5 ul GeneScan™ 500 LIZ® Size Standard) into the appropriate wells with 0.8 ul of PCR product/allelic ladder. In total, six allelic ladders will be run in the recommended and increased load volumes. The run parameters call for a 5 second injection time at 3kV injection voltage. Following analysis of the genotyping results, any samples that can be re-injected for a longer (8 seconds) or shorter time (3 seconds) to improve the quality of the profile obtained will be performed.

Mixture Study

Two different mixture studies were performed, one with gradually increasing concentrations of female DNA and gradually decreasing male DNA (Table 2) and, the other a male/male mixture study with varying ratios of two contributors (Table 3). Three individuals, donor 1, 2 and 11 will be selected and extracts from their buccal swabs will be obtained using the BioRobot® EZ1™. The samples will undergo quantitation with the Plexor® HY System Kit to determine the concentration of the extracts. Each sample will be diluted to 0.75 ng/ul concentration. The samples will each first be diluted 1:10 (bringing the concentration to 0.075 ng/µl) and then be amplified using the AmpFlSTR® Yfiler™ PCR Amplification Kit in duplicate with two positive controls (AmpFlSTR® Control DNA 007) and one negative control

Table 2 –Gradually Increasing Female DNA with Gradually Decreasing Male DNA:

Ratio	DNA in µl to Add	Input Amount of Female DNA - Donor 11	Input Amount of Male DNA - Donor 2
1 to 1	(20 + 20)	0.375 ng	0.375 ng
4 to1	(32 + 8)	0.6 ng	0.15 ng
9 to 1	(36 +4)	0.675 ng	0.075 ng
14 to 1	(56 +4)	0.7 ng	0.05 ng
19 to 1	(76 + 4)	0.7125 ng	0.0375 ng
24 to 1	(96 + 4)	0.72 ng	0.03 ng
49 to 1	(196 + 4)	0.735 ng	0.015 ng
99 to 1	(396 + 4)	0.7425 ng	0.0075 ng

Table 3 - Male/Male Study:

Ratio	DNA in µl to Add	Input DNA Amount of 1st Contributor - Donor 1	Input DNA Amount of 2nd Contributor - Donor 2
19 to 1	(38 + 2)	0.7125 ng	0.0375 ng
9 to 1	(36 +4)	0.675 ng	0.075 ng
2 to 1	(24 + 12)	0.5 ng	0.25 ng
1 to 1	(20 + 20)	0.375 ng	0.375 ng
1 to 2	(12 + 24)	0.25 ng	0.5 ng
1 to 9	(4 + 36)	0.075 ng	0.675 ng
1 to 19	(2 + 38)	0.0375 ng	0.7125 ng

(AmpFlSTR® Control DNA 9947A). A total of 10 µl of sample will be added to the amplification to achieve the optimal input DNA of 0.75 ng. The increased load volume will be used with four allelic ladders and an injection time of 5 seconds at 3kV injection voltage.

Precision Study

The precision study will entail comparisons of individual alleles from each ladder run in the sensitivity, mixture and case type samples study and calculation of the standard deviation of the base pair size per allele. Additionally the average standard deviation for each locus will be calculated.

Contamination Study

Analysis of amplified product in all negative controls and reagent blanks from the sensitivity, mixture, and case type samples studies will done for the contamination study. The

minimum analysis threshold will be determined by evaluating the noise level in the negative controls from the sensitivity study. Samples will be analyzed at 1 RFU, and 5% of the data around the mean peak height will be used to trim out the outlier data. The minimum RFU analysis threshold will be 5 times the mean peak height of the noise.

Case Type Samples Study

Samples such as blood, saliva, semen and epithelial cells will be deposited onto various substrates such as filter paper, swabs, denim jeans, a small piece of wood, a Styrofoam cup, and gum. Included in the samples will be those that typically demonstrate low copy number, such as a fingernail swabbing after a scratching, a licked envelope, the sweatband of a hat, and the nosepieces of a pair of glasses. Swabs will be used to collect samples off the envelope, fingernail, hat, nosepieces and Styrofoam cup. Samples deposited onto the denim and filter paper will be collected by cutting a small portion of the substrate itself. All of the samples will be extracted using the BioRobot® EZ1™ with their appropriate pre-treatment protocol. The samples will be quantified using the Plexor® HY System kit, and the samples will then be diluted to 0.075 ng/ul. The AmpFlSTR® Yfiler™ PCR Amplification Kit will be used to amplify the samples; however they will not be run in duplicate. Along with the samples will be two positive controls (AmpFlSTR® Control DNA 007) and one negative control (containing 1 ul of AmpFlSTR® Control DNA 9947A and 9 ul of nuclease-free water). The increased load volume will be used when preparing the samples for capillary electrophoresis on the 3130*xl*. Following analysis of the genotyping results, any samples that can be re-injected for a longer (8 seconds) or shorter (3 seconds) injection time to improve the quality of the profiles obtained will be performed.

CHAPTER III

RESULTS

Sensitivity

Quantitation results revealed that the dilutions were not at the target of input DNA intended. In order to adjust for this, the appropriate amount of DNA and water were calculated so that the actual amount of input DNA would be close to the intended values (Table 4). Quality profiles were obtained at 0.736 ng (Figure 7)) and 0.534 ng (Figure 8) using the full reaction at 30 cycles.

Table 4 – Adjusted Input DNA Values

	Final Target Concentration	Intended Input DNA (ng)	Actual Concentration After Quantitation	Volume of DNA to Add	Volume of Water to Add	Actual Input DNA (ng)
A	1.5 ng/µl	3	0.977 ng/µl	3 µl	7 µl	2.931
B	0.75 ng/µl	1.5	0.308 ng/µl	4 µl	6 µl	1.232
C	0.375 ng/µl	0.75	0.184 ng/µl	4 µl	6 µl	0.736
D	0.188 ng/µl	0.376	0.114 ng/µl	3 µl	7 µl	0.342
E	0.0938 ng/µl	0.1876	0.0361 ng/µl	3 µl	7 µl	0.1083
F	0.0469 ng/µl	0.0938	0.0227 ng/µl	3 µl	7 µl	0.0681
G	0.0234 ng/µl	0.0468	0.00975 ng/µl	3 µl	7 µl	0.0293
H	0.0117 ng/µl	0.0234	0.00739 ng/µl	3 µl	7 µl	0.0222
I	0.25 ng/µl	0.5	0.178 ng/µl	3 µl	7 µl	0.534
J	0.5 ng/µl	1	0.423 ng/µl	2 µl	8 µl	0.846

Results were interpretable at 2.931 ng (Figure 6) and even down to 0.1083 ng (Figure 10), but at considerably lower RFUs. However, in one sample containing 0.1083 ng of input DNA, a partial profile was obtained using the increased load volume. Partial profiles were observed at 0.0681 ng and 0.0293 ng with numerous alleles below threshold. The lowest amount of input DNA,

0.0222ng, yielded peaks all below threshold and therefore was not interpretable. Table five lists the average number of interpretable alleles at each amount of input DNA.

Table 5 - Average number of alleles called at the respective amount of input DNA. The 9948 control has a total of 17 alleles.

Input DNA (ng)	Average Number of Interpretable Alleles
2.931	17
1.232	17
0.846	17
0.736	17
0.534	17
0.342	17
0.1083	16.3
0.0681	7.5
0.0293	1.5
0.0222	0

Also evident throughout the samples were N-2 stutter observed at the DYS19 (Figure 11) locus and N+3 stutter at the locus DYS392 (Figure 12). All other loci demonstrated stutter that was one repeat unit less than the main peak. However, in the more concentrated samples (2.931ng and 1.232ng) N+4 stutter was occasionally observed at the DYS456 locus (Figures 13 and 14).

The negative control containing 9947A yielded two alleles that fell above threshold. The source of this DNA could not be determined. Since DNA with a known Y-STR profile (9948) was used for the study, and no contamination observed, the results were acceptable for evaluation. Additional controls obtaining water indicated no amplified product was present. The recommended load volume and the increased load volume both produced comparable results.

Mixture Study of Male DNA with Increasing Amounts of Female DNA (Refer to Appendix A)

At the full reaction, the 9:1 dilution, the locus DYS389I dropped out, but was subsequently observed in the 14:1 and 19:1 dilutions. Each of the remaining dilutions indicated that less male DNA was being detected since more female DNA was present in those dilutions. Male alleles fell below threshold at the 49:1 dilution. At the 99:1 dilution amplified male DNA was present at low quantities below the threshold. The data for the 1:1 dilution showed that one duplicate had an N-2 stutter peak called at 10% at the DYS19 locus.

Male-Male Mixture Study with Varying Ratios of DNA from Two Contributors (Refer to Appendix A)

The males selected for the male-male mixture study were selected on the basis of their reference profiles obtained through the PowerPlex® Y kit validation study previously performed by the lab. The loci amplified by PowerPlex® Y for these two individuals shows that many alleles are shared between the two. Subsequent analysis of their reference profiles using the Yfiler™ kit revealed an additional three loci where the two did not share alleles. At the 19:1, 1:19, 9:1, and 1:9 dilutions a few minor alleles were observed from the second male contributor. Major and minor alleles were detected at the 2:1 and 1:2 dilutions. The 1:1 dilution revealed an equal mixture. The occurrence of N-2 stutter was relatively consistent throughout the male-male mixture study at the locus DYS19. Also interesting to note is that N+3 stutter was only observed in the samples where Donor 2 was the major contributor to the mixture.

Precision Study

The base pair sizes of the allelic ladders run along with the sensitivity, mixture, case type samples, and reinjections, 16 in total, were compared at each allele and the standard deviations were determined. One of the allelic ladders run in the sensitivity study fell well below the

threshold and was therefore not used in the calculations toward the precision study. The maximum standard deviation observed for a single allele was 0.113 base pairs at the DYS385 locus. This locus had an average standard deviation of 0.10 base pairs. The lowest average standard deviation was observed at the DYS458 locus with a value of 0.03. The average standard deviations of all other loci fell between this maximum and minimum (Refer to Appendix B).

Based on a comparison of the alleles obtained for the collection of positive controls, ten in total run over the course of the validation studies, complete profiles were obtained. The standard deviations calculated for the base pair sizes of all alleles obtained gave a range from 0.03 to 0.09 base pairs.

Case Type Samples Study

All case type samples which produced profiles were consistent with the expected results (Table 6). Since the initial quantitation results for all samples that underwent differential extraction were very low, new samples were prepared with semen from a different donor. The quantitation results of these were much better in comparison (Table 7). As expected, no samples containing female DNA showed amplified product. The sample taken from donor 6's fingernails after scratching donor 3 did not produce any interpretable results: even after increasing the injection time to 8 seconds (Refer to Appendix C). The "Y" quantitation result for this sample is very low and is most likely the reason for the electropherogram results. Perhaps when the sample was taken donor 6 did not scratch hard enough to get a sufficient amount of donor 3's skin cells. Increasing the injection time to 8 seconds did improve the quality of some profiles, particularly donor 6's buccal swab sample containing 10ul of donor 1's blood (Table 8).

Figure 6 - Electropherogram Data for an Input DNA Amount of 2.931 ng

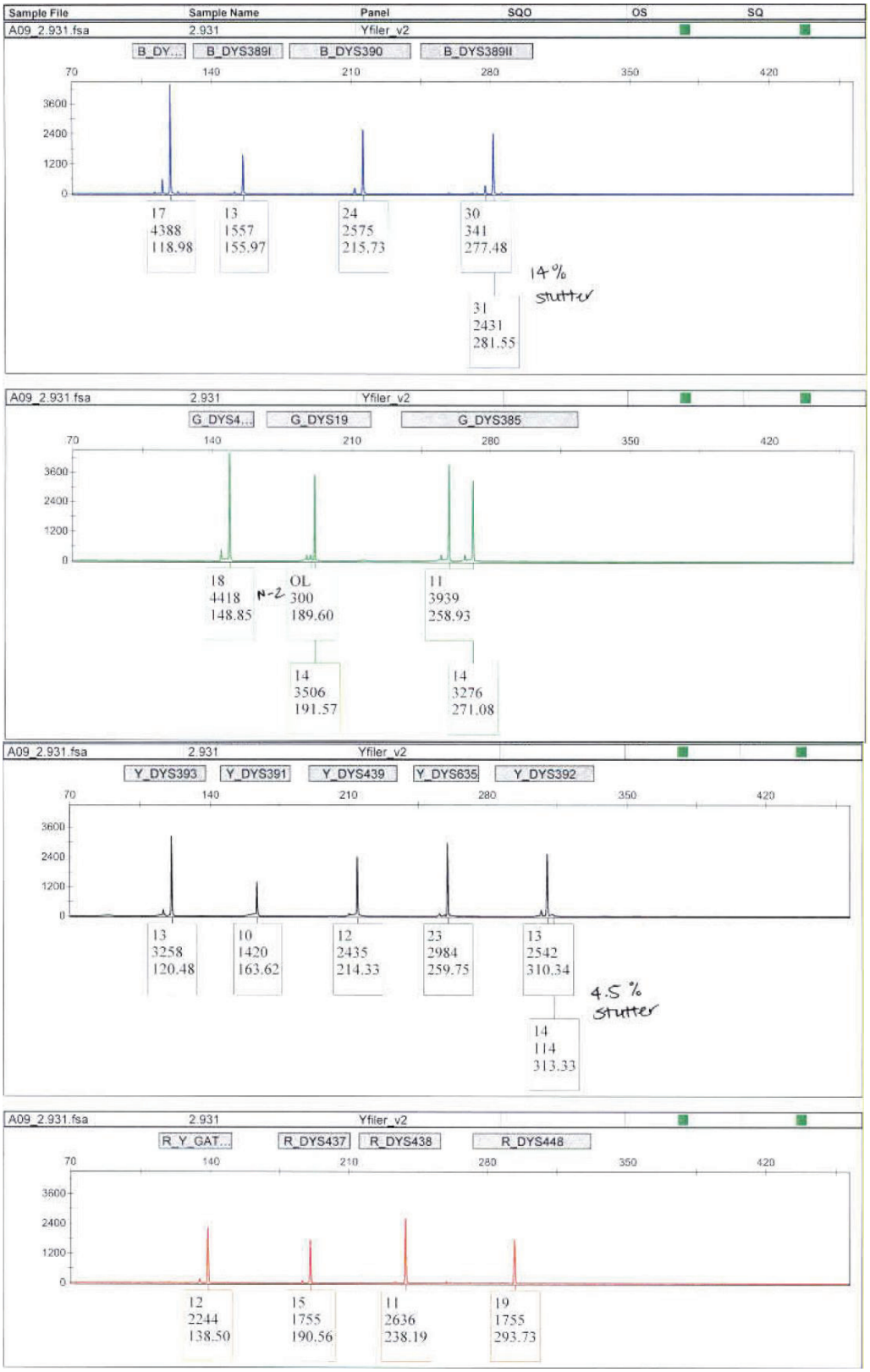


Figure 7 – Electropherogram Data of Optimal Input DNA of 0.736 ng

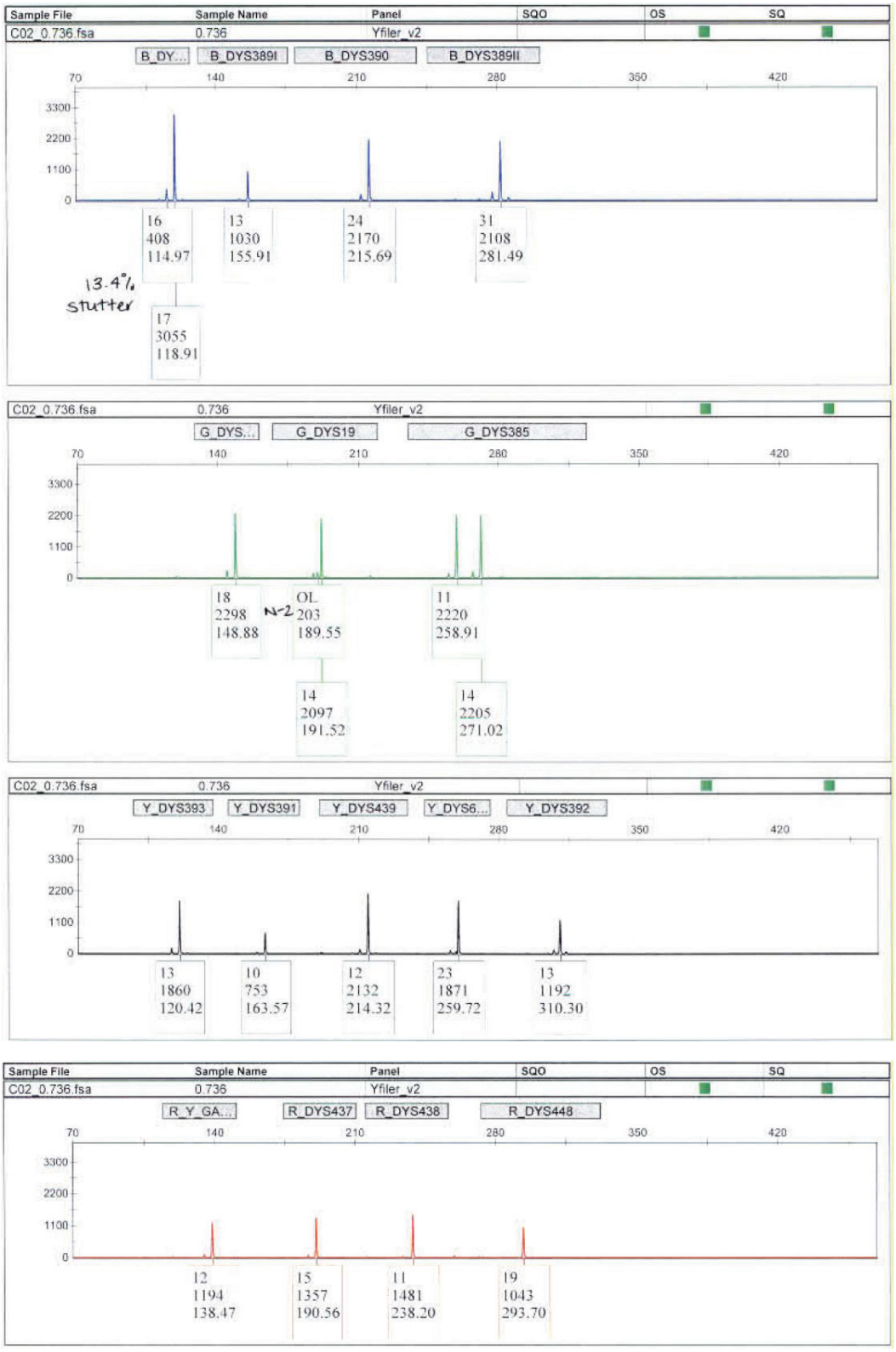


Figure 8 - Electropherogram Data for an Input DNA Amount of 0.534 ng

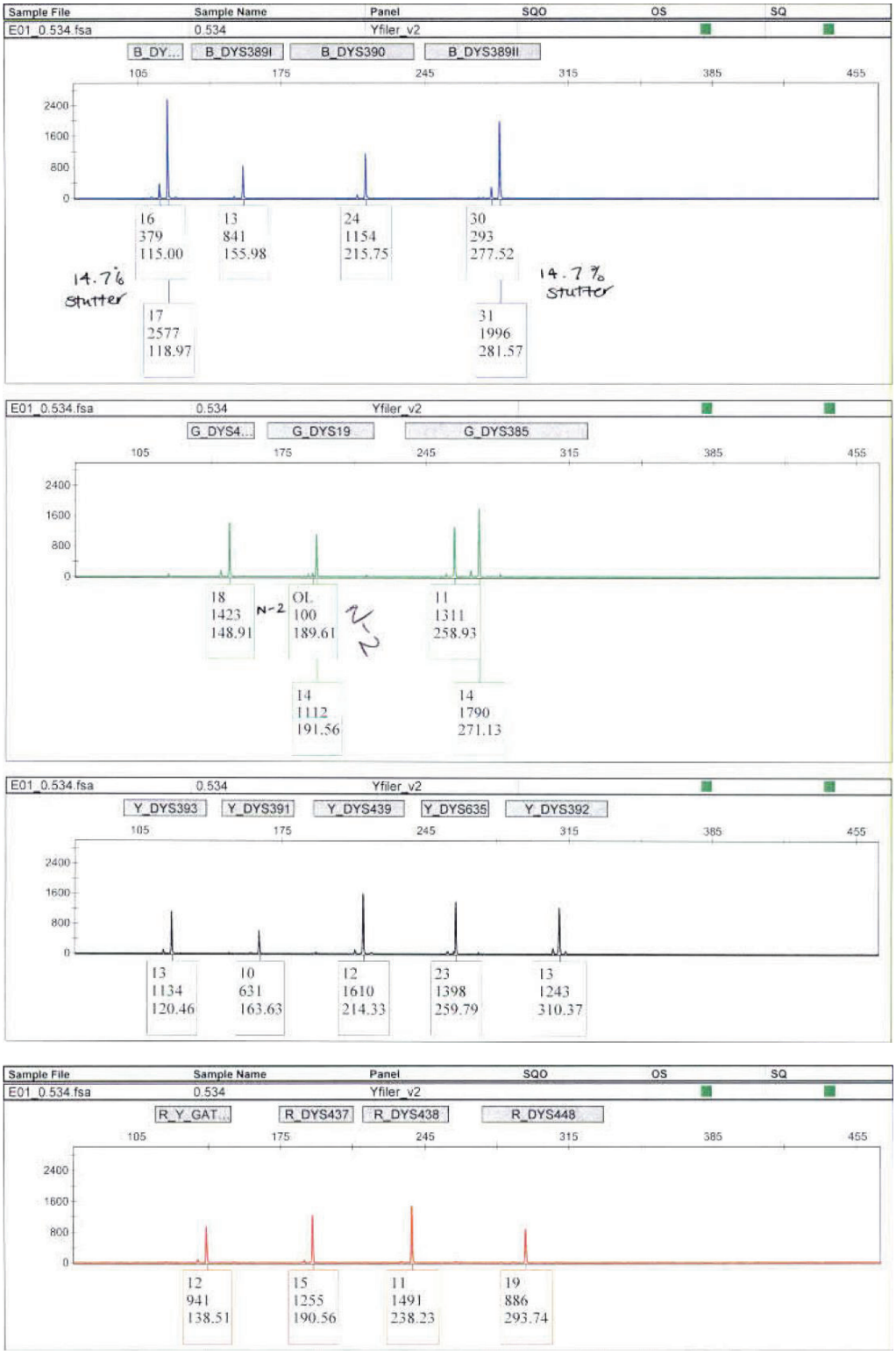
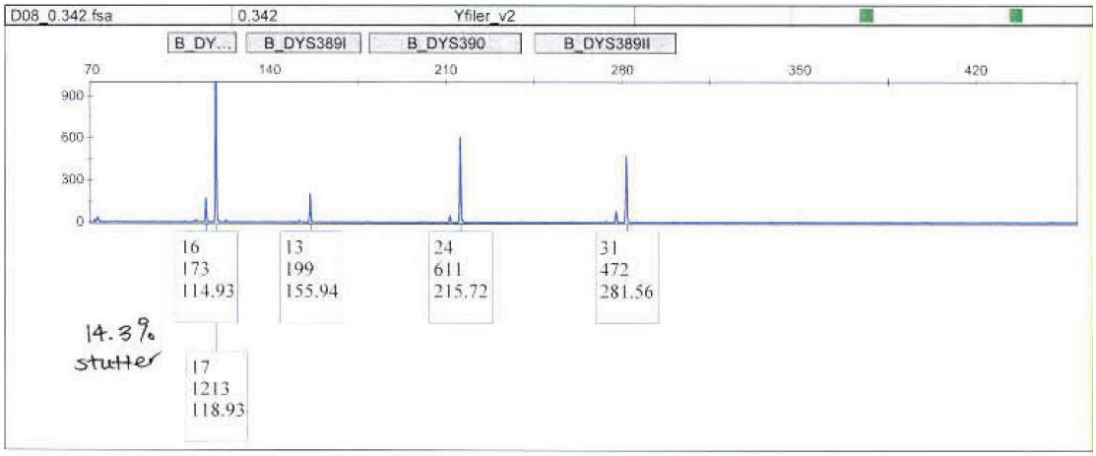
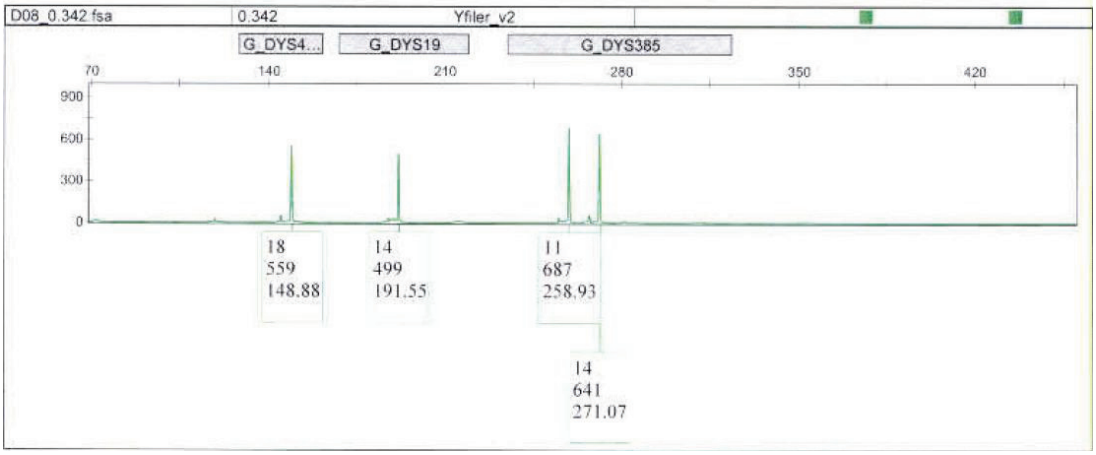
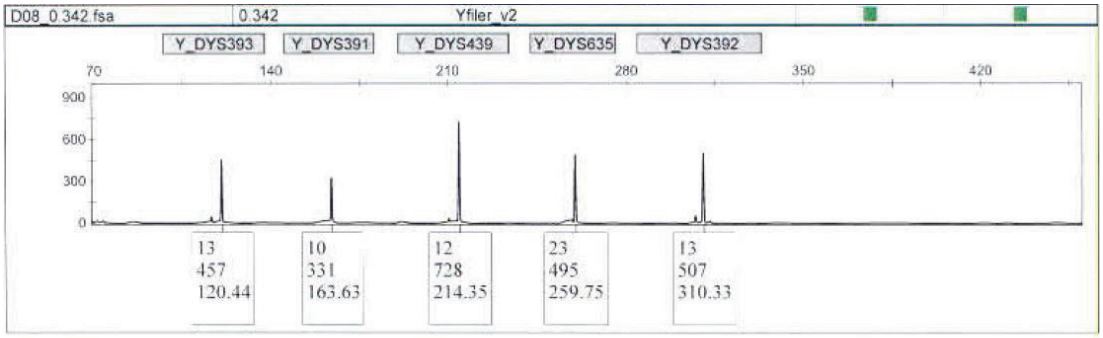


Figure 9 – Electropherogram Data for Input DNA Amount of 0.342 ng







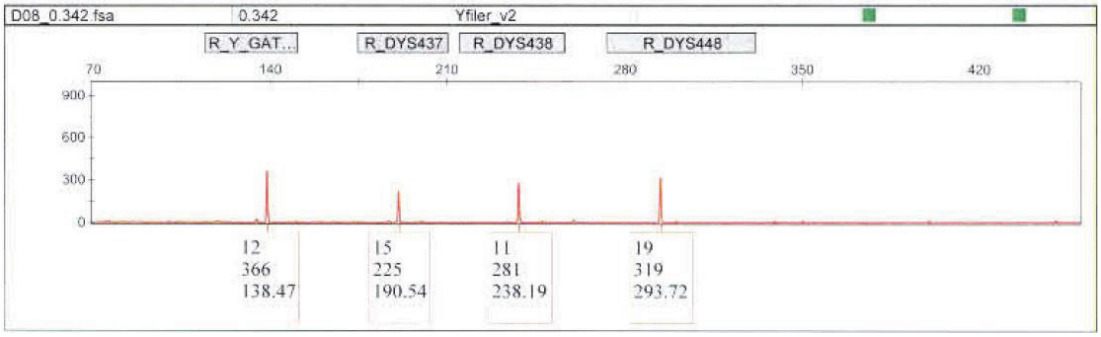


Figure 10 – Electropherogram Data for Input DNA Amount of 0.1083 ng

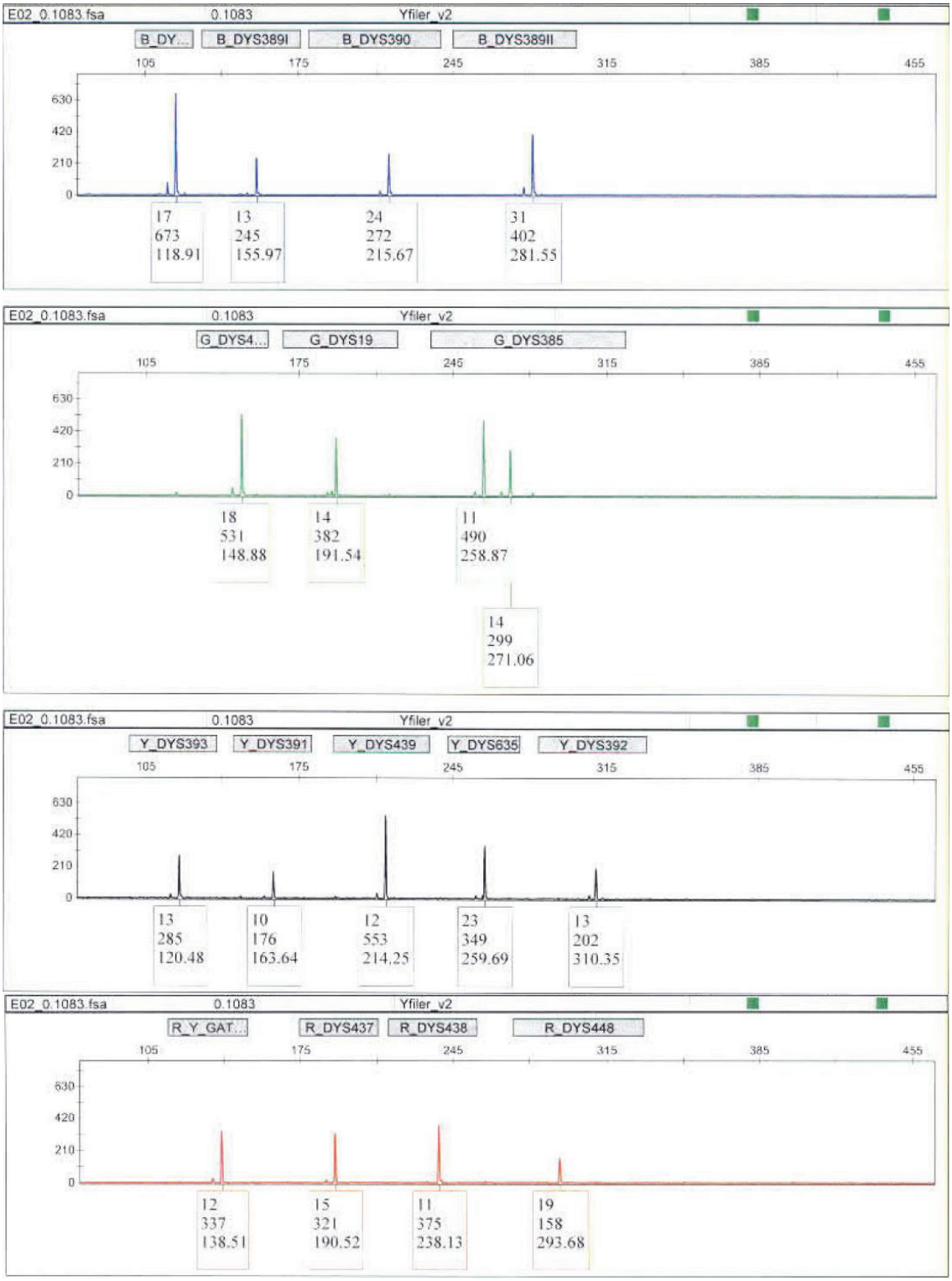


Figure 11 – N-2 stutter peak

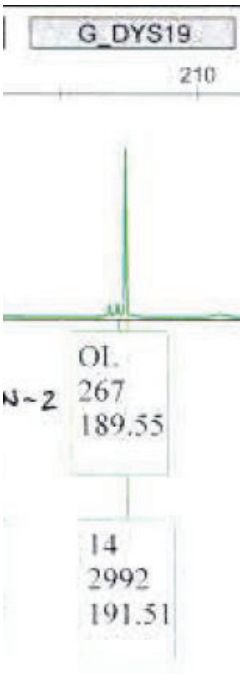


Figure 12 – N+3 stutter peak

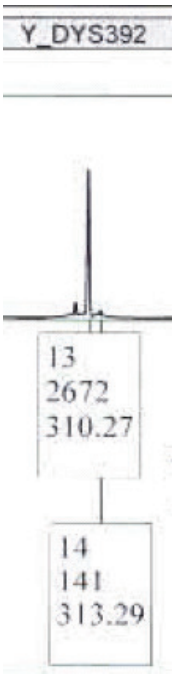


Figure 13 – N+4 stutter peak

observed at 2.931 ng input DNA.

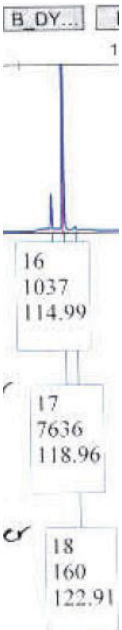


Figure 14 – N+4 stutter peak

observed at 1.232 ng input DNA.

Contamination Study

The negative control containing 9947A produced two interpretable alleles in the sensitivity study. The source of this DNA was not identified. Since known DNA was used for the sensitivity study, and no contamination was present, the results of the study were accepted for evaluation. Additional negative controls containing water were free of amplified product. All negative controls and reagent blanks were analyzed at 10 RFUs. Peak heights greater than 10 RFUs were used to evaluate the baseline. In order to account for outliers, 5% of the data around

Table 6 – Reference profiles for Case Type Samples

Donor 1		Donor 2		Donor 3		Donor 4		Donor 5	
Locus	Profile	Locus	Profile	Locus	Profile	Locus	Profile	Locus	Profile
DYS456	16	DYS456	16	DYS456	15	DYS456	17	DYS456	15
DYS389I	13	DYS389I	13	DYS389I	14	DYS389I	13	DYS389I	13
DYS390	23	DYS390	23	DYS390	24	DYS390	24	DYS390	24
DYS389II	28	DYS389II	29	DYS389II	30	DYS389II	30	DYS389II	29
DYS458	17	DYS458	18	DYS458	16	DYS458	17	DYS458	17
DYS19	14	DYS19	14	DYS19	16	DYS19	14	DYS19	14
DYS385	11,14	DYS385	11,15	DYS385	11,14	DYS385	15,17	DYS385	12,15
DYS393	13	DYS393	13	DYS393	13	DYS393	13	DYS393	13
DYS391	11	DYS391	11	DYS391	10	DYS391	10	DYS391	10
DYS439	11	DYS439	12	DYS439	13	DYS439	12	DYS439	13
DYS635	23	DYS635	25	DYS635	23	DYS635	22	DYS635	23
DYS392	13	DYS392	13	DYS392	13	DYS392	15	DYS392	13
YGATAH4	12	YGATAH4	13	YGATAH4	12	YGATAH4	12	YGATAH4	12
DYS437	15	DYS437	15	DYS437	15	DYS437	14	DYS437	14
DYS438	11	DYS438	11	DYS438	12	DYS438	11	DYS438	12
DYS448	19	DYS448	19	DYS448	20	DYS448	19	DYS448	19

the mean was trimmed: the trimmed mean was 14.21 RFUs. A conservative, minimal RFU threshold was determined to be 5 times the trimmed mean, or a value of 71.03 (Refer to Appendix D).

Injection Time Changes

Injection time changes to three seconds or eight seconds showed differences in RFUs from the standard injection time of five seconds. The largest percent difference for the three second injection time was at the locus DYS390 at a 28.8 RFU difference, while the lowest was at DYS385 for allele eleven with 10.5 percent difference. The overall average percent difference was 20.5%. For the eight second injection time change, the largest RFU difference was in locus DYS456 with 47.9%, while the lowest was at DYS389I with 16.0%. The overall average percent difference was 25.9% (Refer to Appendix E).

Table 7 – Case Type Samples Study: Sample Preparation and Quantitation Results

ITEM #	SAMPLE DESCRIPTION		QUANTITATION RESULTS	
			AUTO (ng/ul)	Y (ng/ul)
1	Donor 6's saliva swabbed onto filter paper		9.81E-01	N/A
2	Blood(Donor 1) on filter paper (10 ul)		2.56E+00	4.10E+00
3	(Semen) Donor 5 on filter paper (10 ul)	N	3.84E+00	3.56E+00
		S	1.56E-01	1.86E-01
4	Blood(Donor 1, 2.5 ul) + Semen(Donor 5, 5 ul) on filter paper	N	1.10E+00	1.35E+00
		S	8.00E-04	2.31E-03
5	Donor 7's saliva on styrofoam cup		4.05E-01	N/A
6	Donor 8's chewed gum		3.98E-01	N/A
7	Licked Envelope from Donor 8		2.40E-01	N/A
8	Donor 6's fingernail swabbing after scratching Donor 3		1.07E-01	5.56E-04
9	Donor 6's buccal swab + Blood(Donor 1, 10 ul)		9.74E+00	2.10E+00
10	Donor 6's buccal swab + Semen(Donor 5, 10 ul)	N	4.84E+01	1.92E+01
		S	4.16E+00	4.27E+00
11	Donor 6's neck swab + Semen(Donor 5, 10 ul)	N	3.02E+01	2.50E+01
		S	3.56E-02	5.09E-02
12	Donor 9's nosepiece swabbing		1.24E-01	N/A
13	Swabbing from sweatband of Donor 4's hat		1.02E-01	1.66E-02
14	Blood(Donor 1, 10 ul) on twig found outside lab		1.66E+00	2.44E+00
15	Blood(Donor 1, 20 ul) on khaki shorts		7.60E+00	1.00E+01
16	Blood(Donor 1, 10 ul) smeared on khaki shorts		3.49E-01	3.21E-01
17	Semen(Donor 10, 10 ul) on khaki shorts	N	1.97E-03	9.73E-04
		S	N/A	6.56E-04
18	Blood(Donor 1, 2.5 ul) + Semen(Donor 10, 5 ul) on khaki shorts	N	2.12E-02	1.58E-02
		S	6.89E-04	N/A
19	Blood(Donor 1, 20 ul) on blue jeans		2.95E-01	3.07E-01
20	Blood(Donor 1, 10 ul) smeared on blue jeans		2.30E-02	1.64E-02
21	Semen(Donor 5, 10 ul) on blue jeans	N	1.70E+01	1.80E+01
		S	5.20E-01	6.50E-01
22	Blood(Donor 1, 2.5 ul) + Semen(Donor 5, 5 ul) on blue jeans	N	2.75E-02	3.75E-02
		S	1.40E-02	2.09E-02

Table 8 – Case Type Samples Study Profiles Obtained with Yfiler™

ITEM #			GENETIC PROFILE															
			DYS456	DYS389I	DYS390	DYS389II	DYS458	DYS19	DYS385	DYS393	DYS391	DYS439	DYS635	DYS392	YGATAH4	DYS437	DYS438	DYS448
1			NO PROFILE OBTAINED															
		Reinj (8sec)	NO PROFILE OBTAINED															
2			16	13	23	28	17	14	11,14	13	11	11	23	13	12	15	11	19
3	N		15	13	24	29	17	14	12,15	13	10	13	23	13	12	14	12	19
	S		15	13	24	29	17	14	12,15	13	10	13	23	13	12	14	12	19
4	N		15,16	13	23,24	28,29	17	14	11,12,14,15	13	10,11	11,13	23	13	12	14,15	11,12	19
	S		NO PROFILE OBTAINED															
5			NO PROFILE OBTAINED															
		Reinj (8sec)	NO PROFILE OBTAINED															
6			NO PROFILE OBTAINED															
		Reinj (8sec)	NO SIZING DATA OBTAINED															
7			NO PROFILE OBTAINED															
		Reinj (8sec)	NO PROFILE OBTAINED															
8			NO PROFILE OBTAINED															
		Reinj (8sec)	NO PROFILE OBTAINED															
9			16															
		Reinj (8sec)	16	13	23	28	17	14	11,14	13	11	11	23	13	12	15	11	19
10	N		15	13	24	29	17	14	12,15	13	10	13	23	13	12	14	12	19
	S		15	13	24	29	17	14	12,15	13	10	13	23	13	12	14	12	19
11	N		15	13	24	29	17	14	12,15	13	10	13	23	13	12	14	12	19
	S		15	13	24	29	17	14	12,15	13	10	13	23	13	12	14	12	19
12			NO PROFILE OBTAINED															
		Reinj (8sec)	NO PROFILE OBTAINED															
13			17				17		15,17	13	10	12	22		12		11	
		Reinj (8sec)	17	13			17		15,17	13	10	12	22	15	12		11	
14			16	13	23	28	17	14	11,14	13	11	11	23	13	12	15	11	19
15			16	13	23	28	17	14	11,14	13	11	11	23	13	12	15	11	19
16			16	13	23	28	17	14	11,14	13	11	11	23	13	12	15	11	19
17	N		POOR QUANTITATION RESULTS - NOT RUN															
	S		POOR QUANTITATION RESULTS - NOT RUN															
18	N		POOR QUANTITATION RESULTS - NOT RUN															
	S		POOR QUANTITATION RESULTS - NOT RUN															
19			16	13	23	28	17	14	11,14	13	11	11	23	13	12	15	11	19
20			16	13		28	17		11	13	11	11	23	13		15	11	19
21	N		15	13	24	29	17	14	12,15	13	10	13	23	13	12	14	12	19
	S		15	13	24	29	17	14	12,15	13	10	13	23	13	12	14	12	19
22	N		15,16	13		28	17	14	11,12,14,15		10,11	11	23	13	12	14	12	19
		Reinj (8sec)	15,16	13		28	17	14	11,12,14,15	13	10,11	11	23	13	12	14,15	12	19
	S		15,16	13			17	14	12	13	10	13	23	13	12			
		Reinj (8sec)	15,16	13		29	17	14	12,15	13	10	13	23	13	12			19

CHAPTER IV

DISCUSSION

Sensitivity

Based on the results obtained for the range of input DNA tested, 0.75 ng was chosen as the target input of template of DNA for optimal results at 30 cycles, full reaction. Since the stutter observed in this sensitivity study was consistent with the reported observed stutter, the manufacturer's recommendation for stutter will be used. Both the recommended and increased load volume produced comparable results. The increased load volume sample preparation method was previously validated at FTL by a prior technical manager so this method will also be used with Yfiler™ to maintain consistency. There is no scientific value in using the increased load volume, and in actuality the increased volumes of Hi-Di™ and size standard required by this method are more costly.

A comparison of the loci DYS389I and DYS391 to all other loci shows that these two loci are lower in RFUs and therefore may fall below threshold with less concentrated samples. Another phenomenon that is typical for samples with less DNA is random stochastic effects. Low copy number samples which contain less than 100 pg of DNA often exhibits peak height imbalance which could lead to a failure in detection of one allele or the other of a heterozygote, or potentially loss of both alleles at a given locus. This becomes obvious in the sensitivity study at 0.0681 ng of input DNA in the locus DYS385 (Figure 15).

Figure 15 - Example of Stochastic Effects depicting Heterozygote Peak Imbalance where allele 11 is the only interpretable allele.

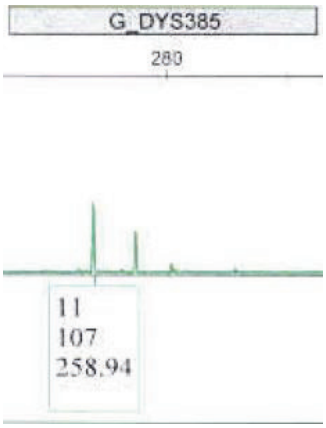


Figure 16 - Example of Heterozygote Peak Imbalance where both peaks are interpretable.

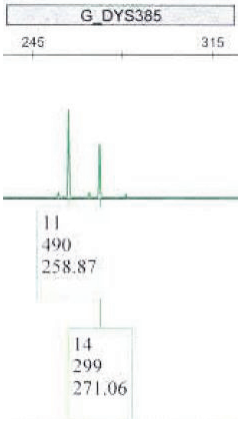


Figure sixteen is another depiction of heterozygote peak imbalance, however both alleles are interpretable and the smaller allele has greater signal intensity over the larger allele. These illustrations show how stochastic effects are expressed randomly.

Stutter is a result of slippage of the polymerase during PCR. Typically stutter is represented as one repeat unit less than the length of the true allele peak. The longer the repeat unit (ie. hexanucleotide, pentanucleotide) the less stutter is observed. The locus DYS392 is a trinucleotide repeat unit, which has been reported to have an additional peak that is three base

pairs longer than the length of the true allele peak (Mulero et al, 2006). The N-3 stutter was not called in the electropherograms, yet its presence is noticeable. In another study N-2 and N-4 stutter was observed at the locus DYS19. The N-2 stutter is the result of a TA repeat in the DYS19 sequence (Gross et al, 2008). In the present validation study only the N-2 stutter was called at DYS19. The N-4 stutter is evident although it was not called. The importance in recognizing stutter is to distinguish if in fact stutter is present or, in the case of a mixture, if an additional contributor is present.

Mixture Study

The results of both mixture studies support the findings observed in the sensitivity study. The gradually increasing female DNA, gradually decreasing male DNA mixture study indicates Yfiler™ is sensitive to male DNA in the presence of overwhelming amounts of female DNA, up to a 49:1 ratio (0.015 ng of male DNA). At the 1:1 ratio and 4:1 ratio, which contained 0.375 ng and 0.15 ng of male DNA respectively, complete profiles were obtained. This is consistent with the sensitivity study results for an input DNA of 0.342 ng and 0.1083 ng where the average number of alleles called was 17 and 16.3 respectively.

In the male/male mixture study a major profile is evident at the 19:1 (0.0375 ng of 2nd male contributor) and 1:19 (0.0375 ng of 1st major contributor) ratios with partial minor alleles observed. At these ratios the number of minor alleles observed corresponds with the data obtained in the sensitivity study at 0.0293 ng input DNA listed in table five. Major and minor alleles were detected at the 9:1 and 1:9 ratios. Similarly at these ratios, where the input DNA for the minor contributor is 0.075 ng, the number of minor alleles detected is comparable to the average number of alleles detected at 0.0681 ng input DNA listed in table five.

Precision Study

The precision observed in the allelic ladder data had a maximum standard deviation of 0.113 base pairs which is within the required precision of 0.5 base pairs. Therefore the conditions between runs on the 3130*xl* are consistent and good precision can be obtained with the Yfiler™ kit. When runs with multiple injections are performed multiple ladders will also be run. Based on evaluation of all the positive controls run, Yfiler™ produces reproducible data.

Case Type Samples Study

Since all samples that produced profiles were consistent with the expected results, Yfiler™ is a reliable method for forensic case type samples. However, the value of this study probably would improve if samples were used that more closely represent forensic casework. Forensic samples typically encountered by a lab have been exposed to sunlight, humidity, and other environmental factors which cause DNA degradation. The information obtained from samples such as these may better indicate how Yfiler™ will respond to actual forensic casework.

Contamination Study

The analysis threshold for all the samples and controls used in these validation studies was 100 RFUs. Based on the calculations for the contamination study it was determined that dropping the threshold to 72 RFUs is acceptable for Yfiler™ controls and samples. FTL has determined that the interpretation threshold of 72 RFUs will only be utilized to investigate possible contamination situations.

It may have been more appropriate to use the data of the sensitivity study in determining the minimum analysis threshold simply because evaluation of the negative controls and reagent blanks at 10 RFU only indicates background noise. The purpose of establishing a minimum

analysis threshold is to be confident that the profile obtained from a questioned sample is representative of the actual reference profile.

Injection Time Changes

The injection time can be changed to three seconds for samples with too much DNA to reduce the RFU values. On the other hand, samples with too little DNA which produce data below threshold can be injected at eight seconds to improve RFU values.

The ultimate goal for increasing or decreasing the injection time is to improve the overall quality of the profile obtained (i.e. complete profiles, less spectral pull up, less off-scale data, ,etc.). Instead of centering the injection time change study on RFU values it would have been better to demonstrate if additional alleles were called with the time modification, specifically for lower level samples injected at eight seconds. Samples which gave complete profiles in the sensitivity study which were reinjected at either three or eight seconds were done primarily to obtain RFU values within an optimal range of 800 – 2000 RFU (as defined by the internship mentor). So in terms of adding any additional information, this was specifically visualized with the lower levels of DNA from the sensitivity study and samples from the case type samples study which were reinjected at eight seconds. The following three tables show the number of alleles called at the normal injection time (5 seconds) and the modified injection times.

Table 9 – Comparison of Alleles Called at 5 Second and 3 Second Injection Times.

Input DNA	Number of Alleles Called 5 Second Injection	Number of Alleles Called 3 Second Injection
2.931ng	17	17
1.232ng	17	17

Table 10 – Comparison of Alleles Called at 5 Second and 8 Second Injection Times.

Input DNA	Number of Alleles Called 5 Second Injection	Number of Alleles Called 8 Second Injection
0.534ng	17	17
0.342ng	17	17
0.1083ng	16.3	5
0.0681ng	7.5	17
0.0293ng	1.5	8
0.0222ng	0	1

Table 11 – Comparison of Alleles Called at 5 Second and 8 Second Injection Times for Case Type Samples

Sample	Number of Alleles Called 5 Second Injection	Number of Alleles Called 8 Second Injection
9	1	17
13	10	12
22N	19	21
22S	12	15

It is unusual that an eight second injection of 0.1083 ng input DNA would result in a lower number of alleles called than at the five second injection. Since amplified product from the sensitivity and case type samples plates run earlier was added to the plate designated for the reinjections, it is possible that sample was taken from the wrong well on the sensitivity plate. Another explanation may be that the 0.1083 ng sample was set for a 3 second injection rather than an eight second injection. Overall, looking at the changes in RFU values and the number of alleles called it is still effective to change the injection times in order to improve the quality of the profiles obtained.

CHAPTER V

CONCLUSIONS

The AmpFlSTR® Yfiler™ PCR Amplification Kit has been validated at the Forensic Testing Laboratories and allows the amplification of 17 STR loci on the Y-chromosome including: DYS456, DYS389I, DYS390, DYS389II, DYS458, DYS19, DYS385, DYS393, DYS391, DYS439, DYS635, DYS392, Y GATA H4, DYS437, DYS438, and DYS448. The first validation study performed was aimed at determining the optimal input DNA to obtain appropriate results. Of the range of input DNA amounts tested, 0.75 ng was determined to be the target input DNA amount. The RFUs exhibited for this sample fell approximately within the optimal range of 800 to 2000 RFUs.

All female DNA samples used throughout the entirety of the validation showed that no female DNA was detected. Even in the presence of overwhelming amounts of female DNA, male DNA is still detectable. The male/male mixture study revealed that the actual amount of contributors included in the mixtures, in this case two individuals, was evident. Although these individuals shared many alleles, the additional loci DYS458, DYS635 and Y GATA H4 that are included in the Yfiler™ kit but not PowerPlex® Y also aided in resolving these contributors.

The validation study also examined how Yfiler™ works with case type samples. Various items were used as substrates for biological materials such as saliva, semen, and blood. All samples containing only female DNA produced no amplified product as expected. In samples containing both male and female DNA only the male DNA was detected. The results also

demonstrated that the appropriate amount of contributors is clear. Evaluation of samples exposed to factors which cause DNA degradation may have given better insight into how Yfiler™ would respond with actual forensic casework.

The required precision on the 3130*xl* is 0.5 base pairs. The precision study calculations obtained from the allelic ladders run over the course of the entire validation study show that Yfiler™ has precision on the 3130*xl*. All standard deviations calculated for each locus fell within the required precision. Evaluation of the positive controls indicates the kit produces reproducible data.

The established analysis threshold used at FTL is 100 RFUs. Based on the results of the contamination study, which examined the negative controls and reagent blanks run over the entire course of the validation study, the minimum analysis threshold was determined to be 72 RFU. Since FTL determines the minimum analysis threshold in this manner, I was instructed to do the same. It may have been more appropriate to use data from the sensitivity study in determining a minimum analysis threshold. The goal of a reduced threshold is to determine if actual PCR products which fell below the analysis threshold will become detected. In addition, increasing or decreasing the injection times can also improve the quality of profiles for samples that have too little or too much DNA respectively.

Overall, the Yfiler™ kit is a reliable method in resolving male DNA. The implementation of this kit at FTL will prove to be useful especially in dealing with complex mixtures where autosomal STR analysis may not be able to provide much information on male contributors. The additional six polymorphic loci included in this kit helps improve the power of discrimination, therefore it may be more informative than the PowerPlex® Y kit currently being used at the lab.

APPENDIX A

YFILER MIXTURE STUDY TABLES AND SELECT ELECTROPHEROGRAMS

Yfiler Reference Profiles of Mixture Study Samples

Locus	Donor 1	Donor 2	Donor 11
DYS456	16	16	
DYS389I	13	13	
DYS390	23	23	
DYS389II	28	29	
DYS458	17	18	
DYS19	14	14	
DYS385	11,14	11,15	
DYS393	13	13	
DYS391	11	11	
DYS439	11	12	
DYS635	23	25	
DYS392	13	13	
YGATAH4	12	13	
DYS437	15	15	
DYS438	11	11	
DYS448	19	19	

Comparison of Alleles Obtained in Male-Male Mixture Study and Corresponding Peak Height Ratios

MALE:MALE / Donor 1:Donor 2				
19 to 1 (Well A4)				
Locus	Alleles Called	PHR		
DYS456	16			
DYS389I	13			
DYS390	23			
DYS389II	28			
DYS458	17			
DYS19	14			
DYS385	11,14			
DYS393	13			
DYS391	11			
DYS439	11			
DYS635	23			
DYS392	13			
YGATAH4	12			
DYS437	15			
DYS438	11			
DYS448	19			

MALE:MALE / Donor 1:Donor 2					
19 to 1 (Well A5)					
Locus	Alleles Called	PHR			Avg
DYS456	16				
DYS389I	13				
DYS390	23				
DYS389II	28				
DYS458	17,18	0.0827			
DYS19	14				
DYS385	11,14				
DYS393	13				
DYS391	11				
DYS439	11				
DYS635	23				
DYS392	13				
YGATAH4	12,13	0.1038			
DYS437	15				
DYS438	11				
DYS448	19				

MALE:MALE / Donor 2:Donor 1				
1 to 19 (Well G4)				
Locus	Alleles Called	PHR		
DYS456	16			
DYS389I	13			
DYS390	23			
DYS389II	28,29	0.1407		
DYS458	17,18	0.2058		
DYS19	14			
DYS385	11,14,15	(14,15)		
		0.151		
DYS393	13			
DYS391	11			
DYS439	12			
DYS635	25			
DYS392	13			
YGATAH4	12,13	0.1553		
DYS437	15			
DYS438	11			
DYS448	19			

MALE:MALE / Donor 2:Donor 1					
1 to 19 (Well G5)					
Locus	Alleles Called	PHR			Avg
DYS456	16				
DYS389I	13				
DYS390	23				
DYS389II	28,29	0.1621			0.151
DYS458	17,18	0.1863			0.196
DYS19	14				
DYS385	11,14,15	(14,15)			
		0.1476			0.149
DYS393	13				
DYS391	11				
DYS439	11,12	0.1487			
DYS635	25				
DYS392	13,14	0.0559			
YGATAH4	12,13	0.1467			0.151
DYS437	15				
DYS438	11				
DYS448	19				

MALE:MALE / Donor 1: Donor 2				
9 to 1 (Well B4)				
Locus	Alleles Called	PHR		
DYS456	16			
DYS389I	13			
DYS390	23			
DYS389II	28,29	0.125		
DYS458	17,18	0.193		
DYS19	14			
DYS385	11,14,15	(14,15)		
		0.140		
DYS393	13			
DYS391	11			
DYS439	11			
DYS635	23,25	0.163		
DYS392	13			
YGATAH4	12,13	0.123		
DYS437	15			
DYS438	11			
DYS448	19			

MALE:MALE / Donor 1: Donor 2					
9 to 1 (Well B5)					
Locus	Alleles Called	PHR			Avg
DYS456	16				
DYS389I	13				
DYS390	23				
DYS389II	28,29	0.143			0.134
DYS458	17,18	0.088			0.140
DYS19	14				
DYS385	11,14,15	(14,15)			
		0.134			0.137
DYS393	13				
DYS391	11				
DYS439	11,12	0.079			
DYS635	23				
DYS392	13				
YGATAH4	12,13	0.168			0.146
DYS437	15				
DYS438	11				
DYS448	19				

MALE:MALE / Donor 2:Donor 1				
1 to 9 (Well F4)				
Locus	Alleles Called	PHR		
DYS456	16			
DYS389I	13			
DYS390	23			
DYS389II	28,29	0.290		
DYS458	17,18	0.196		
DYS19	14			
DYS385	11,14,15	(14,15)		
		0.196		
DYS393	13			
DYS391	11			
DYS439	11,12	0.160		
DYS635	23,25	0.144		
DYS392	13,14	0.058		
YGATAH4	12,13	0.245		
DYS437	15			
DYS438	11			
DYS448	19			

MALE:MALE / Donor 2:Donor 1					
1 to 9 (Well F5)					
Locus	Alleles Called	PHR			Avg
DYS456	16				
DYS389I	13				
DYS390	23				
DYS389II	28,29	0.344			0.317
DYS458	17,18	0.216			0.206
DYS19	14				
DYS385	11,14,15	(14,15)			
		0.201			0.199
DYS393	13				
DYS391	11				
DYS439	11,12	0.192			0.176
DYS635	23,25	0.122			0.133
DYS392	13,14	0.058			0.058
YGATAH4	12,13	0.177			0.211
DYS437	15				
DYS438	11				
DYS448	19				

MALE:MALE / Donor 1:Donor 2				
2 to 1 (Well C4)				
Locus	Alleles Called	PHR		
DYS456	16			
DYS389I	13			
DYS390	23			
DYS389II	28,29	0.630		
DYS458	17,18	0.617		
DYS19	14			
DYS385	11,14,15	(14,15)		
		0.641		
DYS393	13			
DYS391	11			
DYS439	11,12	0.526		
DYS635	23,25	0.724		
DYS392	13			
YGATAH4	12,13	0.377		
DYS437	15			
DYS438	11			
DYS448	19			

MALE:MALE / Donor 1:Donor 2					
2 to 1 (Well C5)					
Locus	Alleles Called	PHR			Avg
DYS456	16				
DYS389I	13				
DYS390	23				
DYS389II	28,29	0.489			0.56
DYS458	17,18	0.497			0.557
DYS19	14				
DYS385	11,14,15	(14,15)			
		0.500			0.571
DYS393	13				
DYS391	11				
DYS439	11,12	0.641			0.584
DYS635	23,25	0.537			0.631
DYS392	13				
YGATAH4	12,13	0.606			0.491
DYS437	15				
DYS438	11				
DYS448	19				

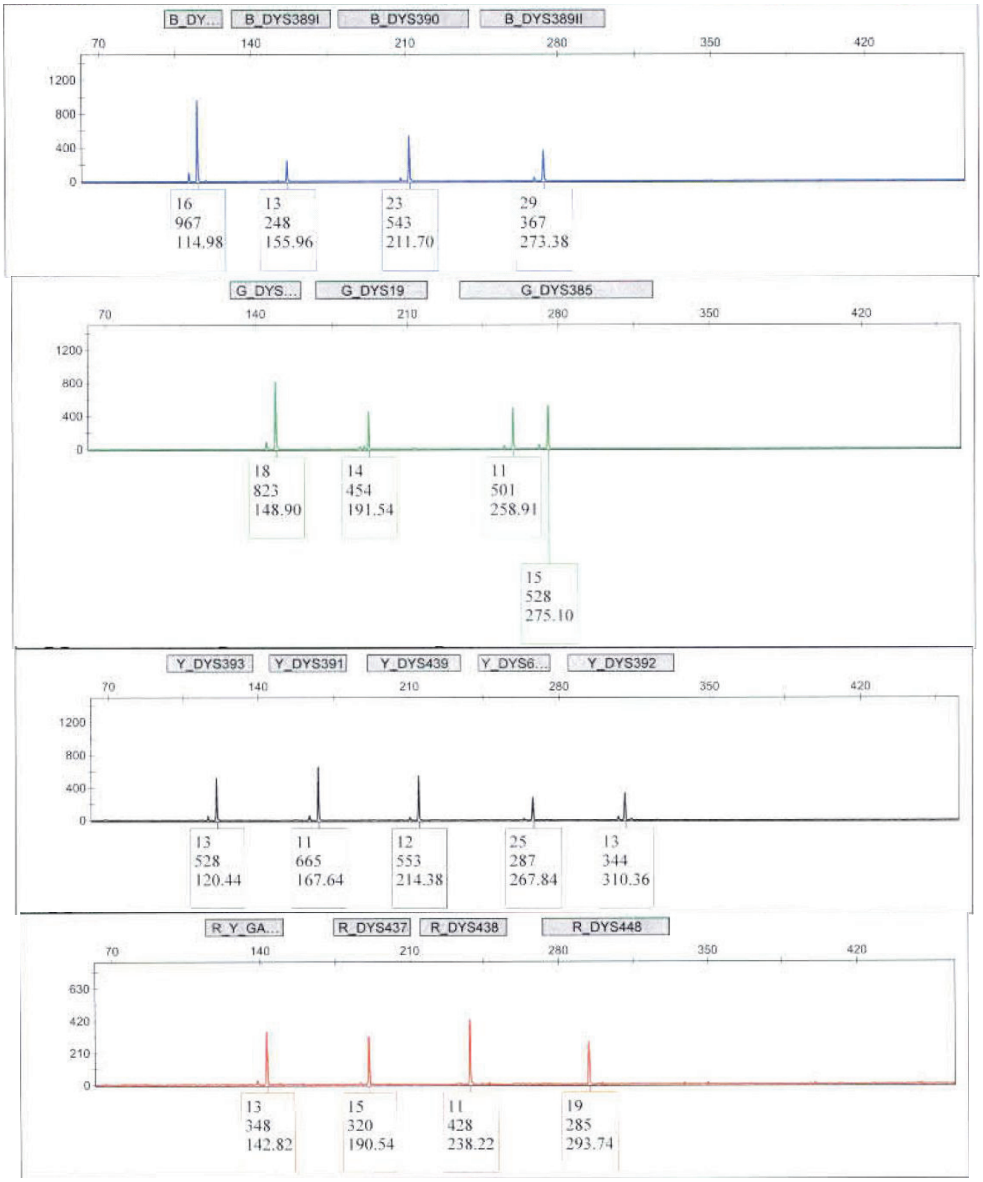
MALE:MALE / Donor 2:Donor 1				
1 to 2 (Well E4)				
Locus	Alleles Called	PHR		
DYS456	15,16	0.134		
DYS389I	13			
DYS390	23			
DYS389II	28,29	0.464		
DYS458	17,18			
		0.642		
DYS19	14			
DYS385	11,14,15	(14,15)		
		0.478		
DYS393	13			
DYS391	11			
DYS439	11,12	0.710		
DYS635	23,25	0.551		
DYS392	13			
YGATAH4	12,13	0.478		
DYS437	15			

MALE:MALE / Donor 2:Donor 1					
1 to 2 (Well E5)					
Locus	Alleles Called	PHR			Avg
DYS456	16				
DYS389I	13				
DYS390	23				
DYS389II	28,29	0.831			0.648
DYS458	16,17,18	(16,17)	(17,18)	(16,18)	
		0.124	0.735	0.091	0.689
DYS19	14				
DYS385	11,14,15	(14,15)			
		0.591			0.534
DYS393	13				
DYS391	11				
DYS439	11,12	0.75			0.73
DYS635	23,25	0.372			0.461
DYS392	13,14	0.060			
YGATAH4	12,13	0.787			0.633
DYS437	15				

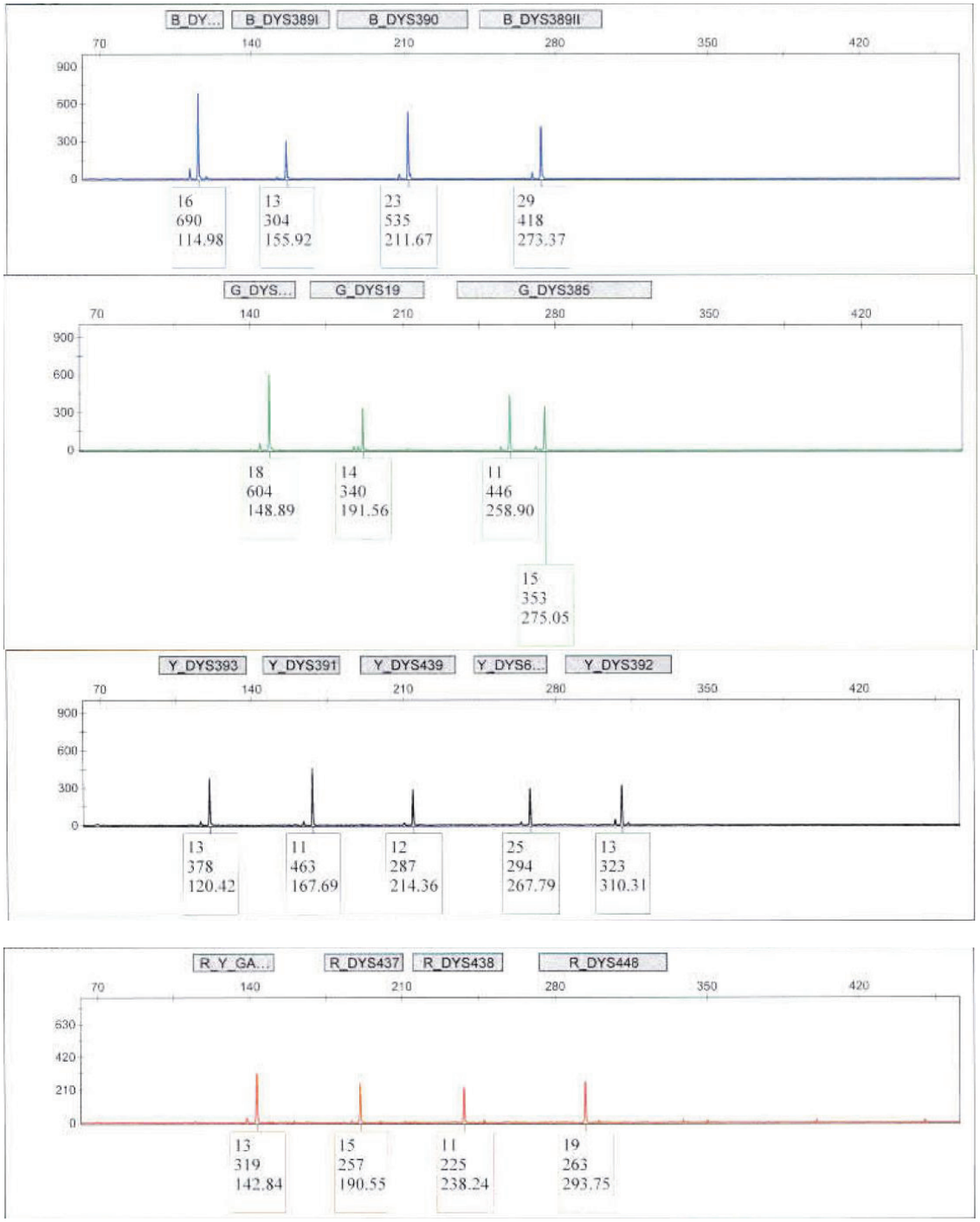
MALE:MALE / Donor 1: Donor 2
1 to 1 (Well D4)
BAD INJECTION

MALE:MALE / Donor 1: Donor 2				
1 to 1 (Well D5)				
Locus	Alleles Called	PHR		
DYS456	16			
DYS389I	13			
DYS390	23			
DYS389II	28,29	0.822		
DYS458	17,18	0.757		
DYS19	14			
DYS385	11,14,15	(14,15)		
		0.659		
DYS393	13			
DYS391	11			
DYS439	11,12	0.682		
DYS635	23,25	0.830		
DYS392	13			
YGATAH4	12,13	0.826		
DYS437	15			
DYS438	11			
DYS448	19			

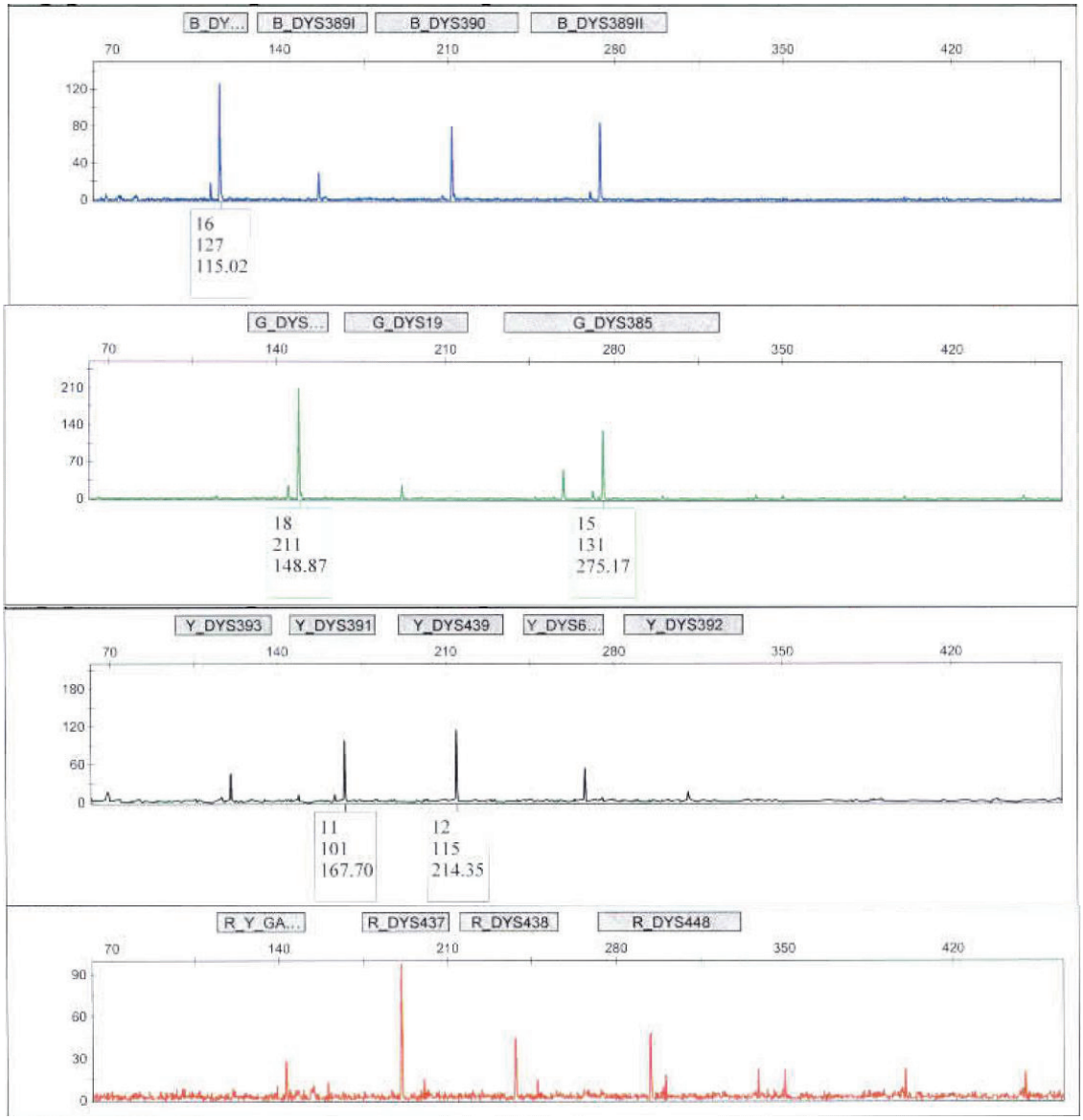
Gradually Increasing Female, Gradually Decreasing Male Mixture Study – 1:1 Ratio (0.375 ng male DNA)



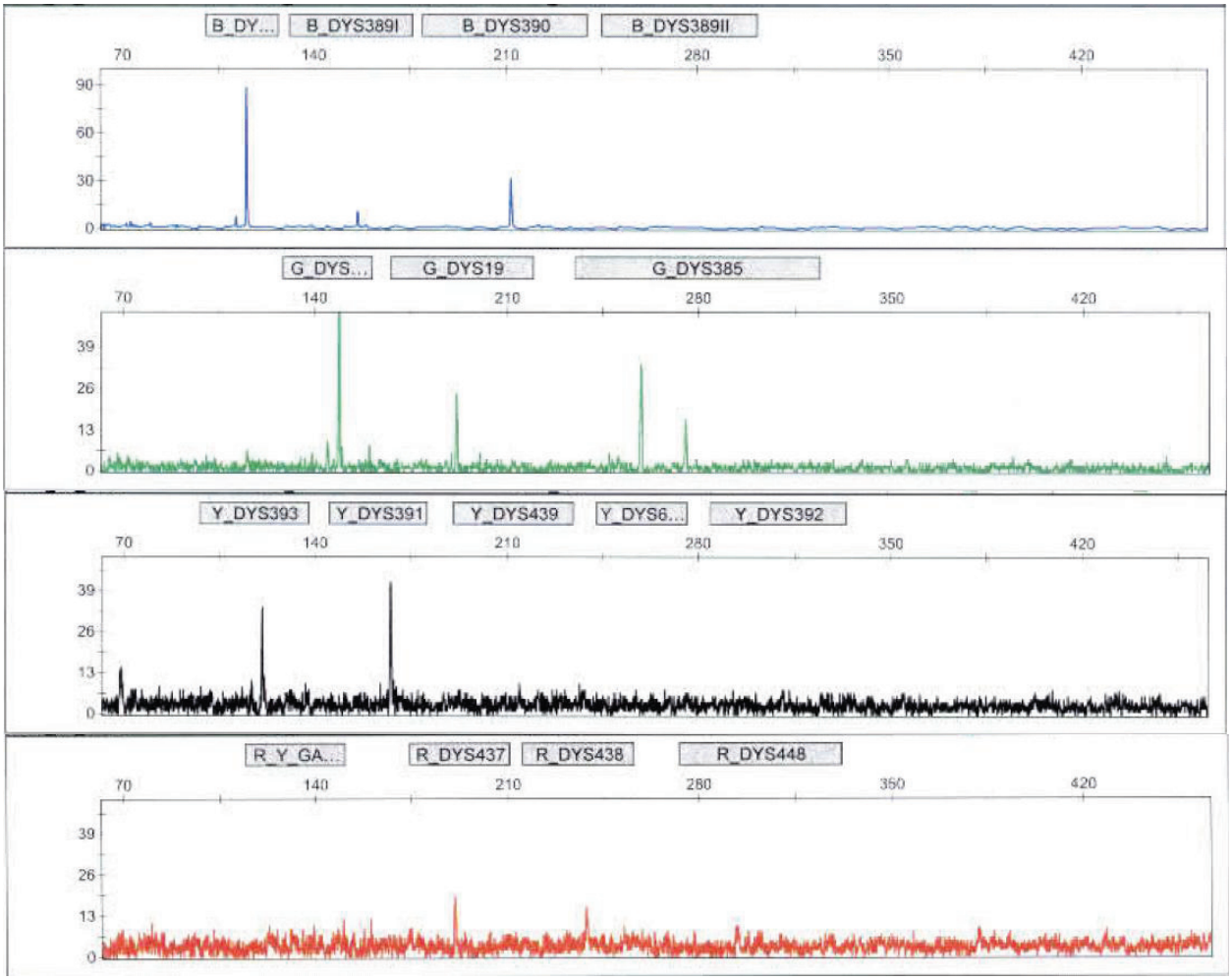
Gradually Increasing Female, Gradually Decreasing Male Mixture Study – 4:1 Ratio (0.15 ng male DNA)



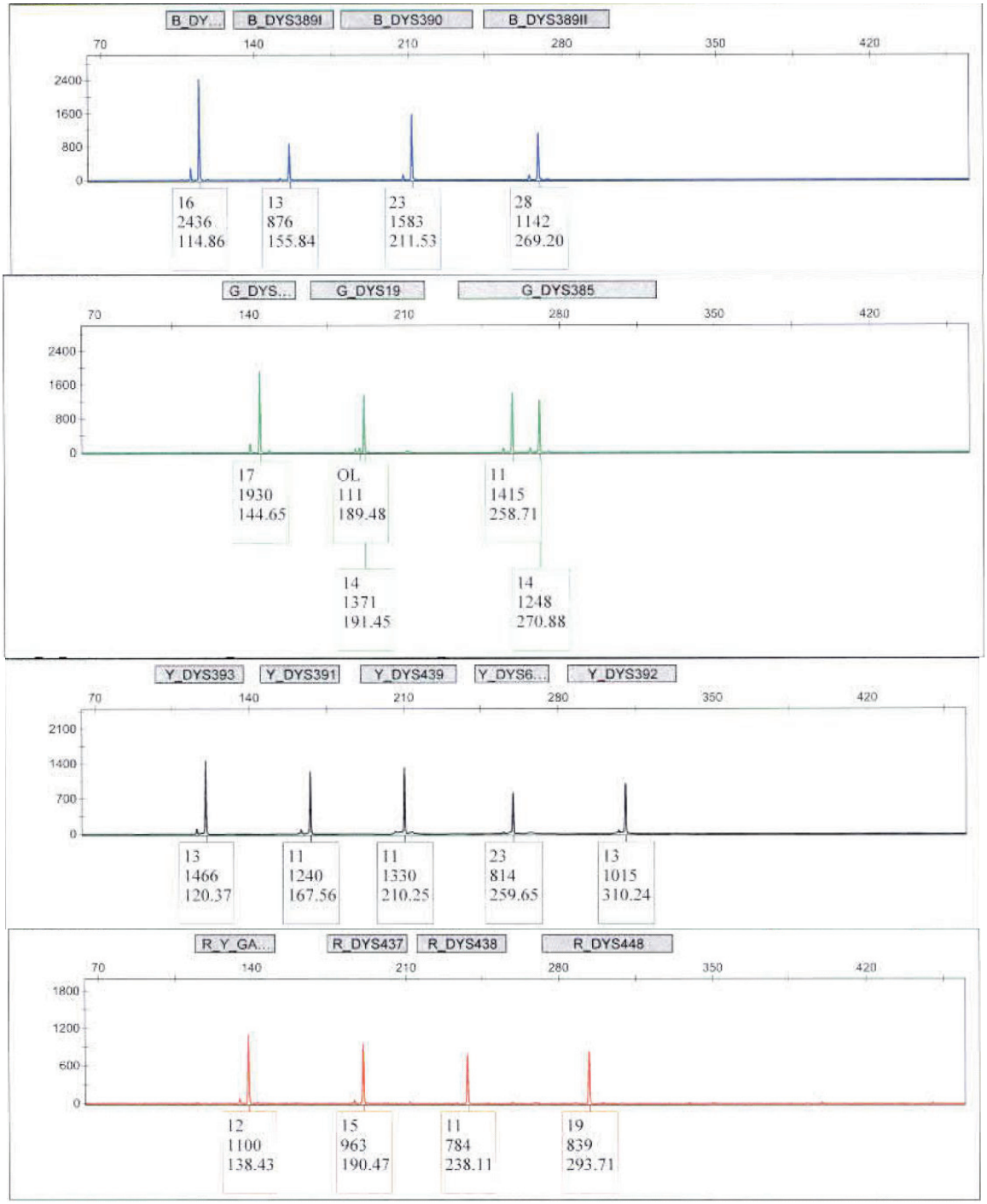
Gradually Increasing Female, Gradually Decreasing Male Mixture Study – 24:1 Ratio (0.03 ng male DNA)



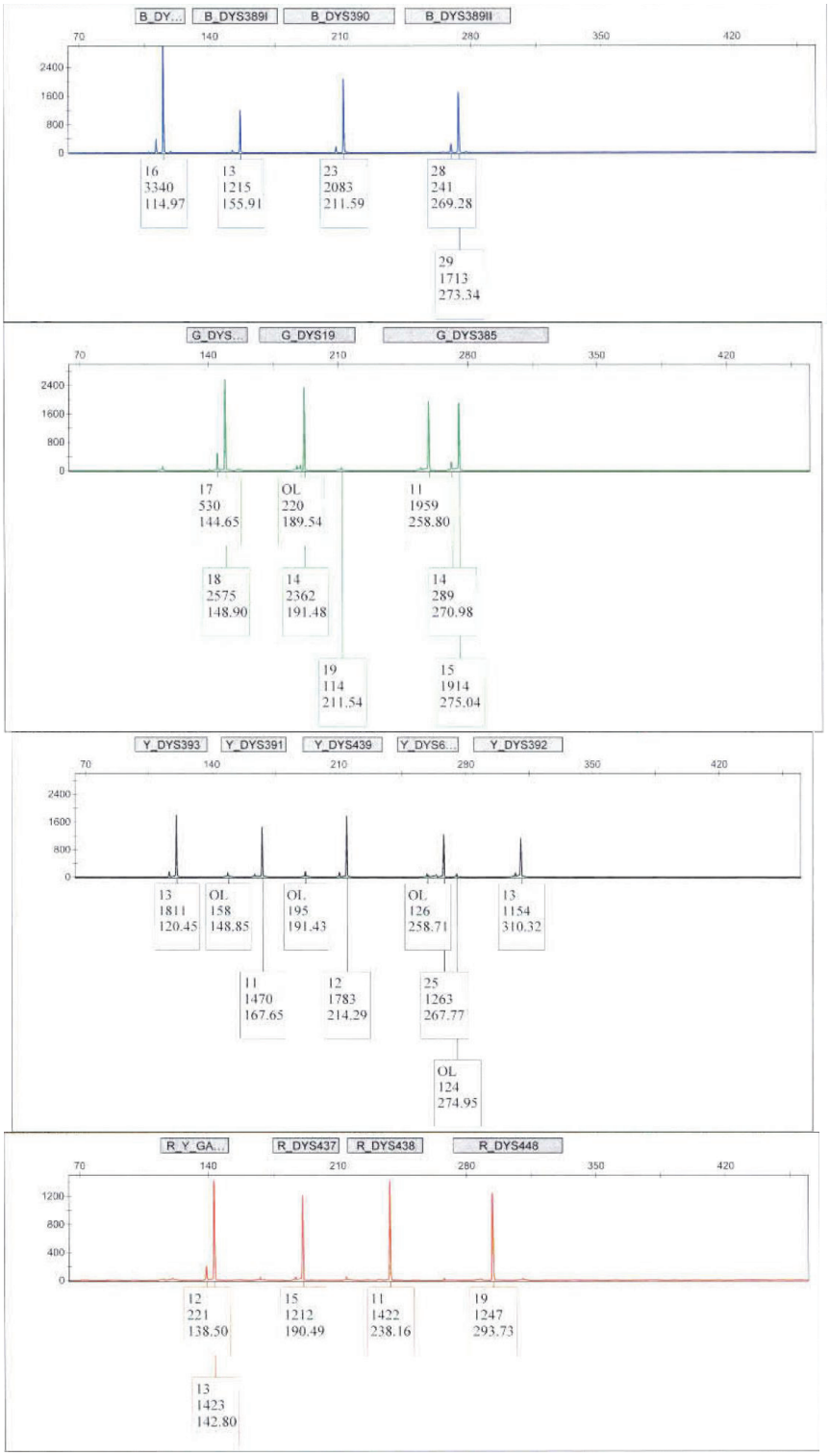
Gradually Increasing Female, Gradually Decreasing Male Mixture Study – 49:1 Ratio (0.015 ng male DNA)



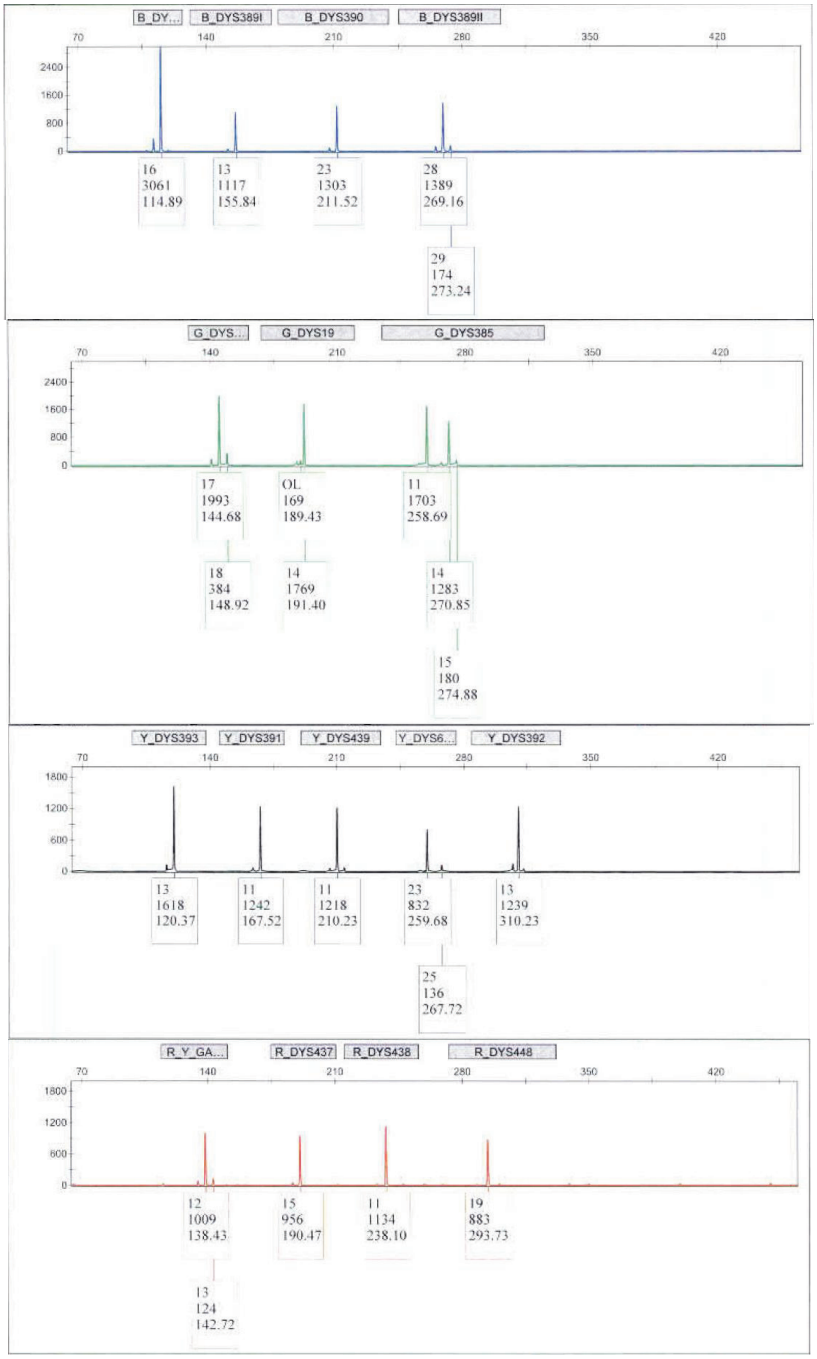
Major Profile from Donor 1 with Minor Alleles from Donor 2 (19:1 Ratio – 0.0375 ng DNA from Donor 2)



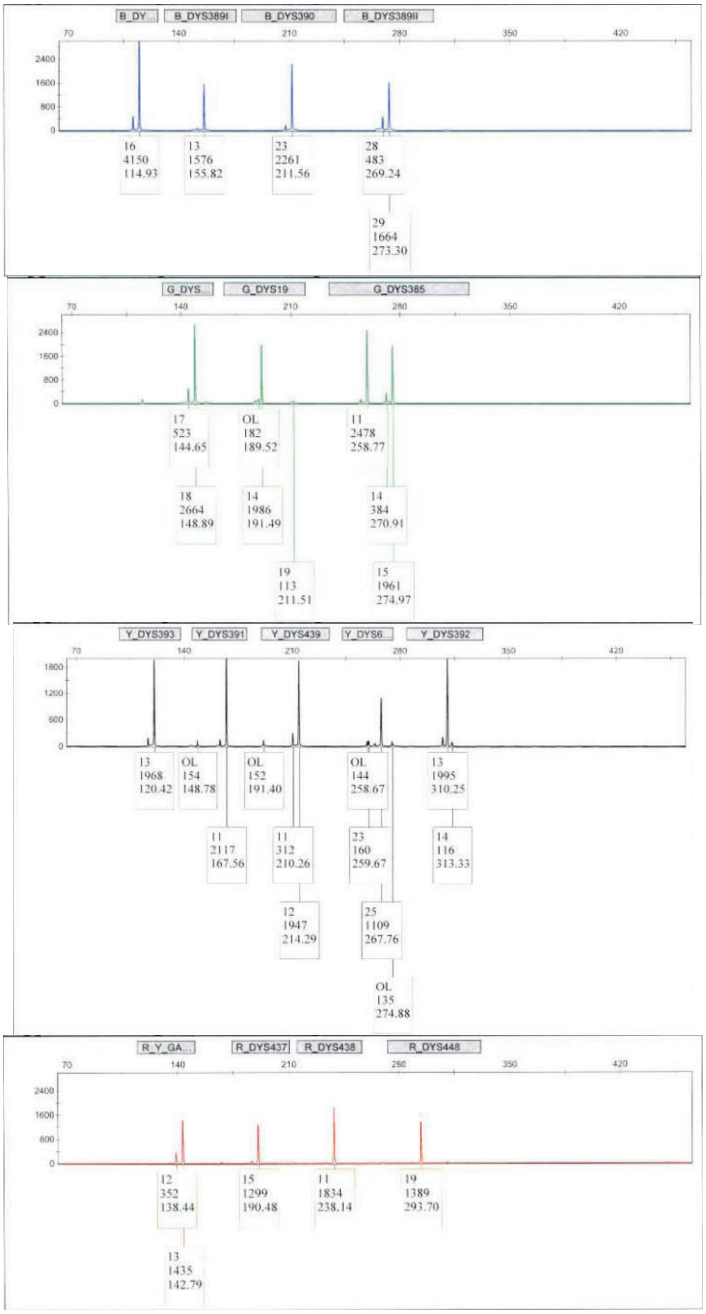
Major Profile from Donor 2 with Minor Alleles from Donor 1 (1:19 Ratio – 0.0375 ng DNA from Donor 1)



Major and Minor Alleles Observed at the 9:1 Ratio (0.075 ng DNA from Donor 2)



Major and Minor Alleles Observed at the 1:9 Ratio (0.075 ng DNA from Donor 1)



APPENDIX B

YFILER PRECISION STUDY TABLE

Y filer Precision Data Allelic Ladder

	Locus DYS456 Alleles					
	13	14	15	16	17	18
SIZE (BP)	103.23	107.15	111.04	115	118.94	122.9
	103.27	107.15	111.06	114.99	118.93	122.9
	103.23	107.12	111.02	114.95	118.89	122.91
	103.23	107.12	111.03	114.96	118.92	122.89
	103.21	107.13	111.02	114.93	118.91	122.87
	103.33	107.22	111.13	115.05	119	122.96
	103.29	107.22	111.12	115.05	119	122.97
	103.16	107.02	110.91	114.87	118.81	122.82
	103.13	107.02	110.93	114.87	118.78	122.77
	103.18	107.05	111	114.93	118.88	122.86
	103.19	107.07	110.97	114.95	118.89	122.87
	103.19	107.1	110.99	114.94	118.87	122.87
	103.18	107.08	110.96	114.9	118.87	122.82
	103.5	106.94	110.85	114.79	118.76	122.75
	103.07	106.97	110.84	114.79	118.77	122.77
	103.07	106.97	110.89	114.83	118.8	122.79
STD DEV	0.103207	0.084437	0.08579	0.08	0.075443	0.06598
AVG	0.082476					

	Locus DYS389I Alleles					
	10	11	12	13	14	15
SIZE (BP)	143.05	147.35	151.69	156.05	160.1	164.19
	143.05	147.35	151.72	156.06	160.14	164.2
	143	147.34	151.7	156.02	160.09	164.19
	143.05	147.35	151.67	156.06	160.09	164.2
	143.04	147.36	151.71	156.05	160.09	164.17
	143.08	147.43	151.76	156.1	160.14	164.2
	143.1	147.4	151.74	156.05	160.1	164.19
	142.9	147.28	151.61	155.89	159.9	163.98
	142.92	147.26	151.57	155.87	159.9	163.95
	142.97	147.3	151.66	155.99	160.05	164.11
	143	147.3	151.64	155.95	160	164.09
	143.03	147.35	151.65	156	160.05	164.09
	143	147.34	151.7	156.01	160.05	164.14
	142.88	147.22	151.54	155.88	159.9	163.97
	142.9	147.25	151.57	155.89	159.9	163.97
	142.9	147.22	151.53	155.87	159.95	164.01
STD DEV	0.071668	0.060759	0.071449	0.07999	0.089757	0.096521
AVG	0.078357					

	Locus DYS390 Alleles									
	18	19	20	21	22	23	24	25	26	27
SIZE (BP)	191.59	195.49	199.52	203.54	207.56	211.68	215.7	219.73	223.76	227.79
	191.65	195.49	199.55	203.53	207.57	211.7	215.69	219.73	223.76	227.75
	191.6	195.47	199.55	203.52	207.54	211.65	215.66	219.72	223.73	227.75
	191.62	195.5	199.55	203.53	207.55	211.67	215.7	219.72	223.75	227.77
	191.63	195.53	199.55	203.54	207.58	211.66	215.7	219.74	223.77	227.76
	191.71	195.61	199.6	203.53	207.56	211.61	215.66	219.62	223.68	227.71
	191.63	195.53	199.57	203.59	207.61	211.73	215.76	219.79	223.82	227.81
	191.41	195.32	199.36	203.33	207.4	211.52	215.54	219.57	223.6	227.57
	191.39	195.28	199.35	203.35	207.4	211.5	215.55	219.56	223.61	227.62
	191.54	195.43	199.46	203.47	207.48	211.6	215.63	219.65	223.73	227.72
	191.5	195.4	199.43	203.43	207.45	211.57	215.63	219.6	223.67	227.65
	191.53	195.42	199.49	203.47	207.51	211.64	215.63	219.67	223.71	227.7
	191.58	195.47	199.49	203.48	207.52	211.66	215.66	219.7	223.7	227.7
	191.43	195.28	199.32	203.34	207.37	211.46	215.5	219.54	223.59	227.6
	191.43	195.34	199.38	203.39	207.41	211.53	215.55	219.58	223.61	227.6
	191.45	195.33	199.38	203.37	207.37	211.53	215.53	219.55	223.61	227.59
STD DEV	0.098164	0.097943	0.090423	0.083962	0.081281	0.07981	0.075584	0.080744	0.072099	0.078036
AVG	0.083805									

	Locus DYS389II Alleles										
	24	25	26	27	28	29	30	31	32	33	34
SIZE (BP)	253.14	257.24	261.23	265.38	269.33	273.29	277.49	281.55	285.56	289.62	293.53
	253.17	257.24	261.26	265.38	269.36	273.33	277.54	281.6	285.58	289.59	293.57
	253.13	257.22	261.26	265.39	269.34	273.34	277.51	281.59	285.53	289.61	293.55
	253.14	257.2	261.27	265.38	269.35	273.32	277.52	281.58	285.55	289.6	293.53
	253.15	257.22	261.24	265.4	269.37	273.35	277.54	281.61	285.53	289.63	293.56
	253.01	257.13	261.16	265.29	269.24	273.19	277.39	281.5	285.47	289.59	293.58
	253.17	257.27	261.27	265.42	269.37	273.33	277.53	281.59	285.55	289.61	293.58
	252.93	257.02	261.06	265.15	269.14	273.09	277.29	281.34	285.34	289.39	293.34
	252.96	257.03	261.05	265.18	269.15	273.13	277.31	281.34	285.32	289.4	293.34
	253.05	257.16	261.18	265.3	269.27	273.24	277.42	281.45	285.44	289.53	293.47
	253.03	257.11	261.14	265.28	269.27	273.21	277.39	281.43	285.43	289.47	293.42
	253.08	257.17	261.17	265.31	269.31	273.26	277.45	281.5	285.5	289.55	293.45
	253.09	257.19	261.2	265.35	269.31	273.27	277.47	281.53	285.5	289.56	293.53
	252.95	257.03	261.07	265.21	269.15	273.1	277.36	281.36	285.37	289.39	293.36
	252.98	257.09	261.11	265.23	269.2	273.13	277.36	281.4	285.39	289.43	293.38
	252.96	257.05	261.05	265.21	269.17	273.13	277.35	281.42	285.39	289.42	293.35
STD DEV	0.085703	0.08416	0.082138	0.086631	0.084732	0.092824	0.084735	0.09659	0.084219	0.091504	0.093372
AVG	0.087873										

	Locus DYS458 Alleles						
	14	15	16	17	18	19	20
SIZE (BP)	132.34	136.34	140.39	144.59	148.84	153.06	157.1
	132.34	136.34	140.39	144.59	148.88	153.09	157.12
	132.33	136.32	140.42	144.56	148.89	153.1	157.12
	132.34	136.29	140.34	144.54	148.83	153.05	157.07
	132.32	136.3	140.38	144.57	148.89	153.12	157.11
	132.39	136.37	140.49	144.62	148.92	153.13	157.12
	132.38	136.38	140.44	144.59	148.89	153.11	157.1
	132.31	136.32	140.43	144.6	148.91	153.12	157.06
	132.32	136.3	140.38	144.58	148.91	153.08	157.05
	132.32	136.34	140.42	144.59	148.87	153.11	157.11
	132.33	136.33	140.39	144.59	148.89	153.06	157.1
	132.35	136.33	140.42	144.59	148.86	153.04	157.08
	132.3	136.28	140.37	144.57	148.91	153.09	157.08
	132.32	136.31	140.4	144.59	148.88	153.08	157.09
	132.29	136.29	140.39	144.6	148.89	153.09	157.09
	132.33	136.35	140.43	144.59	148.85	153.05	157.07
STD DEV	0.025876	0.028745	0.034641	0.018257	0.026133	0.028018	0.021975
AVG	0.026235						

	Locus DYS19 Alleles									
	10	11	12	13	14	15	16	17	18	19
SIZE (BP)	175.7	179.69	183.62	187.59	191.55	195.49	199.47	203.44	207.46	211.44
	175.72	179.72	183.65	187.61	191.56	195.54	199.5	203.44	207.48	211.47
	175.73	179.71	183.63	187.62	191.56	195.52	199.51	203.43	207.45	211.47
	175.71	179.7	183.63	187.63	191.57	195.5	199.5	203.48	207.46	211.44
	175.69	179.7	183.64	187.62	191.58	195.53	199.5	203.5	207.49	211.48
	175.74	179.72	183.65	187.66	191.62	195.61	199.51	203.44	207.43	211.38
	175.71	179.71	183.65	187.62	191.58	195.58	199.52	203.49	207.51	211.54
	175.5	179.49	183.42	187.45	191.41	195.36	199.31	203.33	207.35	211.32
	175.51	179.48	183.44	187.44	191.39	195.38	199.35	203.35	207.35	211.35
	175.65	179.62	183.54	187.55	191.49	195.48	199.41	203.42	207.38	211.4
	175.65	179.6	183.58	187.55	191.5	195.45	199.38	203.39	207.4	211.37
	175.61	179.6	183.57	187.54	191.49	195.47	199.44	203.43	207.42	211.41
	175.65	179.64	183.57	187.58	191.53	195.47	199.44	203.43	207.43	211.42
	175.54	179.52	183.45	187.47	191.43	195.38	199.32	203.29	207.32	211.31
	175.54	179.53	183.45	187.47	191.43	195.38	199.38	203.34	207.36	211.38
	175.6	179.57	183.52	187.52	191.45	195.42	199.33	203.32	207.33	211.33
STD DEV	0.081525	0.086333	0.083564	0.07038	0.070321	0.074521	0.075584	0.063823	0.059875	0.064469
AVG	0.073039									

	Locus DYS385 Alleles																		
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SIZE (BP)	242.72	246.81	250.85	254.9	258.94	262.94	267.04	271.09	275.15	279.2	283.26	287.27	291.38	295.4	299.46	303.51	307.51	311.61	315.71
	242.73	246.81	250.88	254.91	258.93	262.96	267.07	271.09	275.16	279.18	283.25	287.27	291.37	295.39	299.45	303.5	307.55	311.59	315.69
	242.69	246.79	250.83	254.88	258.92	262.97	267.01	271.09	275.13	279.21	283.25	287.24	291.36	295.39	299.46	303.48	307.54	311.65	315.71
	242.72	246.83	250.85	254.87	258.94	263	267.06	271.08	275.14	279.2	283.26	287.23	291.38	295.4	299.41	303.5	307.55	311.6	315.7
	242.75	246.82	250.84	254.91	258.94	262.96	267.07	271.09	275.15	279.17	283.27	287.24	291.39	295.4	299.46	303.5	307.51	311.6	315.7
	242.61	246.66	250.72	254.74	258.81	262.8	266.88	270.98	275.03	279.09	283.15	287.18	291.35	295.34	299.48	303.53	307.58	311.69	315.75
	242.79	246.88	250.88	254.93	258.97	262.97	267.08	271.13	275.18	279.19	283.25	287.27	291.38	295.39	299.46	303.51	307.56	311.62	315.73
	242.51	246.59	250.63	254.67	258.71	262.7	266.8	270.84	274.89	278.94	282.99	286.99	291.09	295.14	299.2	303.23	307.27	311.35	315.44
	242.52	246.63	250.65	254.67	258.69	262.71	266.79	270.86	274.89	278.92	283	286.98	291.07	295.1	299.19	303.21	307.23	311.3	315.43
	242.64	246.74	250.75	254.81	258.82	262.88	266.95	270.98	275.05	279.08	283.12	287.16	291.25	295.29	299.34	303.37	307.41	311.5	315.6
	242.59	246.72	250.7	254.78	258.81	262.85	266.88	270.97	275.01	279.05	283.14	287.13	291.22	295.22	299.27	303.34	307.37	311.46	315.54
	242.65	246.74	250.78	254.82	258.87	262.91	266.96	271.05	275.1	279.1	283.19	287.19	291.29	295.34	299.39	303.43	307.47	311.56	315.61
	242.68	246.77	250.78	254.83	258.89	262.94	267	271.06	275.11	279.17	283.23	287.2	291.35	295.37	299.43	303.48	307.49	311.6	315.66
	242.51	246.63	250.66	254.69	258.72	262.76	266.85	270.9	274.95	279.01	283.07	287.08	291.2	295.22	299.24	303.3	307.36	311.42	315.49
	242.57	246.67	250.68	254.74	258.75	262.77	266.89	270.92	274.95	279.03	283.07	287.06	291.16	295.21	299.26	303.29	307.34	311.44	315.49
	242.54	246.62	250.67	254.71	258.76	262.76	266.87	270.93	274.95	279.01	283.08	287.06	291.19	295.22	299.26	303.33	307.36	311.44	315.53
STD DEV	0.091715	0.089086	0.087899	0.091351	0.094954	0.102794	0.1	0.093014	0.100896	0.096036	0.097082	0.098486	0.109527	0.101776	0.105893	0.109710	0.110386	0.113415	0.109719
AVG	0.100197																		

	Locus DYS393 Alleles								
	8	9	10	11	12	13	14	15	16
SIZE (BP)	100.47	104.47	108.44	112.53	116.41	120.45	124.47	128.47	132.69
	100.51	104.47	108.41	112.55	116.44	120.44	124.47	128.47	132.63
	100.45	104.46	108.4	112.5	116.43	120.43	124.5	128.45	132.66
	100.46	104.48	108.42	112.53	116.42	120.43	124.46	128.46	132.63
	100.46	104.45	108.38	112.51	116.38	120.42	124.47	128.46	132.66
	100.58	104.55	108.49	112.59	116.47	120.47	124.53	128.52	132.71
	100.52	104.53	108.47	112.58	116.46	120.47	124.55	128.55	132.73
	100.39	104.38	108.3	112.39	116.31	120.31	124.39	128.39	132.57
	100.34	104.36	108.25	112.37	116.27	120.29	124.34	128.37	132.53
	100.43	104.39	108.37	112.47	116.36	120.37	124.41	128.43	132.62
	100.43	104.44	108.37	112.47	116.41	120.41	124.44	128.45	132.63
	100.42	104.46	108.38	112.51	116.38	120.41	124.46	128.45	132.65
	100.47	104.45	108.36	112.48	116.38	120.36	124.42	128.44	132.6
	100.33	104.35	108.29	112.41	116.31	120.28	124.38	128.36	132.57
	100.38	104.35	108.26	112.43	116.29	120.32	124.38	128.37	132.54
	100.38	104.35	108.3	112.47	116.33	120.35	124.45	128.42	132.57
STD DEV	0.06632	0.063653	0.071761	0.06395	0.06156	0.063269	0.056569	0.05201	0.058648
AVG	0.061971								

	Locus DYS391 Alleles						
	7	8	9	10	11	12	13
SIZE (BP)	151.23	155.39	159.55	163.65	167.72	171.74	175.79
	151.23	155.38	159.57	163.64	167.73	171.76	175.77
	151.21	155.4	159.58	163.63	167.71	171.73	175.77
	151.23	155.43	159.57	163.63	167.72	171.75	175.8
	151.22	155.38	159.57	163.65	167.71	171.75	175.78
	151.29	155.44	159.63	163.7	167.8	171.79	175.83
	151.28	155.44	159.6	163.65	167.73	171.75	175.81
	151.15	155.28	159.4	163.47	167.49	171.55	175.6
	151.15	155.25	159.39	163.45	167.49	171.53	175.56
	151.2	155.37	159.5	163.56	167.62	171.66	175.7
	151.18	155.34	159.5	163.55	167.63	171.7	175.7
	151.2	155.36	159.52	163.56	167.64	171.7	175.7
	151.25	155.37	159.56	163.61	167.64	171.7	175.7
	151.13	155.27	159.4	163.48	167.54	171.54	175.59
	151.12	155.24	159.41	163.48	167.49	171.55	175.59
	151.12	155.26	159.46	163.53	167.58	171.62	175.65
STD DEV	0.053724	0.068799	0.07897	0.078188	0.098658	0.089508	0.088835
AVG	0.079526						

	Locus DYS439 Alleles							
	8	9	10	11	12	13	14	15
SIZE (BP)	198.32	202.32	206.35	210.27	214.3	218.32	222.35	226.24
	198.36	202.34	206.38	210.28	214.32	218.31	222.39	226.24
	198.34	202.3	206.37	210.29	214.31	218.32	222.38	226.21
	198.32	202.29	206.36	210.25	214.28	218.31	222.33	226.22
	198.38	202.32	206.36	210.3	214.34	218.33	222.41	226.22
	198.39	202.34	206.37	210.23	214.28	218.28	222.25	226.18
	198.37	202.37	206.4	210.33	214.35	218.38	222.46	226.3
	198.23	202.18	206.25	210.18	214.2	218.23	222.25	226.13
	198.26	202.2	206.3	210.2	214.25	218.26	222.31	226.17
	198.28	202.28	206.34	210.21	214.28	218.31	222.34	226.22
	198.28	202.23	206.29	210.21	214.23	218.25	222.32	226.15
	198.27	202.25	206.34	210.23	214.22	218.26	222.3	226.15
	198.27	202.3	206.35	210.25	214.25	218.24	222.34	226.2
	198.2	202.21	206.24	210.18	214.22	218.21	222.26	226.16
	198.27	202.23	206.29	210.22	214.24	218.27	222.3	226.19
	198.24	202.22	206.27	210.17	214.23	218.24	222.26	226.13
STD DEV	0.05714	0.057489	0.0487	0.046833	0.045735	0.04524	0.060467	0.046039
AVG	0.050955							

	Locus DYS635 Alleles						
	20	21	22	23	24	25	26
SIZE (BP)	247.73	251.83	255.82	259.82	263.77	267.87	271.82
	247.77	251.84	255.82	259.85	263.78	267.89	271.87
	247.78	251.82	255.82	259.82	263.82	267.91	271.86
	247.75	251.81	255.79	259.81	263.73	267.89	271.86
	247.77	251.84	255.82	259.84	263.82	267.93	271.86
	247.64	251.65	255.72	259.7	263.69	267.82	271.78
	247.81	251.85	255.85	259.9	263.85	267.95	271.91
	247.69	251.73	255.72	259.76	263.7	267.8	271.74
	247.69	251.75	255.72	259.8	263.72	267.84	271.77
	247.69	251.8	255.76	259.77	263.74	267.86	271.78
	247.69	251.77	255.75	259.78	263.77	267.86	271.8
	247.72	251.77	255.76	259.81	263.71	267.85	271.8
	247.72	251.77	255.78	259.78	263.74	267.85	271.81
	247.67	251.75	255.73	259.77	263.71	267.85	271.75
	247.69	251.75	255.75	259.78	263.71	267.83	271.81
	247.65	251.74	255.74	259.74	263.69	267.8	271.76
STD DEV	0.048836	0.052595	0.043084	0.0469	0.049896	0.042973	0.048836
AVG	0.047589						

	Locus DYS392 Alleles											
	7	8	9	10	11	12	13	14	15	16	17	18
SIZE (BP)	292.31	295.25	298.24	301.14	304.3	307.36	310.32	313.24	316.35	319.42	322.38	325.5
	292.38	295.3	298.27	301.15	304.28	307.41	310.31	313.29	316.33	319.41	322.4	325.52
	292.34	295.3	298.25	301.17	304.29	307.41	310.34	313.32	316.39	319.46	322.48	325.54
	292.29	295.26	298.18	301.11	304.28	307.37	310.31	313.26	316.35	319.43	322.42	325.51
	292.34	295.27	298.24	301.14	304.28	307.37	310.33	313.29	316.38	319.39	322.39	325.53
	292.35	295.24	298.29	301.29	304.39	307.44	310.44	313.31	316.37	319.47	322.44	325.55
	292.36	295.3	298.28	301.19	304.35	307.42	310.38	313.3	316.42	319.49	322.46	325.57
	292.24	295.19	298.15	301.06	304.19	307.27	310.19	313.17	316.25	319.33	322.31	325.44
	292.23	295.15	298.13	301.07	304.17	307.28	310.23	313.18	316.24	319.3	322.3	325.41
	292.31	295.19	298.18	301.12	304.24	307.31	310.27	313.19	316.31	319.33	322.36	325.43
	292.25	295.17	298.15	301.08	304.18	307.28	310.23	313.18	316.28	319.33	322.33	325.43
	292.28	295.24	298.21	301.09	304.23	307.33	310.28	313.23	316.33	319.38	322.38	325.47
	292.3	295.28	298.2	301.15	304.25	307.35	310.31	313.27	316.33	319.38	322.4	325.5
	292.25	295.17	298.14	301.06	304.21	307.31	310.25	313.2	316.25	319.3	322.31	325.42
	292.24	295.16	298.12	301.05	304.19	307.29	310.24	313.19	316.24	319.29	322.35	325.41
	292.22	295.17	298.13	301.09	304.23	307.26	310.25	313.19	316.28	319.32	322.31	325.46
STD DEV	0.051084	0.055076	0.058138	0.06159	0.061739	0.058523	0.063298	0.053194	0.05714	0.065086	0.05608	0.053475
AVG	0.057869											

	Locus YGATAH4 Alleles					
	8	9	10	11	12	13
SIZE (BP)	122.26	126.29	130.35	134.39	138.5	142.84
	122.24	126.27	130.33	134.41	138.52	142.85
	122.25	126.29	130.29	134.42	138.47	142.8
	122.23	126.26	130.28	134.36	138.47	142.8
	122.25	126.27	130.32	134.38	138.52	142.84
	122.27	126.34	130.43	134.49	138.58	142.93
	122.28	126.32	130.39	134.43	138.55	142.89
	122.17	126.15	130.22	134.31	138.43	142.79
	122.16	126.17	130.21	134.33	138.43	142.76
	122.21	126.21	130.29	134.35	138.49	142.81
	122.23	126.27	130.29	134.38	138.5	142.78
	122.24	126.26	130.3	134.37	138.51	142.82
	122.19	126.21	130.3	134.36	138.46	142.79
	122.15	126.17	130.21	134.34	138.44	142.77
	122.13	126.2	130.26	134.34	138.45	142.79
	122.2	126.21	130.3	134.36	138.45	142.8
STD DEV	0.045295	0.055943	0.059802	0.044553	0.043508	0.044852
AVG	0.048992					

	Locus DYS437 Alleles				
	13	14	15	16	17
SIZE (BP)	182.51	186.53	190.53	194.48	198.47
	182.54	186.56	190.55	194.53	198.5
	182.54	186.53	190.56	194.53	198.43
	182.52	186.53	190.52	194.5	198.46
	182.54	186.53	190.54	194.53	198.47
	182.57	186.58	190.67	194.63	198.57
	182.58	186.55	190.57	194.57	198.51
	182.38	186.36	190.42	194.43	198.37
	182.39	186.39	190.44	194.43	198.36
	182.5	186.46	190.51	194.5	198.43
	182.46	186.43	190.49	194.48	198.42
	182.44	186.45	190.5	194.49	198.41
	182.48	186.5	190.54	194.49	198.41
	182.37	186.39	190.45	194.4	198.35
	182.39	186.41	190.47	194.42	198.37
	182.46	186.41	190.44	194.42	198.38
STD DEV	0.069519	0.07099	0.062769	0.061261	0.06156
AVG	0.06522				

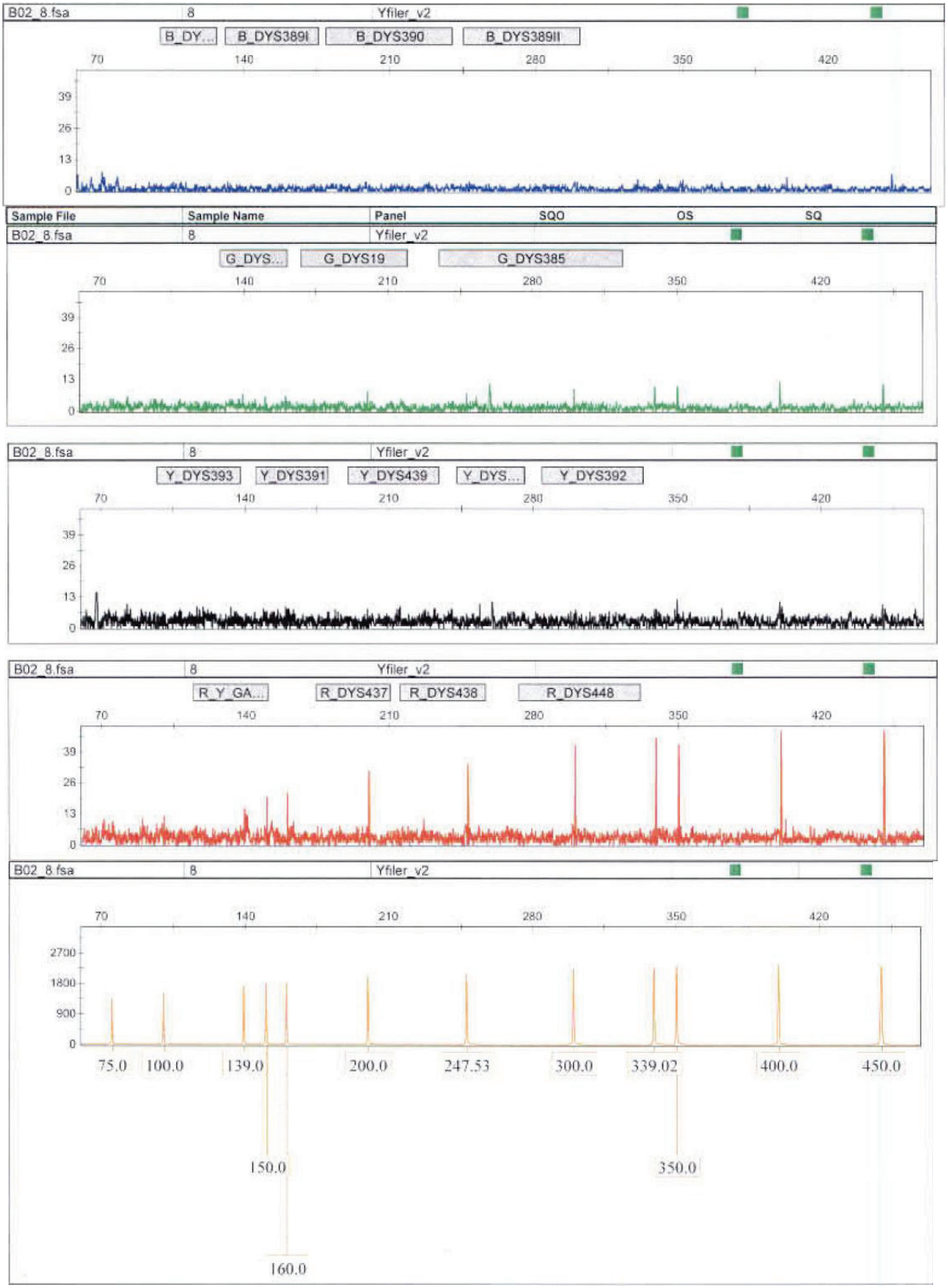
	Locus DYS438 Alleles					
	8	9	10	11	12	13
SIZE (BP)	223.18	228.18	233.19	238.2	243.21	248.27
	223.17	228.16	233.16	238.2	243.24	248.27
	223.15	228.15	233.15	238.15	243.19	248.23
	223.15	228.14	233.17	238.15	243.22	248.25
	223.18	228.17	233.19	238.17	243.2	248.27
	223.08	228.08	233.08	238.14	243.12	248.15
	223.24	228.25	233.25	238.27	243.28	248.35
	223.1	228.07	233.1	238.08	243.11	248.14
	223.11	228.12	233.14	238.15	243.17	248.19
	223.14	228.12	233.15	238.14	243.19	248.24
	223.09	228.09	233.13	238.17	243.17	248.22
	223.15	228.13	233.15	238.14	243.16	248.19
	223.14	228.13	233.16	238.15	243.19	248.24
	223.05	228.04	233.09	238.09	243.1	248.12
	223.08	228.09	233.06	238.08	243.1	248.13
	223.03	228.02	233.07	238.07	243.12	248.13
STD DEV	0.054345	0.056789	0.050465	0.051732	0.052627	0.065546
AVG	0.055251					

	Locus DYS448 Alleles							
	17	18	19	20	21	22	23	24
SIZE (BP)	281.74	287.71	293.73	299.76	305.73	311.8	317.78	323.77
	281.74	287.72	293.79	299.77	305.75	311.82	317.8	323.82
	281.81	287.77	293.78	299.82	305.83	311.88	317.88	323.88
	281.71	287.69	293.71	299.77	305.71	311.83	317.77	323.76
	281.74	287.69	293.74	299.77	305.78	311.83	317.79	323.8
	281.69	287.7	293.77	299.86	305.82	311.92	317.9	323.82
	281.79	287.75	293.83	299.8	305.78	311.92	317.85	323.84
	281.74	287.74	293.74	299.8	305.75	311.86	317.82	323.82
	281.69	287.74	293.74	299.8	305.75	311.81	317.82	323.78
	281.71	287.71	293.78	299.75	305.72	311.81	317.8	323.79
	281.68	287.72	293.71	299.76	305.7	311.8	317.75	323.75
	281.69	287.71	293.74	299.76	305.71	311.8	317.76	323.76
	281.72	287.72	293.76	299.76	305.73	311.84	317.81	323.78
	281.76	287.73	293.81	299.8	305.78	311.88	317.83	323.84
	281.74	287.75	293.77	299.8	305.79	311.84	317.84	323.8
	281.71	287.75	293.75	299.75	305.77	311.84	317.82	323.81
STD DEV	0.036492	0.023381	0.03356	0.030049	0.038966	0.039581	0.040967	0.035
AVG	0.034749							

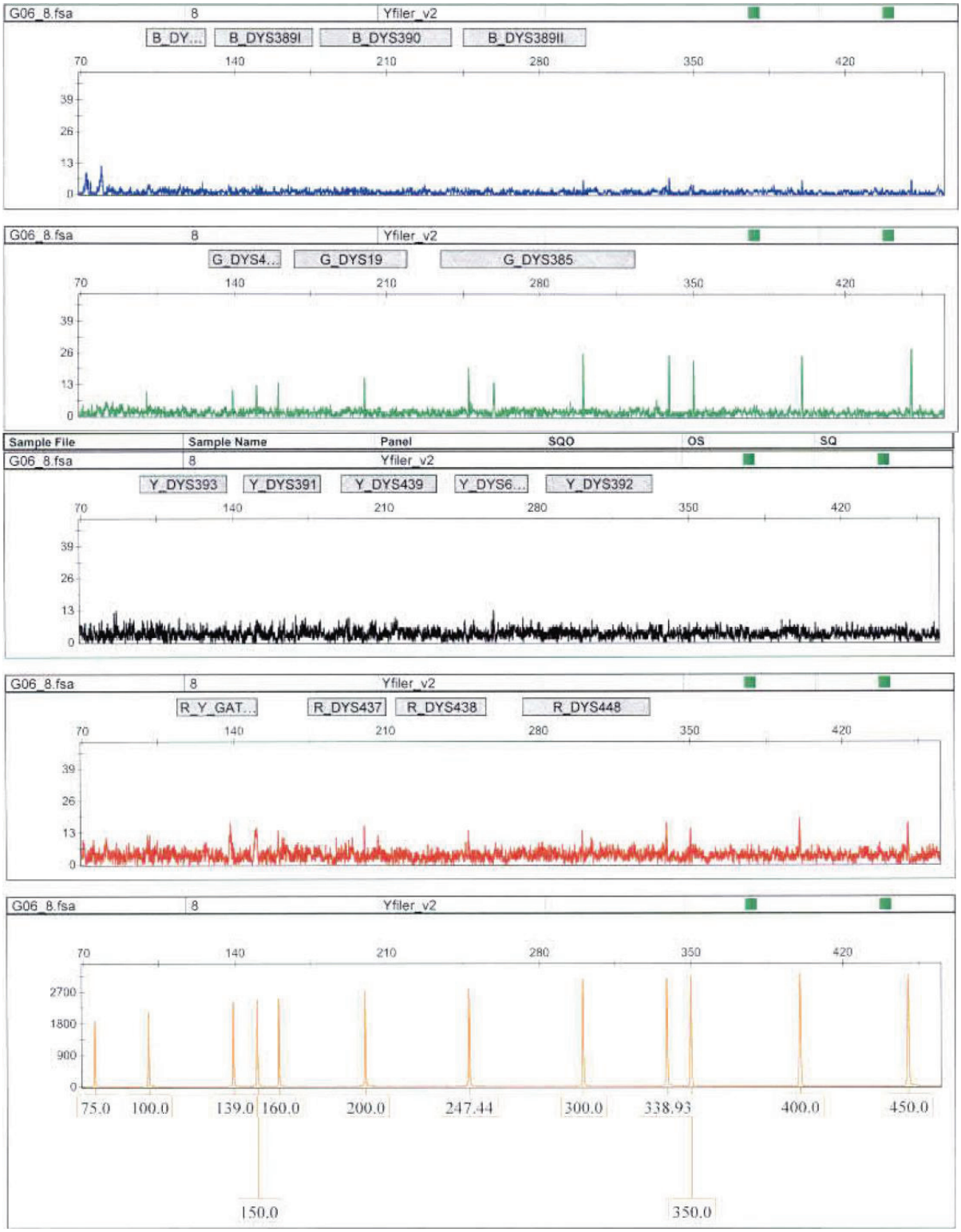
APPENDIX C

YFILER CASE TYPE SAMPLES STUDY EXAMPLE ELECTROPHEROGRAMS

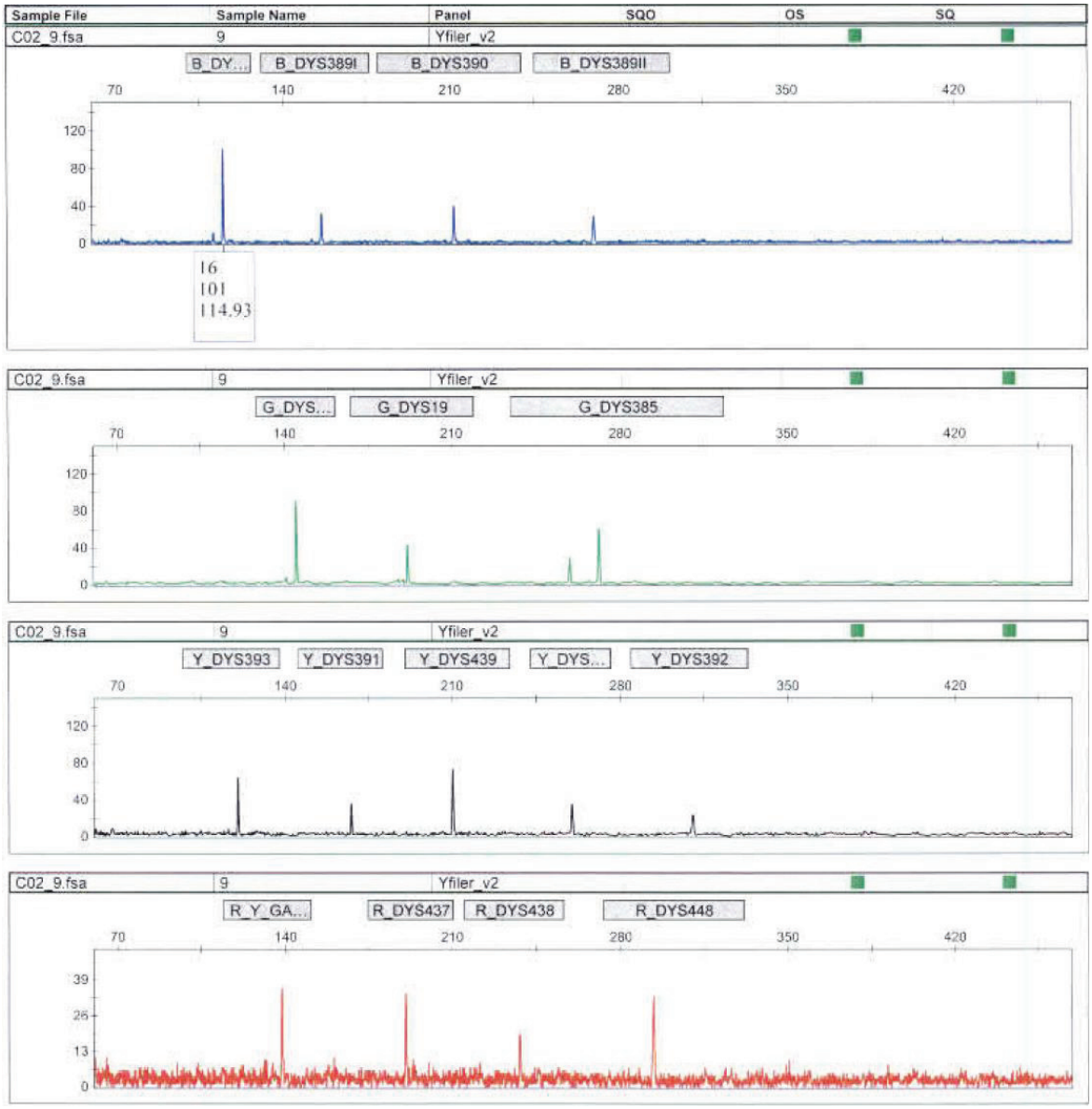
Sample Eight – 5 Second Injection Time, 3kV Injection Voltage, 2800 Second Run Time



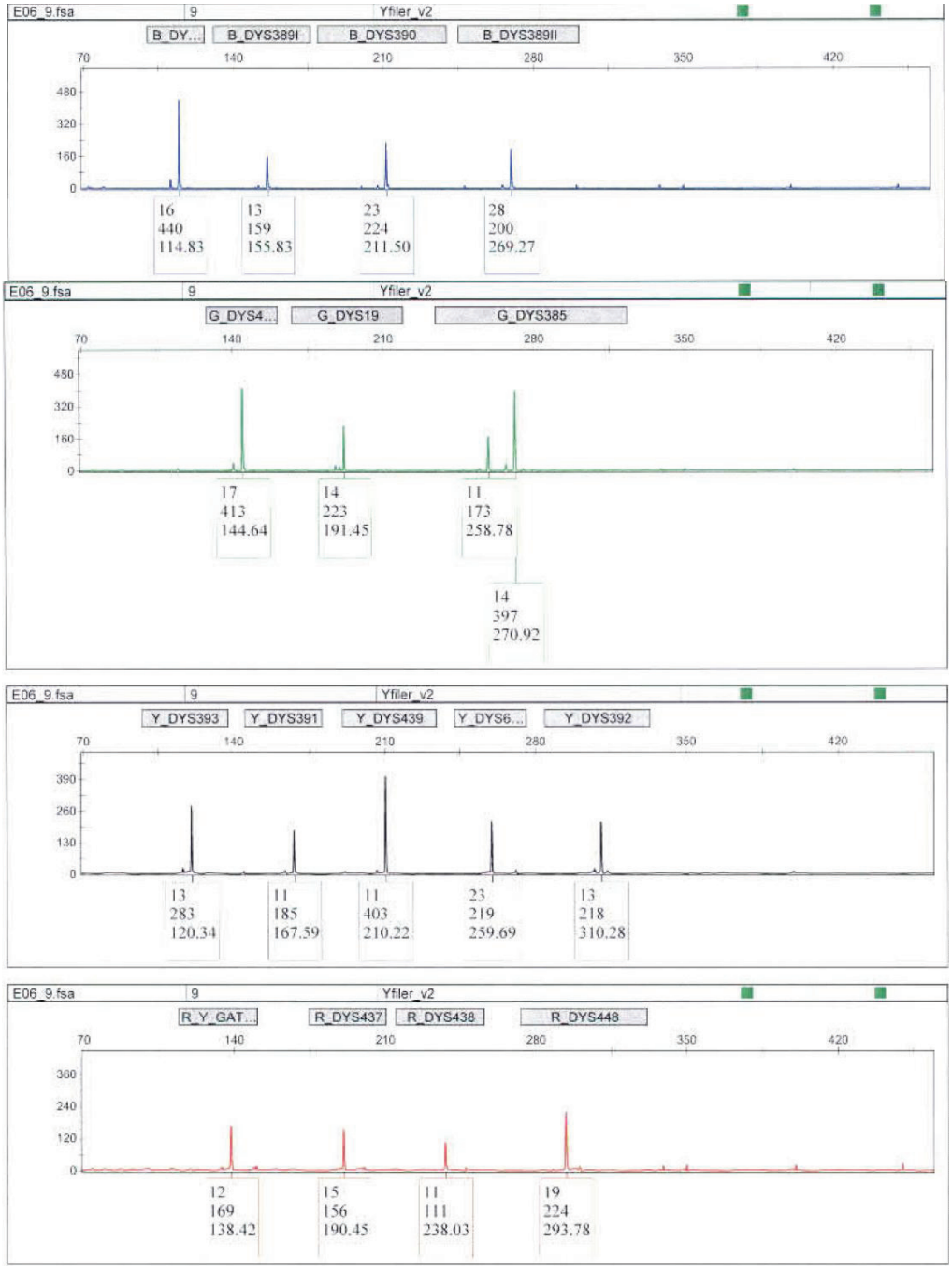
Sample Eight – 8 Second Injection Time, 3kV Injection Voltage, 2800 Second Run Time



Sample Nine – 5 Second Injection Time, 3kV Injection Voltage, 2800 Second Run Time



Sample Nine – 8 Second Injection Time, 3kV Injection Voltage, 2800 Second Run Time



APPENDIX D

YFILER CONTAMINATION STUDY TABLE

Sample Name	Marker	Height 1	Height 2	Height 3	Height 4	Height 5	Height 6	Height 7	Height 8	Height 9
NC	B_DYS456									
NC	B_DYS389I									
NC	B_DYS390									
NC	B_DYS389II									
NC	G_DYS458									
NC	G_DYS19									
NC	G_DYS385									
NC	Y_DYS393	11	11	11						
NC	Y_DYS391									
NC	Y_DYS439									
NC	Y_DYS635									
NC	Y_DYS392	13	10							
NC	R_Y_GATA_H4	11	10	13	11	12				
NC	R_DYS437									
NC	R_DYS438	10	11							
NC	R_DYS448	19								
RBN_CML_60409	B_DYS456									
RBN_CML_60409	B_DYS389I									
RBN_CML_60409	B_DYS390									
RBN_CML_60409	B_DYS389II	10	11							
RBN_CML_60409	G_DYS458									
RBN_CML_60409	G_DYS19									
RBN_CML_60409	G_DYS385	10								
RBN_CML_60409	Y_DYS393	10	11	11						
RBN_CML_60409	Y_DYS391									
RBN_CML_60409	Y_DYS439	10	10	12	10					
RBN_CML_60409	Y_DYS635	10								
RBN_CML_60409	Y_DYS392	10								
RBN_CML_60409	R_Y_GATA_H4	10	12	14	10	11	10	12		
RBN_CML_60409	R_DYS437	10	11	23	10	10				
RBN_CML_60409	R_DYS438	10	22							
RBN_CML_60409	R_DYS448	10	31	11	10					
RBK_CMLLA_52809	B_DYS456									
RBK_CMLLA_52809	B_DYS389I									
RBK_CMLLA_52809	B_DYS390									
RBK_CMLLA_52809	B_DYS389II	15	18							
RBK_CMLLA_52809	G_DYS458									
RBK_CMLLA_52809	G_DYS19									
RBK_CMLLA_52809	G_DYS385									
RBK_CMLLA_52809	Y_DYS393									
RBK_CMLLA_52809	Y_DYS391	10	13	11	10					
RBK_CMLLA_52809	Y_DYS439	18								
RBK_CMLLA_52809	Y_DYS635	11								

Sample Name	Marker	Height 1	Height 2	Height 3	Height 4	Height 5	Height 6	Height 7	Height 8	Height 9
RBK_CMLLA_52809	Y_DYS392	16								
RBK_CMLLA_52809	R_Y_GATA_H4	10	16	15	19	14	21	29		
RBK_CMLLA_52809	R_DYS437	10	26							
RBK_CMLLA_52809	R_DYS438	12	35	10						
RBK_CMLLA_52809	R_DYS448	41	10							
NC	B_DYS456									
NC	B_DYS389I									
NC	B_DYS390									
NC	B_DYS389II									
NC	G_DYS458									
NC	G_DYS19									
NC	G_DYS385									
NC	Y_DYS393									
NC	Y_DYS391	10								
NC	Y_DYS439									
NC	Y_DYS635									
NC	Y_DYS392									
NC	R_Y_GATA_H4	13	10	13	10	10				
NC	R_DYS437	10	13							
NC	R_DYS438	20								
NC	R_DYS448	11	18							
NC	B_DYS456									
NC	B_DYS389I									
NC	B_DYS390									
NC	B_DYS389II									
NC	G_DYS458									
NC	G_DYS19									
NC	G_DYS385									
NC	Y_DYS393	10								
NC	Y_DYS391									
NC	Y_DYS439									
NC	Y_DYS635									
NC	Y_DYS392									
NC	R_Y_GATA_H4	12								
NC	R_DYS437	10								
NC	R_DYS438	11								
NC	R_DYS448	13								
RBK_LA_52709	B_DYS456									
RBK_LA_52709	B_DYS389I									
RBK_LA_52709	B_DYS390									
RBK_LA_52709	B_DYS389II									
RBK_LA_52709	G_DYS458									
RBK_LA_52709	G_DYS19	10								

Sample Name	Marker	Height 1	Height 2	Height 3	Height 4	Height 5	Height 6	Height 7	Height 8	Height 9
RBK_LA_52709	G_DYS385	11	14							
RBK_LA_52709	Y_DYS393	12								
RBK_LA_52709	Y_DYS391	10	10							
RBK_LA_52709	Y_DYS439	10	10							
RBK_LA_52709	Y_DYS635	11	11							
RBK_LA_52709	Y_DYS392	13								
RBK_LA_52709	R_Y_GATA_H4	20	24	35	19	39	15			
RBK_LA_52709	R_DYS437	48	10							
RBK_LA_52709	R_DYS438	24	47							
RBK_LA_52709	R_DYS448	55	10							
RBS_CML_60409	B_DYS456									
RBS_CML_60409	B_DYS389I									
RBS_CML_60409	B_DYS390									
RBS_CML_60409	B_DYS389II									
RBS_CML_60409	G_DYS458	12								
RBS_CML_60409	G_DYS19									
RBS_CML_60409	G_DYS385									
RBS_CML_60409	Y_DYS393	10								
RBS_CML_60409	Y_DYS391									
RBS_CML_60409	Y_DYS439	11								
RBS_CML_60409	Y_DYS635									
RBS_CML_60409	Y_DYS392									
RBS_CML_60409	R_Y_GATA_H4	11	14	10	10	13				
RBS_CML_60409	R_DYS437	20								
RBS_CML_60409	R_DYS438	26								
RBS_CML_60409	R_DYS448	10	28							
RBN_CML_60409	B_DYS456									
RBN_CML_60409	B_DYS389I									
RBN_CML_60409	B_DYS390									
RBN_CML_60409	B_DYS389II									
RBN_CML_60409	G_DYS458	10	10							
RBN_CML_60409	G_DYS19	12								
RBN_CML_60409	G_DYS385	15	17							
RBN_CML_60409	Y_DYS393	10	10	11	10	10				
RBN_CML_60409	Y_DYS391	14	10	10						
RBN_CML_60409	Y_DYS439	10	10	10	11					
RBN_CML_60409	Y_DYS635	10	12	14	10	10				
RBN_CML_60409	Y_DYS392	15	14	10						
RBN_CML_60409	R_Y_GATA_H4	11	16	14	14	33	10	10	24	34
RBN_CML_60409	R_DYS437	47								
RBN_CML_60409	R_DYS438	11	61							
RBN_CML_60409	R_DYS448	10	65							
NC	B_DYS456									

Sample Name	Marker	Height 1	Height 2	Height 3	Height 4	Height 5	Height 6	Height 7	Height 8	Height 9
NC	B_DYS389I									
NC	B_DYS390									
NC	B_DYS389II									
NC	G_DYS458									
NC	G_DYS19									
NC	G_DYS385									
NC	Y_DYS393		10							
NC	Y_DYS391									
NC	Y_DYS439									
NC	Y_DYS635		10							
NC	Y_DYS392									
NC	R_Y_GATA_H4		16	10	13					
NC	R_DYS437		19							
NC	R_DYS438		25							
NC	R_DYS448		26							
RBK_CML_52709	B_DYS456									
RBK_CML_52709	B_DYS389I									
RBK_CML_52709	B_DYS390									
RBK_CML_52709	B_DYS389II									
RBK_CML_52709	G_DYS458									
RBK_CML_52709	G_DYS19									
RBK_CML_52709	G_DYS385									
RBK_CML_52709	Y_DYS393									
RBK_CML_52709	Y_DYS391									
RBK_CML_52709	Y_DYS439									
RBK_CML_52709	Y_DYS635									
RBK_CML_52709	Y_DYS392									
RBK_CML_52709	R_Y_GATA_H4		10	11	13					
RBK_CML_52709	R_DYS437		17							
RBK_CML_52709	R_DYS438		20							
RBK_CML_52709	R_DYS448		22							
NC	B_DYS456									
NC	B_DYS389I									
NC	B_DYS390									
NC	B_DYS389II									
NC	G_DYS458									
NC	G_DYS19									
NC	G_DYS385									
NC	Y_DYS393									
NC	Y_DYS391									
NC	Y_DYS439									
NC	Y_DYS635									
NC	Y_DYS392									

Sample Name	Marker	Height 1	Height 2	Height 3	Height 4	Height 5	Height 6	Height 7	Height 8	Height 9
NC	R_Y_GATA_H4	10	11							
NC	R_DYS437	11								
NC	R_DYS438	17								
NC	R_DYS448	15								
RBN_LA_60409	B_DYS456									
RBN_LA_60409	B_DYS389I									
RBN_LA_60409	B_DYS390									
RBN_LA_60409	B_DYS389II									
RBN_LA_60409	G_DYS458									
RBN_LA_60409	G_DYS19									
RBN_LA_60409	G_DYS385									
RBN_LA_60409	Y_DYS393									
RBN_LA_60409	Y_DYS391									
RBN_LA_60409	Y_DYS439									
RBN_LA_60409	Y_DYS635									
RBN_LA_60409	Y_DYS392									
RBN_LA_60409	R_Y_GATA_H4									
RBN_LA_60409	R_DYS437	15								
RBN_LA_60409	R_DYS438	11								
RBN_LA_60409	R_DYS448	10								
RBK_CMLLA_52909	B_DYS456									
RBK_CMLLA_52909	B_DYS389I									
RBK_CMLLA_52909	B_DYS390									
RBK_CMLLA_52909	B_DYS389II	14								
RBK_CMLLA_52909	G_DYS458									
RBK_CMLLA_52909	G_DYS19									
RBK_CMLLA_52909	G_DYS385	10	11							
RBK_CMLLA_52909	Y_DYS393	10								
RBK_CMLLA_52909	Y_DYS391									
RBK_CMLLA_52909	Y_DYS439	10								
RBK_CMLLA_52909	Y_DYS635	11								
RBK_CMLLA_52909	Y_DYS392	12								
RBK_CMLLA_52909	R_Y_GATA_H4	18	15	32	27	43	11			
RBK_CMLLA_52909	R_DYS437	49								
RBK_CMLLA_52909	R_DYS438	11	53							
RBK_CMLLA_52909	R_DYS448	68								
NC	B_DYS456									
NC	B_DYS389I									
NC	B_DYS390									
NC	B_DYS389II									
NC	G_DYS458									
NC	G_DYS19									
NC	G_DYS385									

Sample Name	Marker	Height 1	Height 2	Height 3	Height 4	Height 5	Height 6	Height 7	Height 8	Height 9
NC	Y_DYS393									
NC	Y_DYS391									
NC	Y_DYS439	11								
NC	Y_DYS635									
NC	Y_DYS392									
NC	R_Y_GATA_H4	17	10							
NC	R_DYS437	16								
NC	R_DYS438	21								
NC	R_DYS448	20								
NC	B_DYS456	84								
NC	B_DYS389I	16								
NC	B_DYS390									
NC	B_DYS389II									
NC	G_DYS458									
NC	G_DYS19									
NC	G_DYS385									
NC	Y_DYS393	10								
NC	Y_DYS391									
NC	Y_DYS439									
NC	Y_DYS635									
NC	Y_DYS392									
NC	R_Y_GATA_H4	12								
NC	R_DYS437	10								
NC	R_DYS438	12								
NC	R_DYS448	13								
RBK_LA_52709	B_DYS456									
RBK_LA_52709	B_DYS389I									
RBK_LA_52709	B_DYS390									
RBK_LA_52709	B_DYS389II									
RBK_LA_52709	G_DYS458									
RBK_LA_52709	G_DYS19									
RBK_LA_52709	G_DYS385									
RBK_LA_52709	Y_DYS393									
RBK_LA_52709	Y_DYS391									
RBK_LA_52709	Y_DYS439									
RBK_LA_52709	Y_DYS635	14								
RBK_LA_52709	Y_DYS392	16	10							
RBK_LA_52709	R_Y_GATA_H4	13	18	14	10					
RBK_LA_52709	R_DYS437	19								
RBK_LA_52709	R_DYS438	20								
RBK_LA_52709	R_DYS448	21								
NC	B_DYS456									
NC	B_DYS389I									

Sample Name	Marker	Height 1	Height 2	Height 3	Height 4	Height 5	Height 6	Height 7	Height 8	Height 9
NC	B_DYS390	15								
NC	B_DYS389II									
NC	G_DYS458									
NC	G_DYS19	23								
NC	G_DYS385	15								
NC	Y_DYS393	23								
NC	Y_DYS391	11	16							
NC	Y_DYS439									
NC	Y_DYS635									
NC	Y_DYS392	10								
NC	R_Y_GATA_H4	10	10							
NC	R_DYS437	10	10							
NC	R_DYS438	10								
NC	R_DYS448	13								
RBS_LA_60409	B_DYS456									
RBS_LA_60409	B_DYS389I									
RBS_LA_60409	B_DYS390									
RBS_LA_60409	B_DYS389II									
RBS_LA_60409	G_DYS458									
RBS_LA_60409	G_DYS19									
RBS_LA_60409	G_DYS385									
RBS_LA_60409	Y_DYS393									
RBS_LA_60409	Y_DYS391									
RBS_LA_60409	Y_DYS439									
RBS_LA_60409	Y_DYS635	10								
RBS_LA_60409	Y_DYS392									
RBS_LA_60409	R_Y_GATA_H4									
RBS_LA_60409	R_DYS437									
RBS_LA_60409	R_DYS438	13								
RBS_LA_60409	R_DYS448	11	12							
NC	B_DYS456									
NC	B_DYS389I									
NC	B_DYS390									
NC	B_DYS389II									
NC	G_DYS458									
NC	G_DYS19									
NC	G_DYS385									
NC	Y_DYS393	34								
NC	Y_DYS391									
NC	Y_DYS439	10								
NC	Y_DYS635	10								
NC	Y_DYS392	12								
NC	R_Y_GATA_H4	10	11							

Sample Name	Marker	Height 1	Height 2	Height 3	Height 4	Height 5	Height 6	Height 7	Height 8	Height 9
NC	R_DYS437	11	12							
NC	R_DYS438	10	13							
NC	R_DYS448	10								
RBK_CMLLA_52809	B_DYS456									
RBK_CMLLA_52809	B_DYS389I									
RBK_CMLLA_52809	B_DYS390									
RBK_CMLLA_52809	B_DYS389II									
RBK_CMLLA_52809	G_DYS458									
RBK_CMLLA_52809	G_DYS19									
RBK_CMLLA_52809	G_DYS385									
RBK_CMLLA_52809	Y_DYS393									
RBK_CMLLA_52809	Y_DYS391									
RBK_CMLLA_52809	Y_DYS439									
RBK_CMLLA_52809	Y_DYS635									
RBK_CMLLA_52809	Y_DYS392	12								
RBK_CMLLA_52809	R_Y_GATA_H4	11								
RBK_CMLLA_52809	R_DYS437	10								
RBK_CMLLA_52809	R_DYS438									
RBK_CMLLA_52809	R_DYS448	11								
RBK_CML_52709	B_DYS456									
RBK_CML_52709	B_DYS389I									
RBK_CML_52709	B_DYS390									
RBK_CML_52709	B_DYS389II	10	13							
RBK_CML_52709	G_DYS458									
RBK_CML_52709	G_DYS19									
RBK_CML_52709	G_DYS385									
RBK_CML_52709	Y_DYS393	10	11							
RBK_CML_52709	Y_DYS391	11	10							
RBK_CML_52709	Y_DYS439	15								
RBK_CML_52709	Y_DYS635	14	32							
RBK_CML_52709	Y_DYS392	21								
RBK_CML_52709	R_Y_GATA_H4	13	15	10	22	21				
RBK_CML_52709	R_DYS437	10	12							
RBK_CML_52709	R_DYS438	17								
RBK_CML_52709	R_DYS448	10	20							
RBN_LA_60409	B_DYS456									
RBN_LA_60409	B_DYS389I									
RBN_LA_60409	B_DYS390	10								
RBN_LA_60409	B_DYS389II	12	16							
RBN_LA_60409	G_DYS458									
RBN_LA_60409	G_DYS19									
RBN_LA_60409	G_DYS385	11	11							
RBN_LA_60409	Y_DYS393									

Sample Name	Marker	Height 1	Height 2	Height 3	Height 4	Height 5	Height 6	Height 7	Height 8	Height 9
RBN_LA_60409	Y_DYS391	10								
RBN_LA_60409	Y_DYS439									
RBN_LA_60409	Y_DYS635	11								
RBN_LA_60409	Y_DYS392									
RBN_LA_60409	R_Y_GATA_H4	21	16	23	22					
RBN_LA_60409	R_DYS437	11	15							
RBN_LA_60409	R_DYS438	12	18							
RBN_LA_60409	R_DYS448	10	24							
RBK_CMLLA_52909	B_DYS456									
RBK_CMLLA_52909	B_DYS389I									
RBK_CMLLA_52909	B_DYS390									
RBK_CMLLA_52909	B_DYS389II									
RBK_CMLLA_52909	G_DYS458									
RBK_CMLLA_52909	G_DYS19									
RBK_CMLLA_52909	G_DYS385	10	11							
RBK_CMLLA_52909	Y_DYS393									
RBK_CMLLA_52909	Y_DYS391									
RBK_CMLLA_52909	Y_DYS439	10	10							
RBK_CMLLA_52909	Y_DYS635									
RBK_CMLLA_52909	Y_DYS392	10								
RBK_CMLLA_52909	R_Y_GATA_H4	11	14	11	11	15				
RBK_CMLLA_52909	R_DYS437	18								
RBK_CMLLA_52909	R_DYS438	24								
RBK_CMLLA_52909	R_DYS448	30								
RBS_CML_60409	B_DYS456									
RBS_CML_60409	B_DYS389I									
RBS_CML_60409	B_DYS390									
RBS_CML_60409	B_DYS389II									
RBS_CML_60409	G_DYS458									
RBS_CML_60409	G_DYS19									
RBS_CML_60409	G_DYS385									
RBS_CML_60409	Y_DYS393									
RBS_CML_60409	Y_DYS391	10								
RBS_CML_60409	Y_DYS439									
RBS_CML_60409	Y_DYS635									
RBS_CML_60409	Y_DYS392									
RBS_CML_60409	R_Y_GATA_H4	11	10	12	13	20				
RBS_CML_60409	R_DYS437	15								
RBS_CML_60409	R_DYS438	11								
RBS_CML_60409	R_DYS448	10	11							
RBS_LA_60409	B_DYS456									
RBS_LA_60409	B_DYS389I									
RBS_LA_60409	B_DYS390									

Sample Name	Marker	Height 1	Height 2	Height 3	Height 4	Height 5	Height 6	Height 7	Height 8	Height 9
RBS_LA_60409	B_DYS389II									
RBS_LA_60409	G_DYS458	10								
RBS_LA_60409	G_DYS19	10								
RBS_LA_60409	G_DYS385	12	14							
RBS_LA_60409	Y_DYS393	10								
RBS_LA_60409	Y_DYS391	11	10	10	10	11	10			
RBS_LA_60409	Y_DYS439	10	10							
RBS_LA_60409	Y_DYS635	10	11							
RBS_LA_60409	Y_DYS392	10	10							
RBS_LA_60409	R_Y_GATA_H4	19	10	19	10	10	16	12	19	
RBS_LA_60409	R_DYS437	11	11	12	13	31	11	10		
RBS_LA_60409	R_DYS438	10	12	30	10					
RBS_LA_60409	R_DYS448	11	11	14	37	10	10			

DATA SET	COUNT	TMEAN5%	5X TM5%
NC-BASELINE(FULL)	352	14.15	70.73

APPENDIX E

YFILER INJECTION TIME CHANGE TABLES

Injection Time Comparison Data – Yfiler Summary_Sensitivity Study

Locus	Allele	3 sec average % Diff
DYS456	17	21.2
DYS389I	13	22.3
DYS390	24	28.8
DYS389II	31	23.9
DYS458	18	22.3
DYS19	14	19.1
DYS385	11	10.5
	14	19.4
DYS393	13	16.9
DYS391	10	18.7
DYS439	12	23.5
DYS635	23	17.7
DYS392	13	21.3
YGATAH4	12	16.6
DYS437	15	24.3
DYS438	11	19.9
DYS448	19	22.6
Total Average		20.5

Locus	Allele	8 sec average % Diff
DYS456	17	47.9
DYS389I	13	16.0
DYS390	24	19.6
DYS389II	31	29.9
DYS458	18	27.2
DYS19	14	40.9
DYS385	11	26.0
	14	38.4
DYS393	13	17.1
DYS391	10	19.4
DYS439	12	36.7
DYS635	23	19.4
DYS392	13	20.2
YGATAH4	12	26.2
DYS437	15	17.5
DYS438	11	19.0
DYS448	19	18.3
Total Average		25.9

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