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CONCORDANCE STUDY OF FORENSIC CASEWORK SAMPLES USING THE AMPFLSTR© COFILER™ KIT, AMPFLSTR® IDENTIFILER™ KIT, AMPFLSTR® PROFILER PLUS™ KIT AND PowerPlex™ 16 Kit

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INTERNSHIP PRACTICUM REPORT

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By

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INTRODUCTION

In 1994, the Federal DNA Identification Act authorized the FBI to create a national DNA Identification Index for law enforcement agencies. The implementation of the Combined DNA Indexing System (CODIS) has taken DNA testing to a new level. CODIS enables law enforcement agencies to electronically store and compare DNA profiles against profiles within state and national indexes. Sample genotypes from convicted offenders of violent crimes, missing persons and their families and crime-scene samples can now be input into this database. Crime laboratories from different states and regions are now able to communicate and potentially link crimes. CODIS ultimately helps law enforcement agencies and forensic laboratories identify repeat, unknown and predatory offenders. However, many forensic DNA laboratories are burdened with growing case backlogs due to minimal staff and support personnel, making CODIS input practically impossible. In conjunction with casework, the forensic DNA analyst is responsible for, but not limited to, courtroom testimony, case peer reviews, DNA profile entry into CODIS, internal and external training, proficiency testing, internal and external auditing, equipment calibration and method validation, quality assurance and quality control duties and a host of numerous time consuming, yet necessary, tasks. These additional tasks leave the average forensic DNA analyst with minimal time to perform routine DNA casework. As a result, many cases are not processed or analyzed for years after the actual crime occurred.

Forensic DNA identity testing has greatly evolved over the past few decades. Initially, forensic scientists used immunologically based antigen-antibody interactions to categorize and identify individuals. These tests provided minimal discriminatory power. Immunological testing was gradually phased out and Restriction Fragment Length Polymorphism (RFLP) testing gained wide acceptance. Polymerase Chain Reactions (PCR) techniques, such as, D1S80, DQα1 and Polymarker, quickly replaced this tedious and time-consuming RFLP technology. Around 1995, Short Tandem Repeat (STR) DNA analysis was introduced to and accepted by the forensic community. STR's are short, tandemly repeated segments of DNA comprised of 3 to 7 basepairs. These segments are distributed throughout the human genome and are a rich source of polymorphism. STR's are more advantageous because of their discrete nature, practical intolerance to degradation and small size. A vast majority of forensic laboratories across the United States and the world use commercially available amplification kits such as, AmpFℓSTR® COfiler™ and AmpFℓSTR® Profiler Plus™, in the rapid replication of DNA molecules. These kits are equipped with the essential buffers, nucleotides, DNA Polymerase and locus specific primers necessary for the amplification of specific STR loci.

Although the AmpFℓSTR® COfiler™ and AmpFℓSTR® Profiler Plus™ kits have proven to be highly discriminating, sensitive and reliable, two separate amplifications are required. The two amplifications essentially equate to doubled analysis time and sample consumption. The acceptance and utilization of a single amplification system, such as, the PowerPlex™ 16 or the AmpFℓSTR® Identifiler™ kits may help streamline the work of the forensic DNA scientist and potentially decrease amplification and fragment

analysis time. Additionally, the acceptance of a single megaplex amplification system would provide less hands-on time, therefore, potentially decreasing the chance for human error. In order to be applicable to forensic DNA casework, this single megaplex amplification system must at least provide the same degree of efficiency, reliability and sensitivity already present in the AmpFℓSTR® COfiler™ and AmpFℓSTR® Profiler Plus™ kits.

It was proposed that 1.) The PowerPlex™ 16 and the AmpFℓSTR® Identifiler™ kits would provide concordant typing results with the widely used and accepted AmpFℓSTR® COfiler™ and AmpFℓSTR® Profiler Plus™ kits. 2.) A single megaplex kit, such as, PowerPlex™ 16 and the AmpFℓSTR® Identifiler™ would provide a more cost effective, efficient, sensitive and reliable alternative for forensic DNA laboratories.

The PowerPlex™ 16, AmpFℓSTR® Identifiler™, AmpFℓSTR® Profiler Plus™ and AmpFℓSTR® COfiler™ Kits allow for the co-amplification of the amelogenin gender determining marker and the thirteen core CODIS STR loci: D3S1358, FGA, vWA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, THO1, TPOX and CSF1PO. PowerPlex™ 16 adds the Penta D and Penta E loci and the AmpFℓSTR® Identifiler™ adds the D2S1338 and D19S433 loci. Manufactures of these systems have a suggested input DNA sample range of 0.5-2.5 ng, but have been successfully used to type samples containing less than 0.5 ng of DNA.

In this study, several questions were addressed: First, "Are all four STR multiplex kits concordant in their reproducibility, reliability, sensitivity and efficiency?" Second, "Is one particular STR megaplex kit more applicable to routine forensic

casework?" and Third, "In a mixed DNA sample can individuals, whether male or female, be differentiated?"

This paper describes a casework concordance study using adjudicated nonprobative sexual assault, mixed DNA and reference blood samples. Amplifications, on all samples, were performed using the AmpFℓSTR® COfiler™, Profiler Plus™ and Identifiler™ and PowerPlex™ 16 Kits and genotyping results were obtained using GeneScan® and Genotyper® software.

MATERIALS AND METHODS

Sample Sources

Semen-containing vaginal swabs and victim reference samples from four adjudicated non-probative sexual assault cases were obtained from the Fort Worth Police Forensic Science Laboratory.

Blood samples from four male and three female individuals were obtained using either the Lancet and fingerstick method or the blooddraw method. Blood samples were spotted onto white sterile cloth using a disposable transfer pipette and allowed to air-dry overnight.

Buccal epithelial samples were obtained from three female and three male individuals. The inside of each cheek was swabbed with a sterile cotton-tip applicator.

DNA Extraction

An approximate 3 x 3 mm^2 cutting of each bloodstain and one-half of each buccal sample was added to a 1.7mL microcentrifuge tube containing 700μL of Tris-EDTA buffer (TE^{-4} Buffer). Buccal and blood samples were allowed to soak for 30 minutes at room temperature. The bloodstain swatch cutting and buccal swabs were then removed to a Spin-X basket and spun in a microcentrifuge at maximum speed (10,000 x g) for 5 minutes. Approximately 625-650μL of the supernatant was decanted and 150μL of 5% Chelex® 100 was added to each microcentrifuge tube. Chelex® 100 is a resin which acts as a chelating agent that removes metal ions from the extraction solution, thus preventing

DNA degradation. All buccal and blood samples were incubated at 56°C for 30 minutes and then at 100°C for 8 minutes (12).

Vaginal swabs containing semen were subjected to the chelex differential extraction method (6). The differential extraction method results in two DNA fractions from the sperm-containing sample, one referred to as an epithelial cell fraction and the second a sperm cell fraction. Initially, one-half of each vaginal swab was cut and added to a 1.7 mL microcentrifuge tube containing 700μL of TE^{-4} Buffer (10mM Tris-HCl- 0.1 mM EDTA). The samples were incubated at room temperature. The cotton swab was removed and placed into a Spin-X basket and spun at maximum speed for one minute. All but 50μL of the supernatant was discarded, without disturbing the cell pellet. The epithelial cells were lysed with 150μL of TE^{-4} and 2μL Proteinase K (10 mg/ml), at 56°C for one hour. The samples were then centrifuged for 5 minutes at maximum speed (10,000 x g). Approximately 150 μL of the supernatant (epithelial fraction) was removed to a new microcentrifuge tube containing 50 μL of 20% Chelex® 100. The resultant sperm cell pellet was washed five times in 0.5mL of sperm wash buffer (10mM Tris-HCl- 10 mM EDTA- 50mM NaCl- 2% SDS, pH 8.0). Washing included adding 0.5mL of sperm wash buffer to the sperm cell pellet followed by a 5-minute (maximum speed) centrifugation. The residual supernatant was discarded after each wash. After washing, the sperm cell pellet was resuspended in 1 mL of sterile nanopure water and spun at maximum speed for 5 minutes. About 950μL of the supernatant was removed, leaving a 50 μL sperm cell solution. To each sperm cell pellet, 150μL of 5% Chelex® 100, 2μL of

Proteinase K and 7μL of Dithiothreitol (1M) was added. The sperm and epithelial fractions were incubated at 56°C for 1 hour and then at 100°C for 8 minutes.

DNA Quantitation

The rapid quantitation of human DNA was performed using Applied Biosystem's QuantiBlot® Human DNA Quantitation Kit using slot blot hybridization and colorimetric detection (4). Human DNA between 0.15 to 10.0 ng can be detected using the QuantiBlot® Kit. For each DNA sample approximately 5 μL of DNA and 150μl of spotting solution (0.4N NaOH, 25 mM EDTA, 0.00008% Bromothymol Blue) was added into a new 0.5 mL microcentrifuge tube.

DNA standards (5 μL) of the following quantities: 10.0, 5.0, 2.5, 1.25, 0.625, 0.3125 and 0.15625 ng of control DNA, provided in the kit, were also added to 150 μl of spotting solution in a new 0.5 mL microcentrifuge tube. The pre-wetted positively charged nylon membrane (Biodyne® B Nylon Membrane, 0.45 μm) was placed into a slot blot vacuum chamber and the entire volume of each DNA sample and standard was loaded into separate wells. Under low air pressure (15-20 mm Hg) samples were slowly drawn through the membrane, immobilizing the DNA onto the nylon membrane.

The membrane was transferred to a hybridization tray containing 100 mL of Pre-Warmed Hybridization solution (5X SSPE, 0.5% w/v SDS) and 5 mL of 30% H_2O_2. The hybridization tray was incubated in a 50°C rotating water bath (50 to 60 rpm) for 15 minutes. The DNA and respective standards were hybridized to a biotinylated D17Z1 oligonucleotide probe (20μL) in 30 ml of Pre-Warmed Hybridization solution. The hybridization tray was incubated in a 50°C rotating water bath (50 to 60 rpm) for 20

minutes. The membrane was rinsed in 100 ml of Pre-Warmed Wash Solution (1.5X SSPE, 0.5% w/v SDS). A stringent wash was performed using 30 mL of Pre-Warmed wash solution and 180 μL of the Horseradish Peroxidase-Streptavidan enzyme conjugate. The hybridization tray was incubated in a 50°C rotating water bath (50 to 60 rpm) for 10 minutes. The membrane was rinsed with Pre-Warmed Wash Solution (15 minutes on an desktop Orbital shaker at 100 to 125 rpm) followed by a 100 mL rinse in citrate buffer (0.1 M Sodium Citrate, pH 5.0).

The colorimetric development solution was prepared by mixing 30 mL of Citrate Buffer, 1.5 mL of Chromogen:TMB and 30 μL of 3% H_2O_2. The developing solution was added to the membrane in the hybridization tray and covered with aluminum foil in order to block out any exogenous light. The hybridization tray was placed on the orbital shaker (50 to 60 rpm) for 25 minutes. The solution was decanted and the chemical reaction was halted by the addition of 100 mL of sterile, deionized nanopure water.

The quantity of human DNA in each sample was determined by comparing their signal intensity to that of known DNA standards. Each sample was quantitated in duplicate.

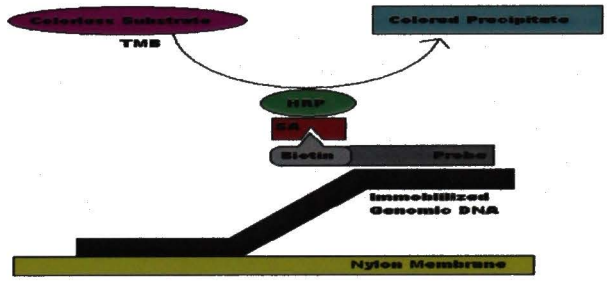


Figure 1: A schematic representation of the colorimetric detection of DNA using 3,3',5,5'-tetramethylbenzidine (Chromogen:TMB), Horseradish Peroxidase Streptavidin (HRP-SA) Enzyme Conjugate and a primate specific biotinylated DNA probe.

DNA Amplification

The PowerPlex™ 16, AmpFℓSTR® Identifiler™, AmpFℓSTR® Profiler Plus™, and the AmpFℓSTR® COfiler™ Kits allow for the co-amplification of the amelogenin gender determining marker and thirteen STR loci: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, THO1, TPOX, AND CSF1PO. PowerPlex™ 16 adds the Penta D and Penta E loci and AmpFℓSTR® Identifiler™ adds the D2S1338 and D19S433 loci. These systems have a suggested input DNA sample range of 0.5-2.5 ng. The manufactures of the multiplex kits suggest that samples containing less than 0.5 ng of genomic DNA have been successfully typed.

Locus	AmpFℓSTR® COfiler™	AmpFℓSTR® Profiler Plus™	AmpFℓSTR® Identifiler™	PowerPlex™ 16
D3S1358				
vWA				
FGA				
THO1				
TPOX				
CSF1PO				
D5S818				
D13S317				
D7S820				
D8S1179				
D21S11				
D18S51				
D16S539				
AMELOGENIN				
D2S1338				
D19S433				
PENTA E				
PENTA D				

Table 1: Loci contained within the AmpFℓSTR® and PowerPlex™ 16 Multiplex and Megaplex Systems.

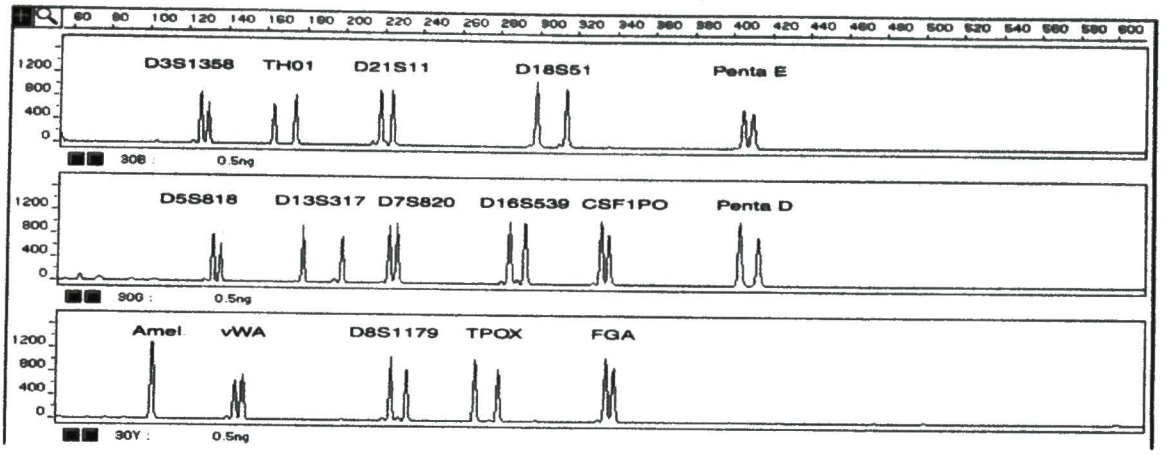


Figure 2- Genotyper electropherogram of the PowerPlex™ 16 Allelic Ladder, indicating allele designations. The top horizontal scale represents the relative size of each allele.

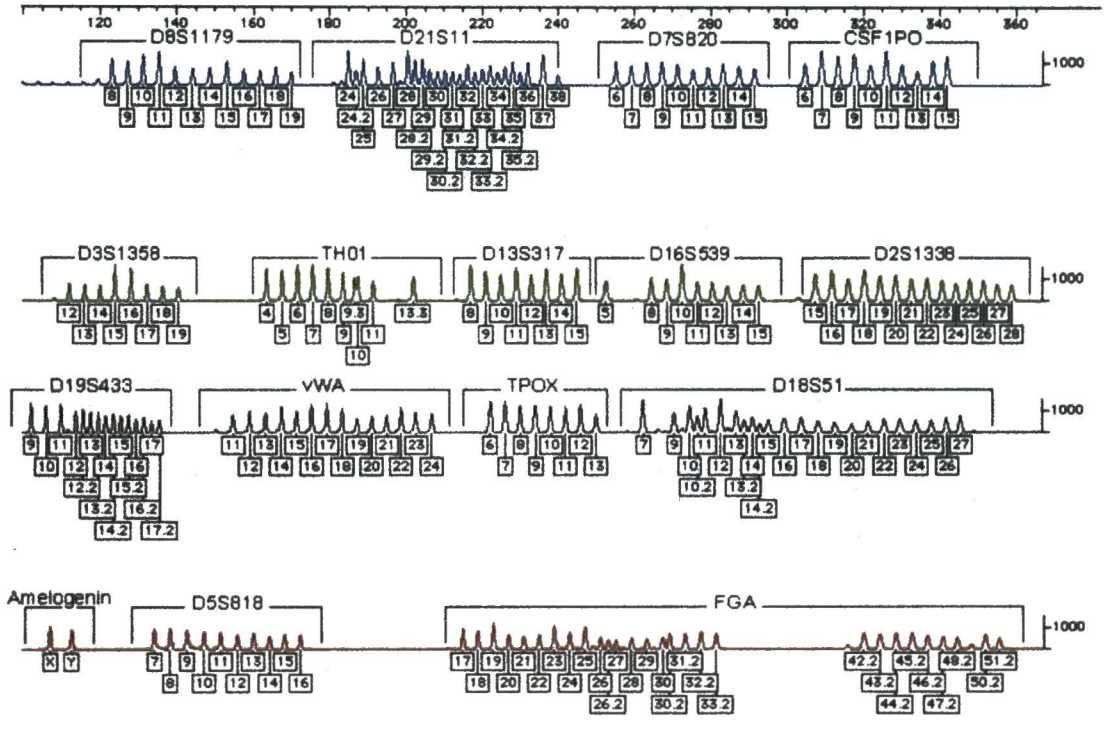


Figure 3- Genotyper electropherogram of the AmpFlSTR® Identifiler™ Allelic Ladder, indicating allele designations; the top horizontal scale represents the relative size of each allele.

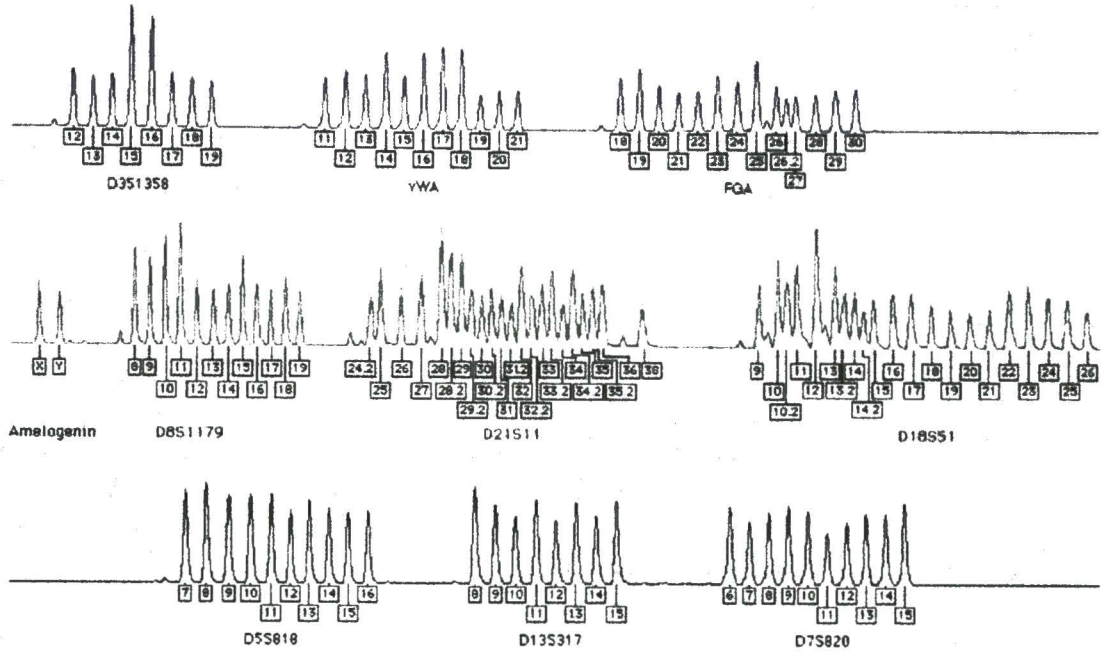


Figure 4- Genotyper electropherogram of the AmpFlSTR® Profiler Plus™ Allelic Ladder, indicating allele designations; the top horizontal scale represents the relative size of each allele.

Amplifications using either the AmpFℓSTR® Identifiler™ and PowerPlex™ 16 Systems, were performed in 0.2mL thin-walled MicroAmp® reaction tubes on the GeneAmp® 9700 PCR System, with the 9600 emulation mode. Amplifications using both the AmpFℓSTR® COfiler™ and Profiler Plus™ Systems, were performed on the GeneAmp® 2400 PCR System.

PCR System	PCR Instrument System	Kit Specific Times and Temperatures					
		Initial Incubation Step	Step Cycle-28			Final Extend Hold	Final Step Soak
			Melt	Anneal	Extend		
Profiler Plus	GeneAmp PCR System 2400	95° C 11min. 1 cycle	94°C 1 min.	59°C 1 min.	72°C 1 min.	60°C 45 min.	4° C ∞
COfiler	GeneAmp PCR System 2400	95° C 11min. 1 cycle	94°C 1 min.	59°C 1 min.	72°C 1 min.	60°C 45 min.	4° C ∞
Identifiler	GeneAmp PCR System 9700	95° C 11min. 1 cycle	94°C 1 min.	59°C 1 min.	72°C 1 min.	60°C 60 min.	4° C ∞

Table 2: Thermal Cycling Parameters for the AmpFℓSTR® Identifiler™, Profiler Plus™ and COfiler™ Amplification Systems. (1)(2)(3)

PCR System	Kit Specific Times and Temperatures						
	Initial Incubation Step	Second Incubation Step	Step Cycle-10			Final Extension (Hold)	Final Step (Soak)
			Melt	Anneal	Extend		
PPlex 16	95° C 11min. 1 cycle	96° C 1 min. 1 cycle	94°C 30sec.	60°C 30 sec.	70°C 45 sec.	60°C 30 min.	4° C ∞
GeneAmp PCR System 9700			Step Cycle-18				
			90°C 30sec.	60°C 30 sec.	70°C 45 sec.		

Table 3: Thermal Cycling Parameters for the PowerPlex 16™ Amplification System. (7)

The AmpFℓSTR Profiler Plus™, Identifiler™ and COfiler™ amplification kits contain locus-specific primer sets, AmpliTaq® Gold DNA Polymerase and a PCR Reaction Mix, containing nucleotides and buffers. Using PowerPlex™ 16 and AmpFℓSTR® Profiler Plus™, Identifiler™ and COfiler™ PCR Amplification Kit reagents and suggested protocols, a target DNA template amount of 1.0 ng was amplified in a 25μL reaction volumes. Although previous work has shown concordant and reliable results using either a 10μL or 50μL reaction volume, in this study each PCR reaction was carried out in a 25μL total reaction volume. The following reagent volumes were added to each DNA sample: 10.5μL of PCR Reaction Mix, 0.5μL of AmpliTaq Gold DNA Polymerase and 5.5μL of the locus-specific primer sets. The reagent volumes for the PowerPlex™ 16 system are as follows: 2.5μL of Gold STHR 10X Buffer, 2.5μL of the PowerPlex™ 16 10X locus-specific primer pair, 16.7 μL of Nuclease-Free water and 0.8 μL of AmpliTaq Gold® DNA Polymerase.

Amplification parameters were those recommended in each AmpFℓSTR® User's Manual and/or Bulletin (Table 2): initial incubation (95°C for 11 minutes), followed by twenty-eight cycles of denaturation (94°C for 1 minute), primer annealing (59°C for 1 minute) and extension (72°C for 1 minute). A final extension was performed at 60°C for 45 minutes (AmpFℓSTR® Profiler Plus™ and COfiler™) or for 60 minutes (AmpFℓSTR® Identifiler™).

Amplification parameters were those recommended in each PowerPlex 16 User's Manual and/or Bulletin (Table 3): initial incubation (95°C for 11 minutes) and a second incubation (96°C for 1 minute), followed by ten cycles of denaturation (94°C for 30 seconds), primer annealing (60°C for 30 seconds) and extension (70°C for 45 seconds) followed by twenty-two cycles of denaturation (90°C for 30 seconds), primer annealing (60°C for 30 seconds) and extension (70°C for 45 seconds). A final extension was performed at 60°C for 30 minutes.

All samples were held at 4°C until removed from their respective thermal cyclers. All amplification experiments were repeated in triplicate.

Data Collection and Software Installation

Data Collection Software v2.1, Genotyper® Software v2.5.2 and GeneScan Analysis Software Version 3.1.2 were installed, according to manufacturers direction, onto G4-PowerMac computers supporting the ABI Prism® 310 Genetic Analyzer. The GeneScan® v.3.1.2 Analysis software generated electropherogram graphics, allele size, peak height and peak area. The Genotyper® v.2.5.2 software converted the fragment size of the fluorescently tagged amplicon products into allele designations. Genotyping is performed by comparing the amplicon fragment lengths to that of the kit specific allelic ladders. Only alleles above 75 relative fluorescent units (RFU) were interpreted.

Capillary Electrophoresis

Matrix Standard Preparation

Multicomponent analysis refers to the process of color separation that is essential in the accurate and precise analysis of multicolor fluorescent PCR systems. PCR amplicon fragments of various lengths were labeled with different fluorescent dyes. Although each fluorescent dye exhibits a distinct and precise emission maximum, some spectral overlap occurs between the various dyes. The matrix determines and corrects for the spectral overlap. (10) (13)

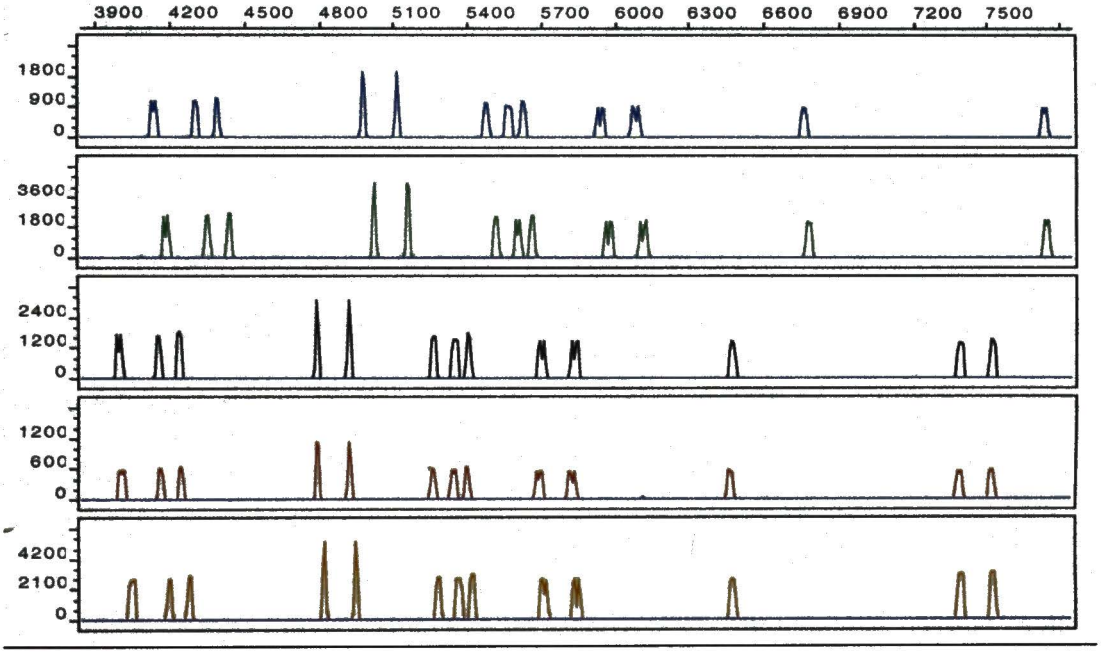


Figure 5: Electropherogram of AmpFℓSTR® Identifiler™ matrix standards

AmpFlSTR® Profiler Plus™ and COfiler™

Four matrix standard samples (5-FAM™, JOE™, NED™ and ROX™) were prepared by combining 1 µL of each sample with 25 µL of deionized formamide. One

tube was prepared for each matrix standard sample. The samples were denatured at 95°C for 3 minutes, then snap cooled on ice-cold glycerin.

AmpFlSTR® Identifiler™

Five matrix standard samples (6-FAM™, VIC™, NED™, PET™ and ROX™) were prepared by combining 2 μL of each sample with 25 μL of deionized formamide. One tube was prepared for each matrix standard sample. The samples were denatured at 95°C for 3 minutes, then snap cooled on ice-cold glycerin.

PowerPlex 16™

Four matrix standard samples (FL™, JOE™, TMR™ and CXR™) were prepared by combining 1 μL of each sample with 25 μL of deionized formamide. One tube was prepared for each matrix standard sample. The samples were denatured at 95°C for 3 minutes, and then snap cooled on ice-cold glycerin. (11)

The amplicon cocktails were isokinetically injected through POP-4™ polymer, which acted as a molecular sieve. Matrix standards and DNA samples were evaluated using the following run and injection parameters:

Table 4: ABI Prism 310 Genetic Analyzer run and injection parameters for the AmpFℓSTR Profiler Plus™, AmpFℓSTR® Identifiler™, AmpFℓSTR® COfiler™ and PowerPlex™ 16 Amplification kits (1)(2)(3)(7)

	AmpFlSTR® Profiler Plus™	AmpFlSTR® COfiler™	AmpFlSTR® Identifiler™	PowerPlex™ 16
Injection (sec.)	5	5	5	4
Injection kV	15	15	15	15
Run kV	15	15	15	15
Run °C	60	60	60	60

Run Time	24	24	28	30
Module	GS STR POP4 (1mL) F	GS STR POP4 (1mL)F	GS STR POP4(1mL)G5	GS STR POP4 (1mL)A

Table 4 continued

Sample and Ladder Preparation

AmpFℓSTR® Profiler Plus™ and COfiler™

Amplicon samples and allelic ladders were prepared by combining 1 μL of each sample with 24 μL of deionized formamide and 1 μL of the GS-500 (ROX) internal sizing standard. One tube was prepared for each amplicon sample. The samples were denatured at 95°C for 3 minutes, then snap cooled on ice-cold glycerin.

AmpFℓSTR® Identifiler™

Amplicon samples and allelic ladders were prepared by combining 1.5 μL of each sample with 24.5 μL of deionized formamide and 1 μL of the GeneScan-500 (LIZ) internal sizing standard. One tube was prepared amplicon sample. The samples were denatured at 95°C for 3 minutes, followed by snap cooling on ice-cold glycerin.

PowerPlex 16™

Amplicon samples and allelic ladders were prepared by combining 1 μL of each sample with 24 μL of deionized formamide and 1 μL of the ILA-600 internal sizing standard. One tube was prepared for each matrix standard sample. The samples were denatured at 95°C for 3 minutes, then snap cooled on ice-cold glycerin.

Statistical Analysis

The mean and standard deviation values, for each STR locus, were calculated for heterozygote peak heights using the Microsoft Excel software. A single factor Analysis of Variance (ANOVA) test was also performed using Microsoft Excel in order to test for variations in heterozygote peak height ratios between the AmpFℓSTR® Identifiler™, AmpFℓSTR® Profiler Plus™, AmpFℓSTR® COfiler™ and PowerPlex™ 16 Kits. Results of the ANOVA were derived from single source buccal swab samples at 1.25ng of template DNA. Significance was determined at a 95% confidence level (α=0.05). (9)

RESULTS

Cost Comparison

Although many Forensic DNA crime laboratories are substantially funded through federal grant programs, local legislature or private funds, the cost of running and maintaining a forensic DNA laboratory can be highly inflated and overwhelming. Ultimately, cost plays a major part in the type of equipment and methods used in a forensic laboratory (Table 5).

	AmpFℓSTR® Profiler Plus™ and COfiler™	AmpFℓSTR® Identifiler™	PowerPlex™ 16
Kit Cost	$3750	$2750	$2775 $8995
Total Number of Reactions	100 or 200	200	100 or 400
Total Reaction Volume	50 or 25 µL	25 µL	25 µl
Cost per Reaction	$37.50 or $18.75	$13.75	$27.75 or $22.49
PCR Run Time (Ramp time not included)	2.58 hours	2.83 hours	1.52 hours

Table 5: Cost and efficiency breakdown of the AmpFℓSTR® and PowerPlex™ Systems

The AmpFℓSTR® Identifiler system provided the most cost effective amplification system. However, the run time for the AmpFℓSTR® Identifiler system is longer than the PowerPlex™ 16 and AmpFℓSTR® Profiler Plus™ and COfiler™, making the actual determination of cost savings more difficult to determine.

DNA Quantitation

DNA quantitation was performed with Applied Biosystem's QuantiBlot® Human Quantitation System using colorimetric detection. Quantitation results were obtained by visually comparing the signal intensity of the DNA test samples to the signal intensity of the DNA standards (Figure 6: Columns 1 and 6). The stock concentration of DNA Calibrator 1 and Calibrator 2 was 0.7 ng/μL and 0.1 ng/μL, respectively. Therefore, the signal intensity of DNA Calibrator 1 should fall between the 5.0 ng and 10.0 ng DNA standard and the signal intensity of DNA Calibrator 2 should fall between the 0.3125 ng and 0.625 ng DNA standard. Visual inspection of both membranes (Figures 6 and 7) illustrated that the signal intensities of both DNA Calibrators visually fell between the respective DNA standards. Due to the large volume of samples needing to be quantitated, two QuantiBlot® reactions were performed. Each sample was blotted twice to ensure that reproducible quantitation results were obtained.

DNA concentrations ranged between ~30ng/5μL to 0.625 ng/5 μL (Table 6). Several reference blood samples did not produce any signal intensity (Columns 3 and 5). Buccal and blood samples which did not display a signal intensity were excluded from this study.

DNA concentrations obtained from the adjudicated sexual assault cases ranged between ~7.5 ng/ 5 mL to 2.5 ng/5mL. Quantitation results were not obtained from the epithelial and sperms fractions in Case 4. The signal intensities of the DNA standards and DNA test samples were very low.

	1	2	3	4	5	6
A	10.0 ng	**DNA Calibrator 1**	Buccal 3 10.0ng	Blood 2 0.625ng	Blood 2 0.0ng	0.0 ng
B	5.0 ng	**DNA Calibrator 2**	Buccal 4 10.0ng	Blood 2 0.625ng	Blood 2 0.0ng	0.1562 ng
C	2.5 ng		Buccal 4 10.0ng	Blood 3 0.0ng	Blood 3 0.0ng	0.3125 ng
D	1.25 ng	Buccal 1 0. 625ng	Buccal 5 10.0ng	Blood 3 0.0ng	Blood 3 0.0ng	0.625 ng
E	0.625 ng	Buccal 1 0.625ng	Buccal 5 10.0ng	Blood 4 0.0ng		1.25 ng
F	0.3125 ng	Buccal 2 ~30.0ng	Buccal 6 10.0ng	Blood 4 0.0ng		2.5 ng
G	0.1562 ng	Buccal 2 ~30.0ng	Buccal 6 10.0ng	Blood 1 0.0ng	**DNA Calibrator 1**	5.0 ng
H	0.0 ng	Buccal 30.0ng	Blood 1 0.0ng	Blood 1 0.0ng	**DNA Calibrator 2**	10.0 ng

Table 6: DNA results using Applied Biosystems QuantiBlot Human DNA Quantitation Kit for the sensitivity and simulated mixed blood samples; DNA concentration per 5 μL.

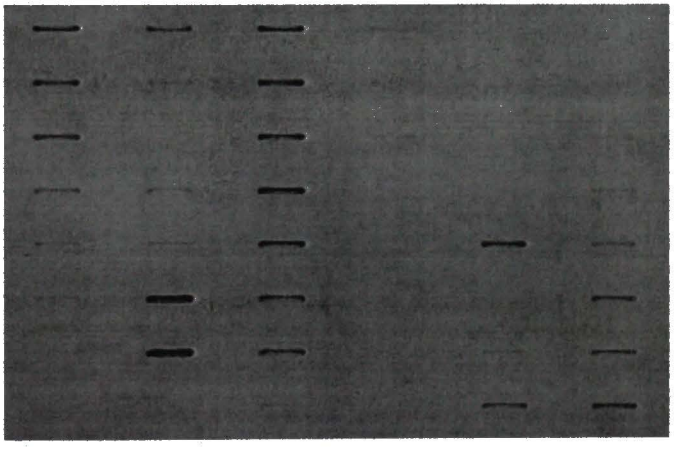


Figure 6: QuantiBlot® results for the sensitivity and simulated mixed blood samples; DNA concentration per 5 μL.

	1	2	3	4	5	6
A	10.0 ng	**DNA Calibrator 1**	Case-2 Epi. 2.5ng	Sperm Blank		0.0 ng
B	5.0 ng	**DNA Calibrator 2**	Case-2 Epi. 2.5ng	Sperm Blank		0.15625 ng
C	2.5 ng		Case-2 Sperm 5.0ng	Epithelial Blank		0.3125 ng
D	1.25 ng		Case-2 Sperm 5.0ng	Epithelial Blank		0.625 ng
E	0.625 ng	Case-1 Epi. 2.5ng	Case-3 Epi. 7.5ng	Case-4 Epi. 0.0ng		1.25 ng
F	0.3125 ng	Case-1 Epi. 5.0ng	Case-3 Epi. 7.5ng	Case-4 Epi. 0.0ng		2.5 ng
G	0.15625 ng	Case-1 Sperm 5.0ng	Case-3 Sperm 2.5ng	Case-4 Sperm 0.0ng	**DNA Calibrator 1**	5.0 ng
H	0.0 ng	Case-1 Sperm 2.5ng	Case-3 Sperm 2.5ng	Case-4 Sperm 0.0ng	**DNA Calibrator 2**	10.0 ng

Table 7: DNA results using Applied Biosystems QuantiBlot Human DNA Quantitation Kit for the adjudicated nonprobative sexual assault cases; DNA concentration per 5 μL. Epi = epithelial fraction, Sperm = sperm fraction

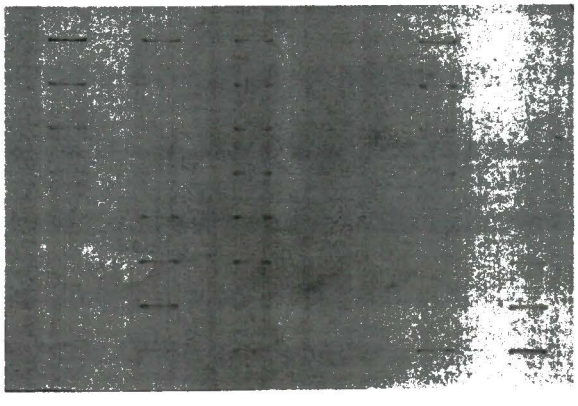


Figure 7: QuantiBlot® results for the adjudicated nonprobative sexual assault cases; DNA concentration per 5 μL.

Adjudicated Non-Probative Sexual Assault Samples

Sexual assault cases constitute a majority of the cases that are routinely examined by DNA analysts. Therefore, the analysis of such cases was possibly the most practical, applicable and logical for this study. Examinations were based on vaginal swab samples taken from sexual assault kits and known liquid blood samples obtained from the victims. Initially four adjudicated cases were extracted, quantitated and amplified using the PowerPlex™ 16, AmpFℓSTR® COfiler™ and Profiler Plus™ and the AmpFℓSTR® Identifiler™ Kits. Three of the four cases did not have a suspect, therefore, only victims reference samples were used for comparison. Case 4 did not render any quantitation results nor were DNA profiles obtained from either the male or female fractions. Results were based on the three remaining nonprobative cases.

Genotyping results obtained from the three adjudicated cases, using the PowerPlex™ 16, AmpFℓSTR® COfiler™ and Profiler Plus™ and the AmpFℓSTR® Identifiler™ Kits, were indeed concordant. In each case, the victim's reference sample was consistent with the non-sperm, epithelial fraction from the vaginal swab. The differential extraction method attempts to separate cells of different types: non-sperm and sperm. Using all four amplification systems, complete separation was observed in cases 1 and 2, and carry-over from the victim was observed in the sperm fraction in case 3, resulting in a minor DNA profile (8).

	PowerPlex 16			Identifiler			COfiler/Profiler Plus		
STR Loci	K1	Q1	Q2	K1	Q1	Q2	K1	Q1	Q2
D5S818	12,12	12	11,12	12,12	12,12	11,12	12,12	12,12	11,12
D13S317	11,11	11	11,11	11,11	11,11	11,11	11,11	11,11	11,11
D7S820	11,12	11,12	10,12	11,12	11,12	10,12	11,12	11,12	10,12
D16S539	11,13		13,13	11,13	11,13	13,13	11,13	11,13	13,13
CSF1PO	11,11	11		11,11	11,11	11,12	11,11	11,11	11,12
D3S1358	15,15		16	15,15	15,15	16,17	15,15	15,15	16,17
THO1	7,7	7	6,8	7,7	7,7	6,8	7,7	7,7	6,8
D21S11	29,30	29	30,35	29,30	29,30	30,35	29,30	29,30	30,35
D18S51	14,22			14,22	14,22	17,21	14,22	14,22	17,21
vWA	15,16	15,16	16,17	15,16	15,16	16,17	15,16	15,16	16,17
D8S1179	12,14	12,14	13,14	12,14	12,14	13,14	12,14	12,14	13,14
TPOX	8,9	8,9	9,9	8,9	8,9	9,9	8,9	8,9	9,9
FGA	21,21	21,21	22,27	21,21	21,21	22,27	21,21	21,21	22,27
AMELO.	XX	XX	XY	XX	XX	XY	XX	XX	XY
D2S1338				21,23	21,23	19,21			
D19S433				13,14	13,14	11,12			
Penta D	12,13	12,13	7,12						
Penta E	10,17	10,17	8						

Table 8: STR typing results for Case 1 using the PowerPlex™ 16, AmpFℓSTR® COfiler™ and Profiler Plus™ and the AmpFℓSTR Identifiler™ Kits; K1 = victim reference DNA profile, Q1 = epithelial fraction DNA profile, Q2 = sperm fraction DNA profile, AMELO = amelogenin gender marker.

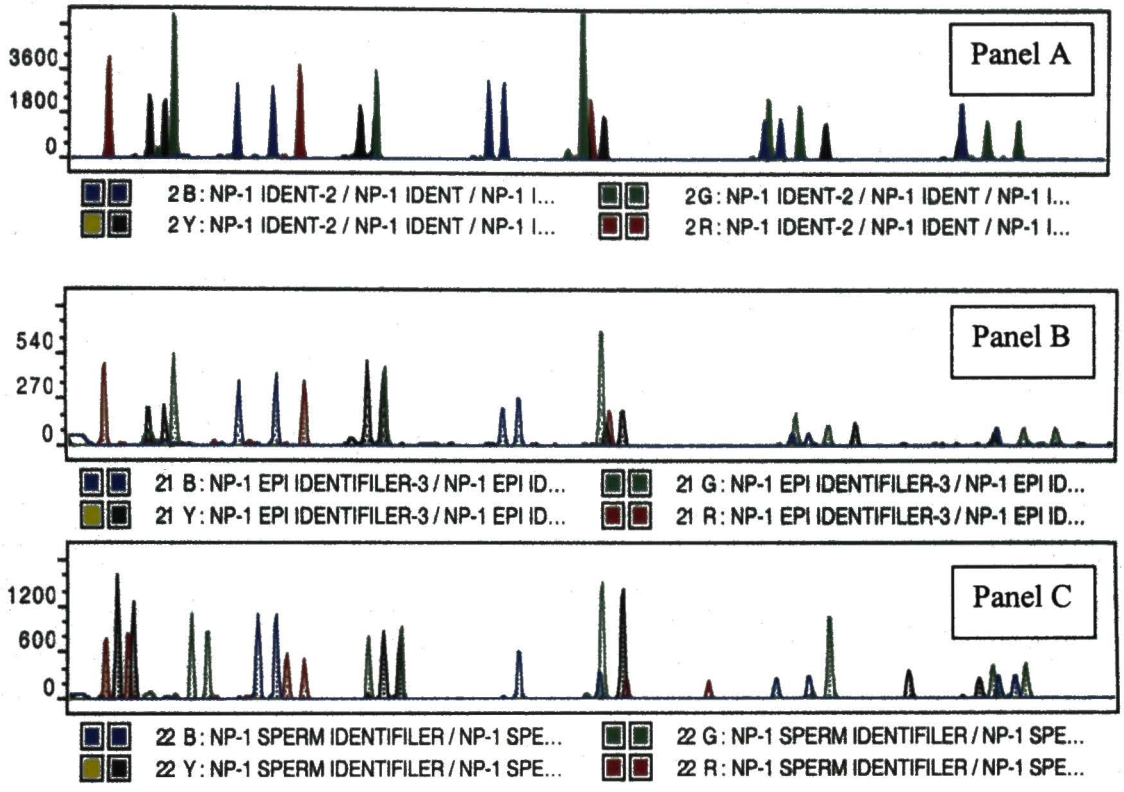


Figure 8 – Case 1. STR DNA profiles were generated using the AmpFℓSTR® Identifiler™ Kit. The profile generated from the epithelial (non-sperm) fraction (Panel B) matched that of the victim (Panel A). A DNA profile was obtained from the sperm fraction (Panel C) of the vaginal swabs, which was inconsistent with the profiles of the victim and non-sperm fraction. The victim was excluded as a contributor of the sperm fraction. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (basepairs).

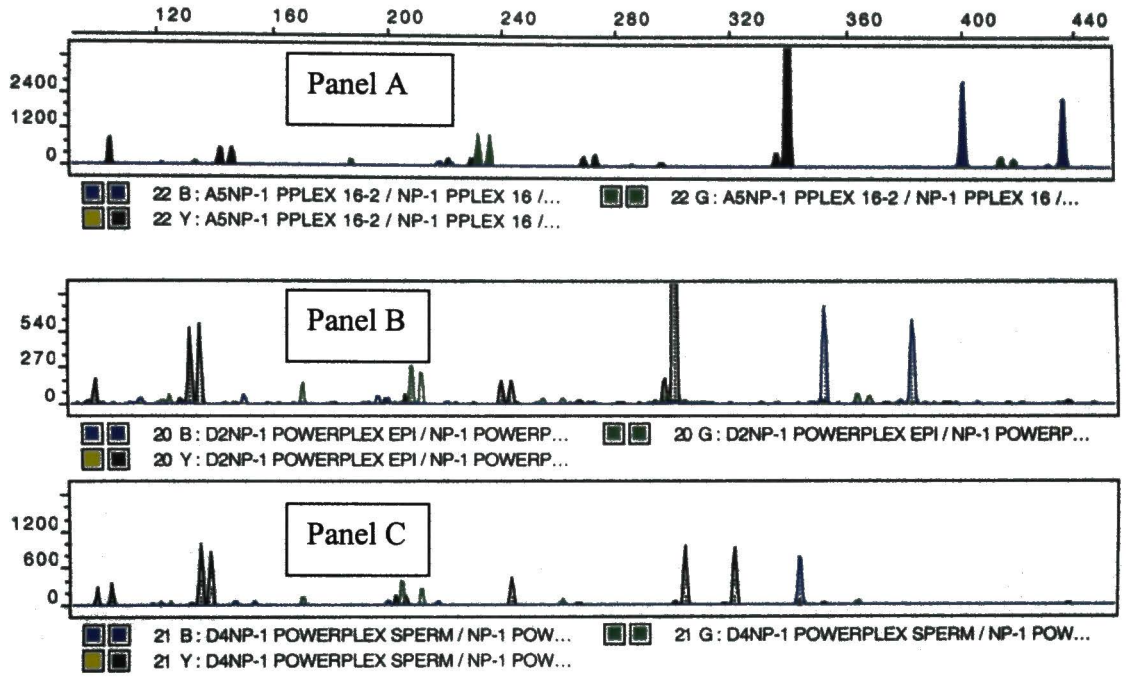


Figure 9 – Case 1. STR DNA profiles were generated using the PowerPlex™ 16 System. The profile generated from the epithelial (non-sperm) fraction (Panel B) matched that of the victim (Panel A). A DNA profile was obtained from the sperm fraction (Panel C) of the vaginal swabs, which was inconsistent with the profiles of the victim and non-sperm fraction. The victim was excluded as a contributor of the sperm fraction. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (basepairs).

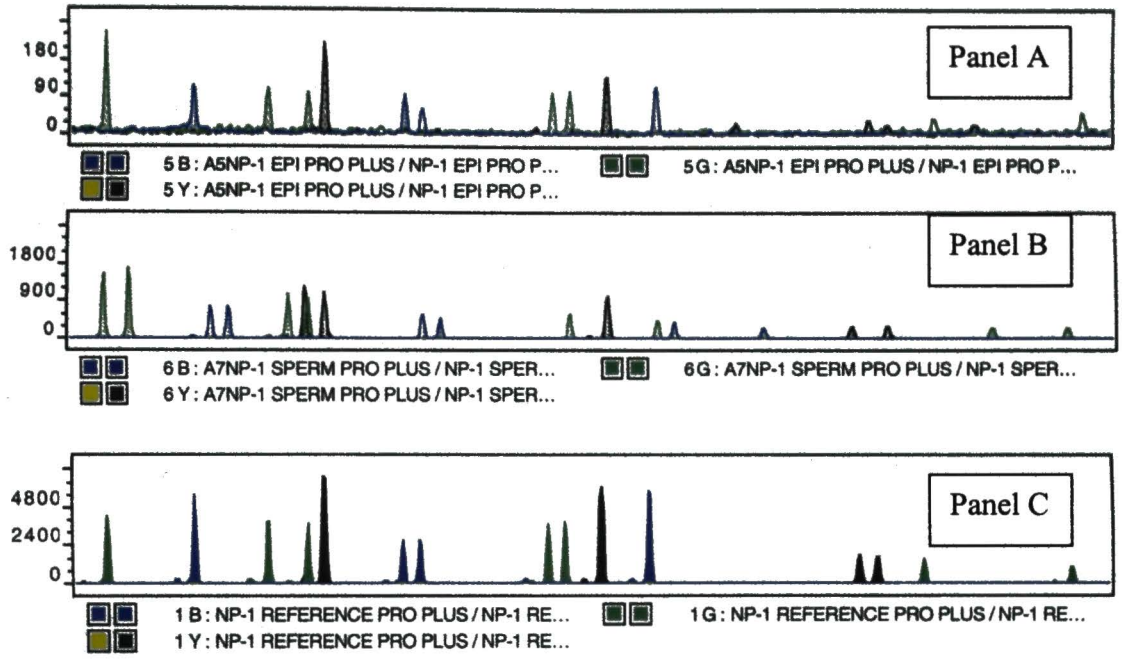


Figure 10 – Case 1. STR DNA profiles were generated using the AmpFℓSTR® Profiler Plus™ System. The profile generated from the epithelial (non-sperm) fraction (Panel A) matched that of the victim (Panel C). A DNA profile was obtained from the sperm fraction (Panel B) of the vaginal swabs, which was inconsistent with the profiles of the victim and non-sperm fraction. The victim was excluded as a contributor of the sperm fraction. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (basepairs).

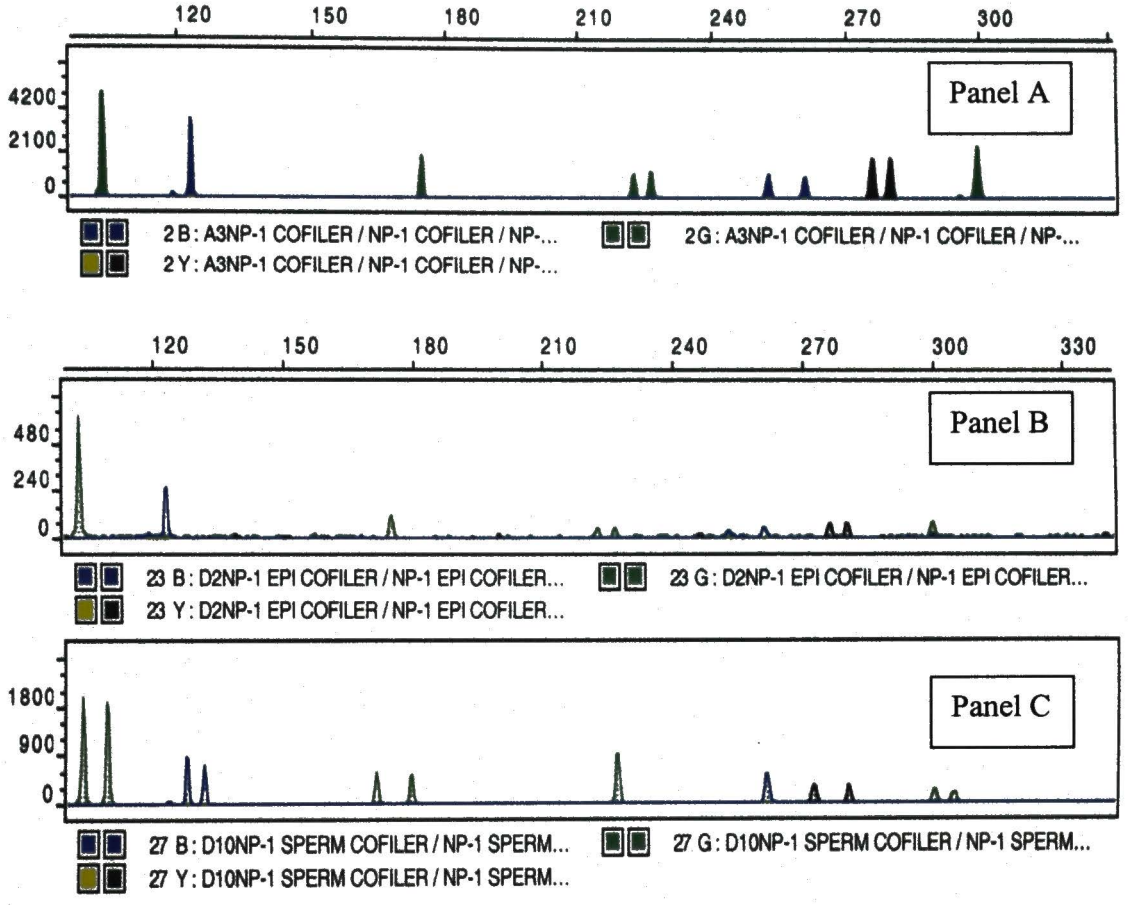


Figure 11 – Case 1. STR DNA profiles were generated using the AmpFℓSTR® COfiler™ System. The profile generated from the epithelial (non-sperm) fraction (Panel B) matched that of the victim (Panel A). A DNA profile was obtained from the sperm fraction (Panel C) of the vaginal swabs, which was inconsistent with the profiles of the victim and non-sperm fraction. The victim was excluded as a contributor of the sperm fraction. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (basepairs).

	PowerPlex 16			Identifiler			COfiler/Profiler Plus		
STR Loci	K1	Q1	Q2	K1	Q1	Q2	K1	Q1	Q2
D5S818	8,12	8,12	11,13	8,12	8,12	11,13	8,12	8,12	11,13
D13S317	12,12	12,12	12,13	12,12	12,12	12,13	12,12	12,12	12,13
D7S820	11,11	11,11	10,11	11,11	11,11	10,11	11,11	11,11	10,11
D16S539	11,13	11,13	11,11	11,13	11,13	11,11	11,13	11,13	11,11
CSF1PO	11,13	11,13	10,12	11,13	11,13	10,12	11,13	11,13	10,12
D3S1358	15,17	15,17	14,15	15,17	15,17	14,15	15,17	15,17	14,15
THO1	6,8	6,8	7,7	6,8	6,8	7,7	6,8	6,8	7,7
D21S11	28,28	28,28	30,31	28,28	28,28	30,31	28,28	28,28	30,31
D18S51	15,17	15,17	16,16	15,17	15,17	16,16	15,19	15,19	16,16
VWA	13,15	13,15	15,18	13,15	13,15	15,18	13,15	13,15	15,18
D8S1179	14,14	14,14	14,14	14,14	14,14	14,14	14,14	14,14	14,14
TPOX	10,10	10,10	8,10	10,10	10,10	8,10	10,10	10,10	8,10
FGA	22,23	22,23	22,25	22,23	22,23	22,25	22,23	22,23	22,25
AMELO.	XX	XX	XY	XX	XX	XY	XX	XX	XY
D2S1338				16,20	16,20	19,20			
D19S433				13,14	13,14	13,14			
Penta D	9,9	9,9	5,8						
Penta E	7,14	7,14	12,12						

Table 9: Allele designations for Case 2 using the PowerPlex™ 16, AmpFlSTR® COfiler™ and Profiler Plus™ and the AmpFlSTR Identifiler™ Kits; K1 = victim reference DNA profile, Q1 = epithelial fraction DNA profile, Q2 = sperm fraction DNA profile, AMELO = amelogenin gender marker.

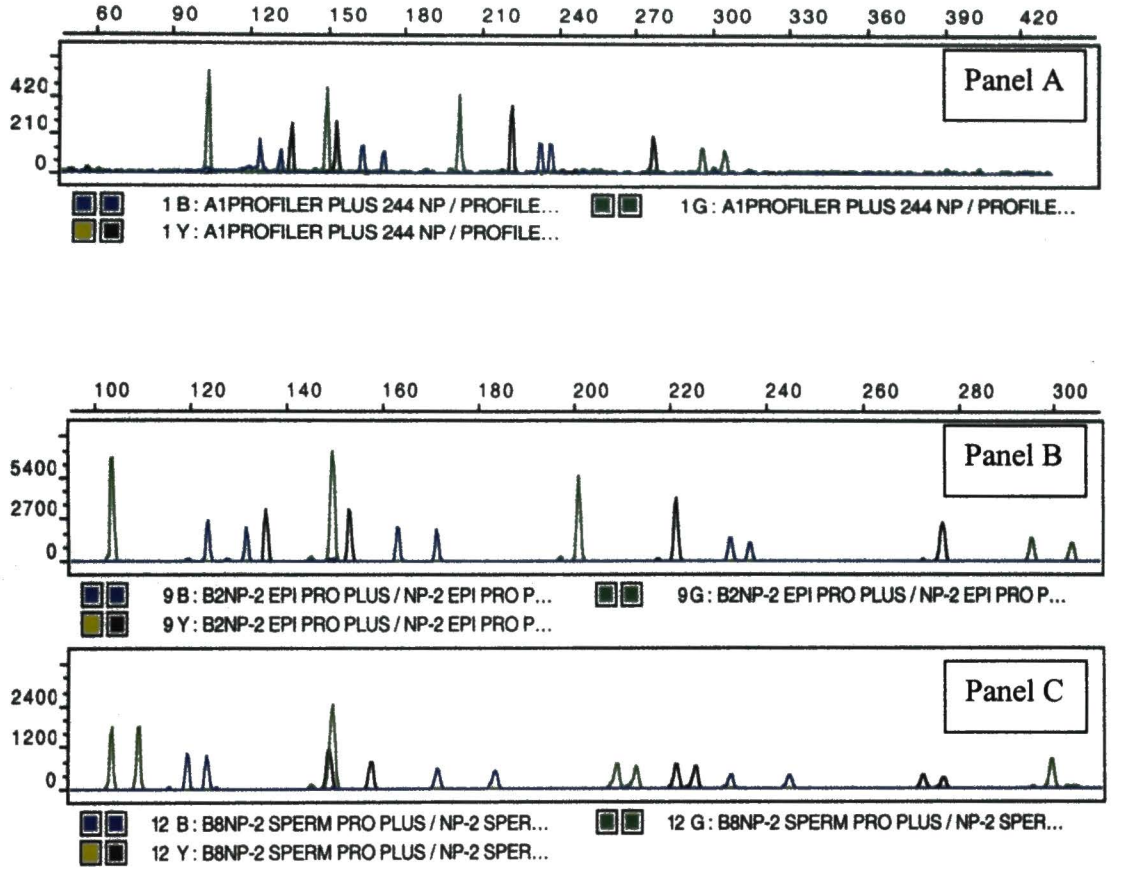


Figure 12 – Case 2. STR DNA profiles were generated using the AmpFℓSTR® Profiler Plus™ System. The profile generated from the epithelial (non-sperm) fraction (Panel B) matched that of the victim (Panel A). A DNA profile was obtained from the sperm fraction (Panel C) of the vaginal swabs, which was inconsistent with the profiles of the victim and non-sperm fraction. The victim was excluded as a contributor of the sperm fraction. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (basepairs).

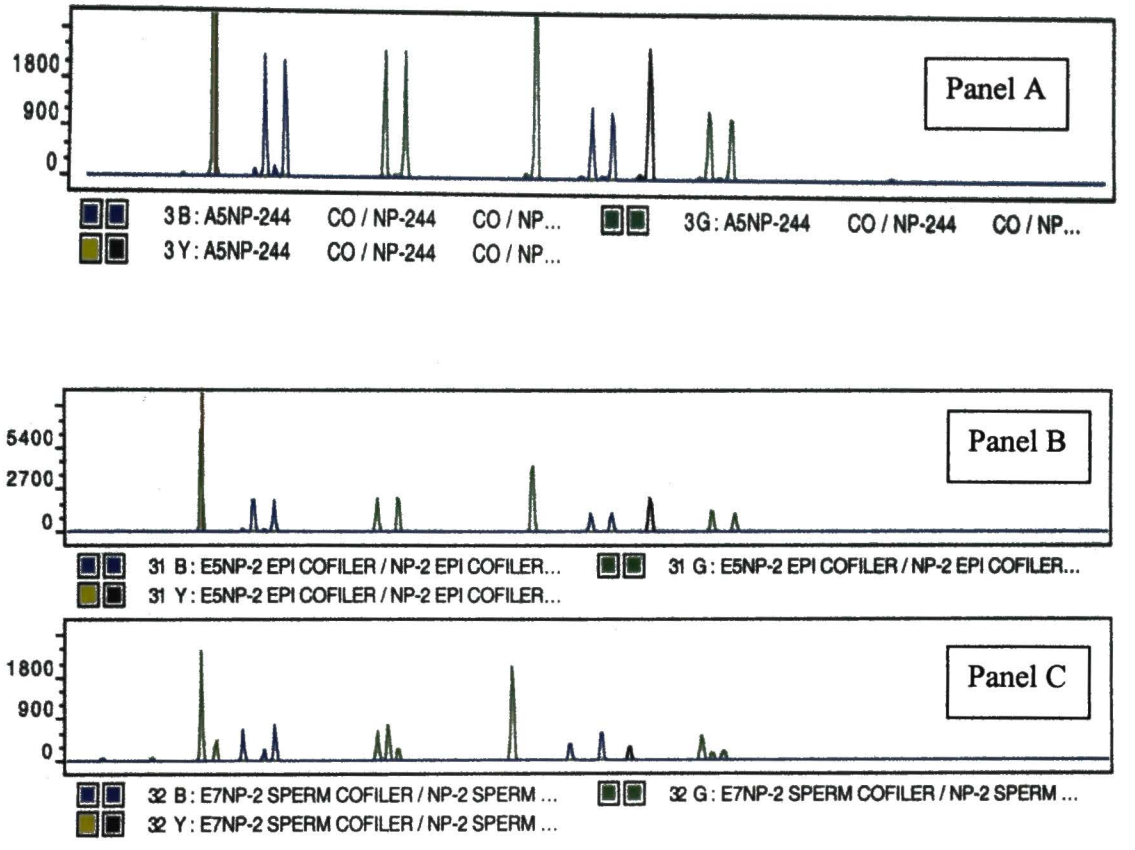


Figure 13 – Case 2. STR DNA profiles were generated using the AmpFℓSTR® COfiler™ System. The profile generated from the epithelial (non-sperm) fraction (Panel B) matched that of the victim (Panel A). A DNA profile was obtained from the sperm fraction (Panel C) of the vaginal swabs, which was inconsistent with the profiles of the victim and non-sperm fraction. The victim was excluded as a contributor of the sperm fraction. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (basepairs).

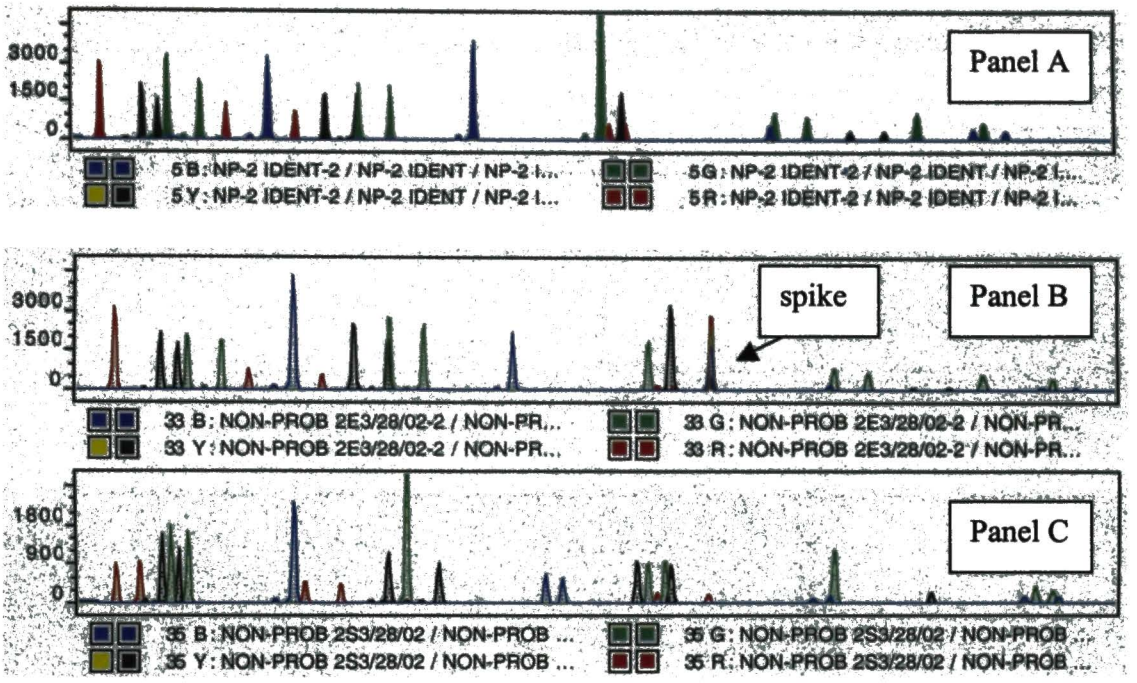


Figure 14 – Case 2. STR DNA profiles were generated using the AmpFℓSTR® Identifiler™ System. The profile generated from the epithelial (non-sperm) fraction (Panel B) matched that of the victim (Panel A). A DNA profile was obtained from the sperm fraction (Panel C) of the vaginal swabs, which was inconsistent with the profiles of the victim and non-sperm fraction. The victim was excluded as a contributor of the sperm fraction. A spike peak is present in panel B. Spikes are usually caused by voltage changes during electrophoresis or by particulates within the POP-4 polymer. Spike peaks may be interpreted as alleles if care is not taken in analyzing the electrophoresis data. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (basepairs).

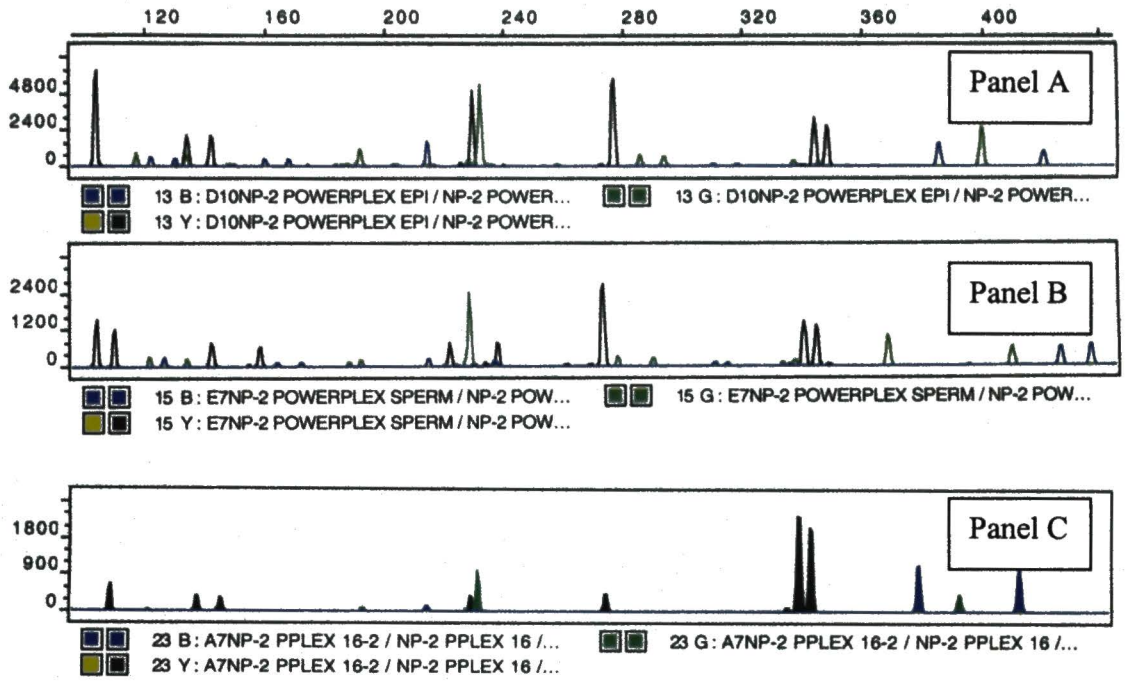


Figure 15 – Case 2. STR DNA profiles were generated using the PowerPlex™ 16 System. The profile generated from the epithelial (non-sperm) fraction (Panel A) matched that of the victim (Panel C). A DNA profile was obtained from the sperm fraction (Panel B) of the vaginal swabs, which was inconsistent with the profiles of the victim and non-sperm fraction. The victim was excluded as a contributor of the sperm fraction. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (basepairs).

	PowerPlex 16			Identifiler			COfiler/Profiler Plus		
STR Loci	K1	Q1	Q2	K1	Q1	Q2	K1	Q1	Q2
D5S818	8,9	8,9	9,12 (8,11)	8,9	8,9	9,12 (8)	8,9	8,9	9,12 (8)
D13S317	12,13	12,13	11,12	12,13	12,13	11,12 (13)	12,13	12,13	11,12 (13)
D7S820	8,10	8,10	10,10 (8)	8,10	8,10	10,10	8,10	8,10	10,10 (8)
D16S539	10,13	10,13	9,12	10,13	10,13	9,12	10,13	10,13	9,12
CSF1PO	12,12	12,12	10,11	12,12	12,12	10,11	12,12	12,12	10,11
D3S1358	14,14	14,14	16,16	14,14	14,14	16,16 (14,15)	14,14	14,14	16,16 (14)
THO1	6,8	6,8	7,9	6,8	6,8	7,9 (6)	6,8	6,8	7,9 (6,8)
D21S11	28,30	28,30	28,33.2	28,30	28,30	28,33.2 (30)	28,30	28,30	28,33.2 (30)
D18S51	15,15	15,15	15,16	15,15	15,15	15,16	15,15	15,15	15,16
VWA	15,15	15,15	15,19	15,15	15,15	15,19	15,15	15,15	15,19
D8S1179	14,15	14,15	12,16 (14,15)	14,15	14,15	12,16 (14,15)	14,15	14,15	12,16 (14,15)
TPOX	6,9	6,9	9,9	6,9	6,9	9,9	6,9	6,9	9,9
FGA	22,23	22,23	21,22 (23)	22,23	22,23	21,22	22,23	22,23	21,22
AMELO.	XX	XX	XY	XX	XX	XY	XX	XX	XY
D2S1338				16.19	16,19	19,22			
D19S433				11,14.2	11,14.2	11,12.2 (14.2)			
Penta D		2.2,8	2.2,11						
Penta E		12,17	15,17						

Table 10: Allele designations for Case 3 using the PowerPlex™ 16, AmpFlSTR® COfiler™ and Profiler Plus™ and the AmpFlSTR Identifiler™ Kits; K1 = victim reference DNA profile, Q1 = epithelial fraction DNA profile, Q2 = sperm fraction DNA profile, AMELO = amelogenin gender marker.

In case 3, all minor allele components were consistent with the victim and did not appear to have originated from another sperm contributor. The minor component, or carry-over into the sperm fraction, in Case 3 is summarized in table (Table 11). AmpFℓSTR® Identifiler™, COfiler™ and Profiler Plus™ exhibited the most concordant allele designations. However, at the D3S1358 locus the Identifiler™ system detected the 15-allele and the COfiler™ system detected the 8-allele at the THO1 locus. The Identifiler™ kit also detected the 14.2- microvariant at the D19S431 locus. The PowerPlex™ 16 system detected the least amount of minor alleles. The PowerPlex™ 16 system was able to detect the 23-allele at the FGA locus, unlike the AmpFlSTR® systems. Allele designations for all sperm fractions were concordant in Case 1 and Case 3.

In Case 1, allelic dropout, preferential and differential amplification was observed in both the epithelial and sperm fractions using the PowerPlex™ 16 system (Figure 9 & Table 8). However alleles within the tetranucleotide repeat loci, excluding D2S1338 and D19S433, were concordant with the AmpFℓSTR® systems.

STR Loci	PowerPlex™ 16	Identifiler™	COfiler™	Profiler™ Plus
D5S818	8,11	8		8
D7S820	8		8	8
D13S317		13		13
D3S1358		14,15	14	14
THO1		6	6,8	
D21S11		30		30
D8S1179	14,15	14,15		14,15
FGA	23			
D19S431		14.2		

Table 11: Case 3. Carry-over from the epithelial fraction into the sperm fractions; the carry-over alleles are consistent with that of the victim

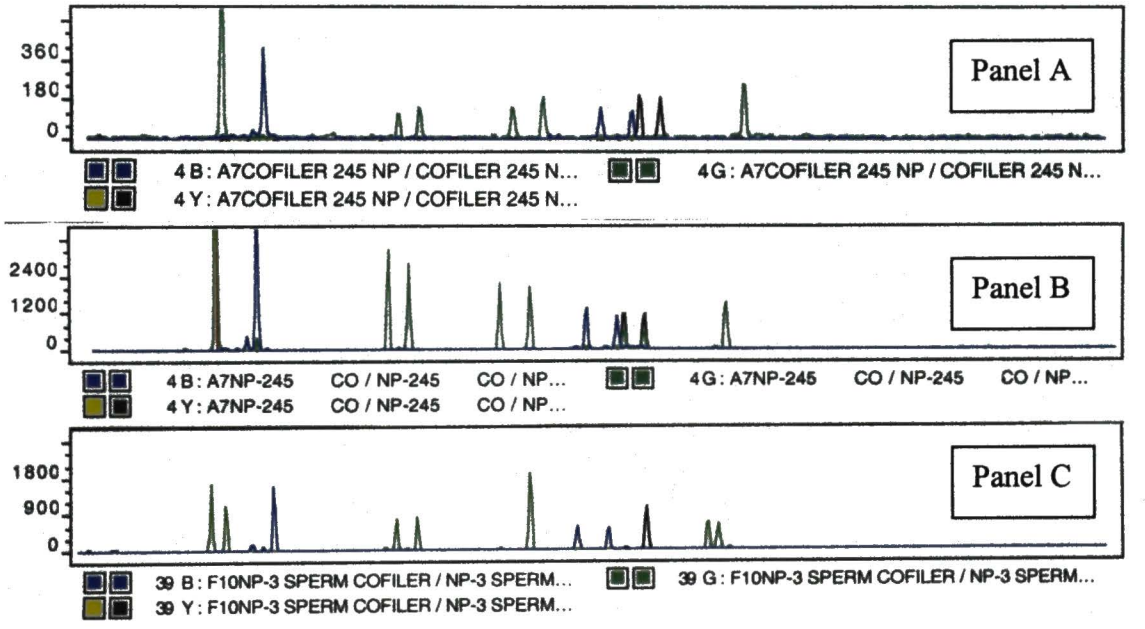


Figure 16 – Case 3. STR DNA profiles were generated using the AmpFℓSTR® COfiler™ 16 System. The profile generated from the epithelial (non-sperm) fraction (Panel B) matched that of the victim (Panel A). A mixed DNA profile was obtained from the sperm fraction (Panel B) of the vaginal swabs. The minor contribution was consistent with the profiles of the victim and non-sperm fraction. Therefore, the victim was included as a potential contributor to the minor component of the sperm fraction.

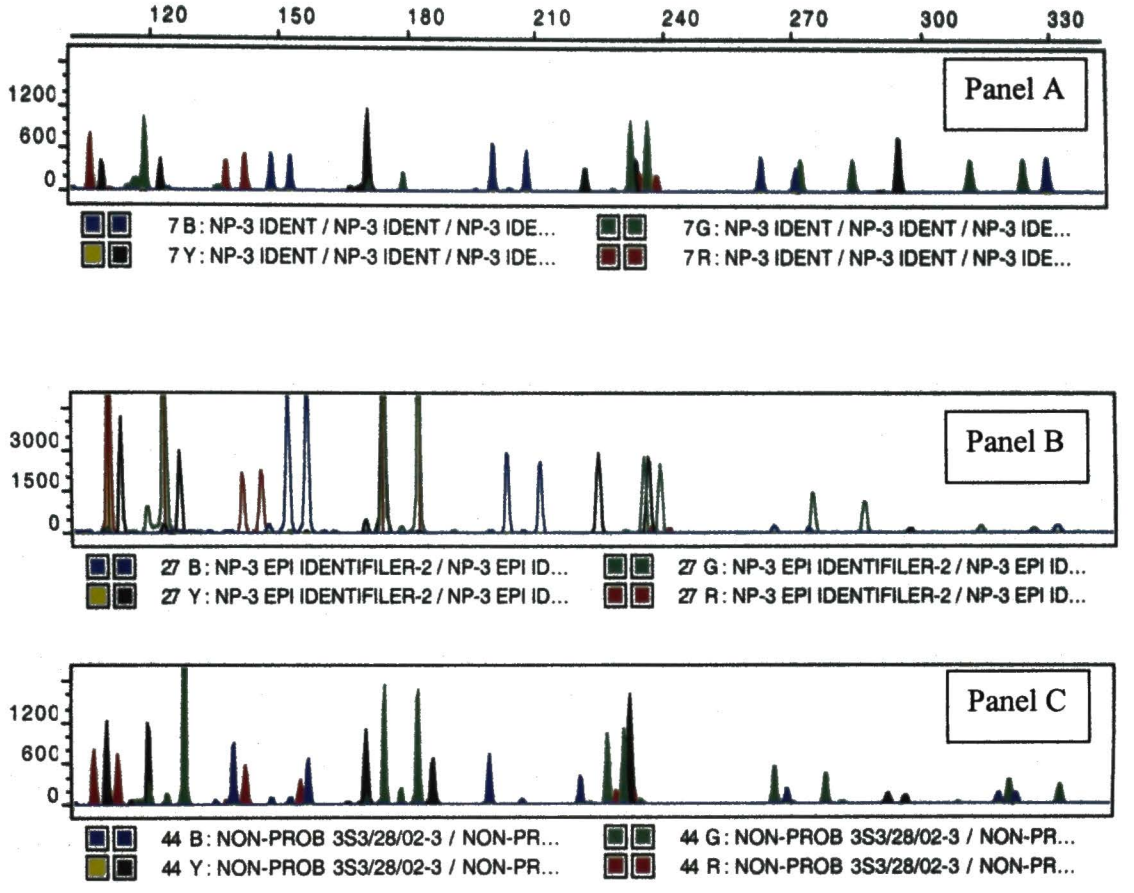


Figure 17 – Case 3. STR DNA profiles were generated using the AmpFℓSTR® Identifiler™ System. The profile generated from the epithelial (non-sperm) fraction (Panel B) matched that of the victim (Panel A). A mixed DNA profile was obtained from the sperm fraction (Panel B) of the vaginal swabs. The minor contribution was consistent with the profiles of the victim and non-sperm fraction. Therefore, the victim was included as a potential contributor to the minor component of the sperm fraction. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (basepairs).

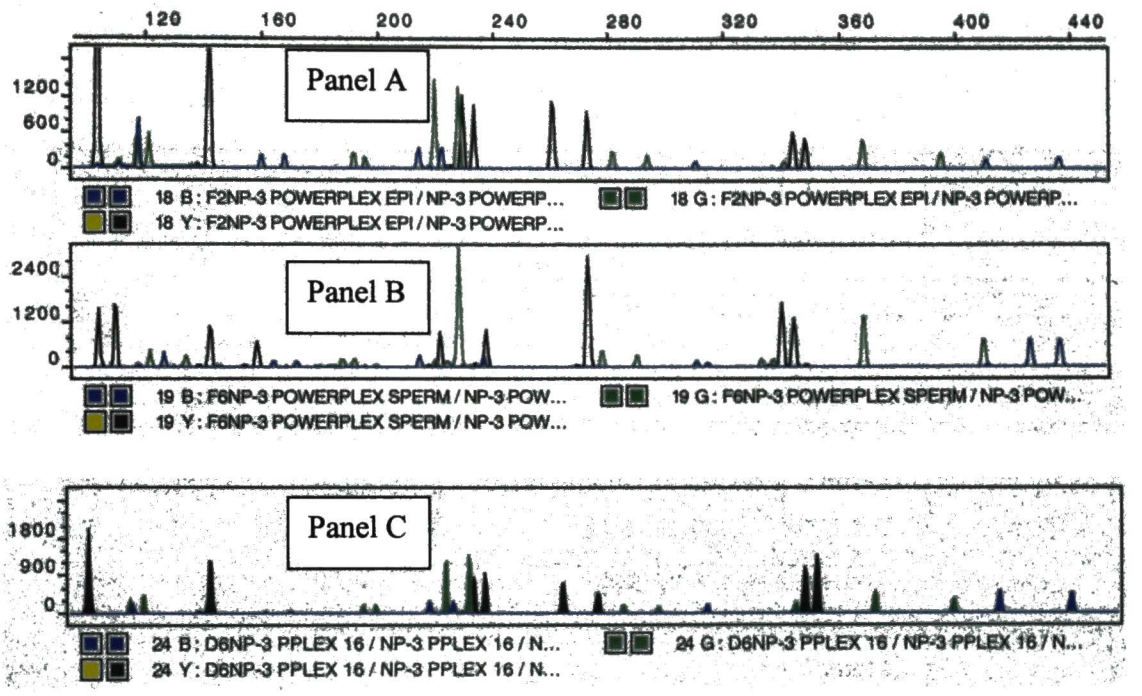


Figure 18– Case 3. STR DNA profiles were generated using the PowerPlex™ 16 System. The profile generated from the epithelial (non-sperm) fraction (Panel A) matched that of the victim (Panel C). A mixed DNA profile was obtained from the sperm fraction (Panel B) of the vaginal swabs. The minor contribution was consistent with the profiles of the victim and non-sperm fraction. Therefore, the victim was included as a potential contributor to the minor component of the sperm fraction. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (basepairs).

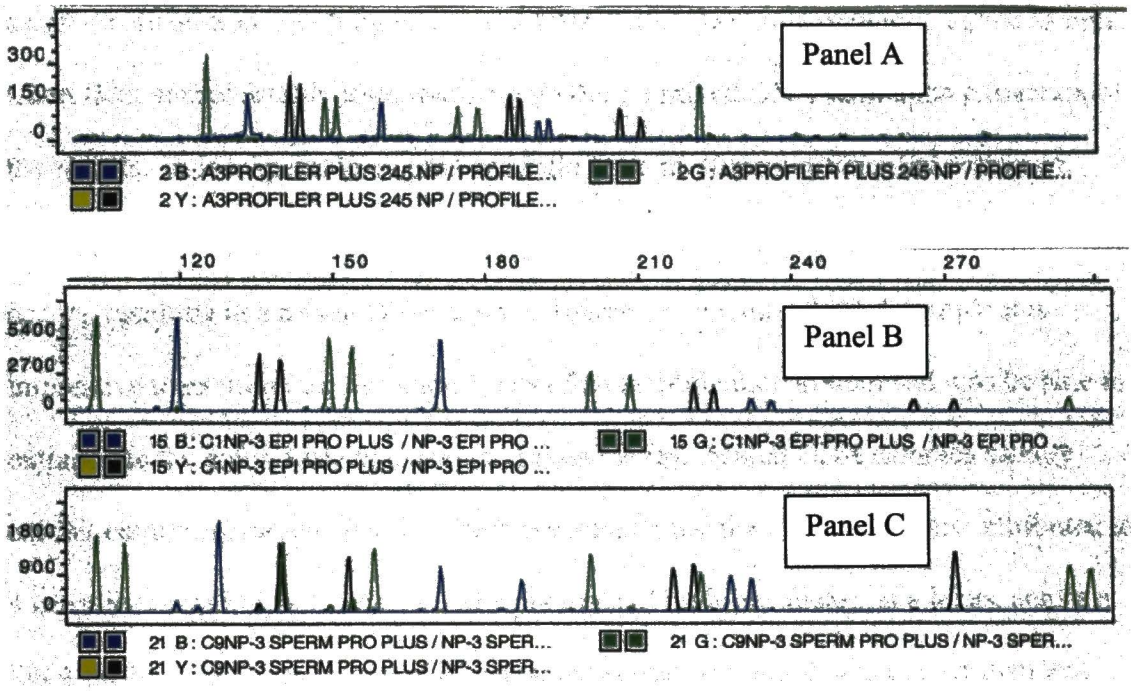


Figure 19– Case 3. STR DNA profiles were generated using the AmpFlSTR® Profiler Plus™ System. The profile generated from the epithelial (non-sperm) fraction (Panel B) matched that of the victim (Panel A). A mixed DNA profile was obtained from the sperm fraction (Panel C) of the vaginal swabs. The minor contribution was consistent with the profiles of the victim and non-sperm fraction. Therefore, the victim was included as a potential contributor to the minor component of the sperm fraction. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (basepairs).

Mixed DNA Samples

Crimes do not occur in a sterile vacuum. In many cases both the victim and assailant are injured, resulting in a mixed DNA stain. Semen-containing vaginal samples taken from sexual assault cases routinely produce a mixed DNA stain upon extraction of the sample. A low sperm to vaginal cell ratio or an inefficient differential extraction technique is usually the cause of a mixed DNA sample. Sometimes cross contamination occurs, resulting in a mixed DNA sample. Therefore, in a mixed DNA sample it is imperative to examine the detection limits of an amplification system and also be able to extrapolate the individual components, if possible (8). Mixed DNA samples usually exhibit certain characteristics: 1.) The presence of more than two alleles, not attributed to a germline mutation, at a locus 2.) The presence of multiple alleles, at a locus, across a DNA profile 3.) The presence of a peak at the stutter position that is greater than that expected in a single source sample and 4.) Imbalance in heterozygote peak heights

A sample containing DNA from two sources can be comprised (at a single locus) of any of the seven genotypes combinations (1):

- Heterozygote + Heterozygote, no overlapping alleles (four peaks)
- Heterozygote + Heterozygote, one overlapping allele (three peaks)
- Heterozygote + Heterozygote, two overlapping alleles (two peaks)
- Heterozygote + Homozygote, no overlapping alleles (three peaks)
- Heterozygote + Homozygote, overlapping alleles (two peaks)
- Homozygote + Homozygote, no overlapping alleles (two peaks)
- Homozygote + Homozygote, overlapping alleles (one peak)

Table 12 – Mean Peak Height Ratios for the AmpFℓSTR® and PowerPlex™ 16 systems; Ratios were calculated for heterozygote samples with peak heights > 75 RFU; n = 6 observations

STR Loci	PowerPlex™ 16	Identifiler™	COfiler™	Profiler Plus™
D5S818	90.3	83.0		94.8
D13S317	89.3	89.6		83.3
D72820	88.6	89.5	93.6	89.3
D16S539	72.5	89.6	92.9	
CSF1PO	82.5	71.3	87.0	
D3S1358	88.9	83.2	85.0	95.8
THO1	92.1	88.0	88.4	
D21S11	87.1	92.6		93.5
D18S51	88.8	82.5		96.8
vWA	78.6	85.5		92.1
FGA	77.8	85.0		89.6
TPOX	81.0	93.7	93.9	
D8S11	91.2	89.1		98.4
PENTA D	84.5			
PENTA E	85.1			
D2S1338		74.0		
D19S433		88.2		

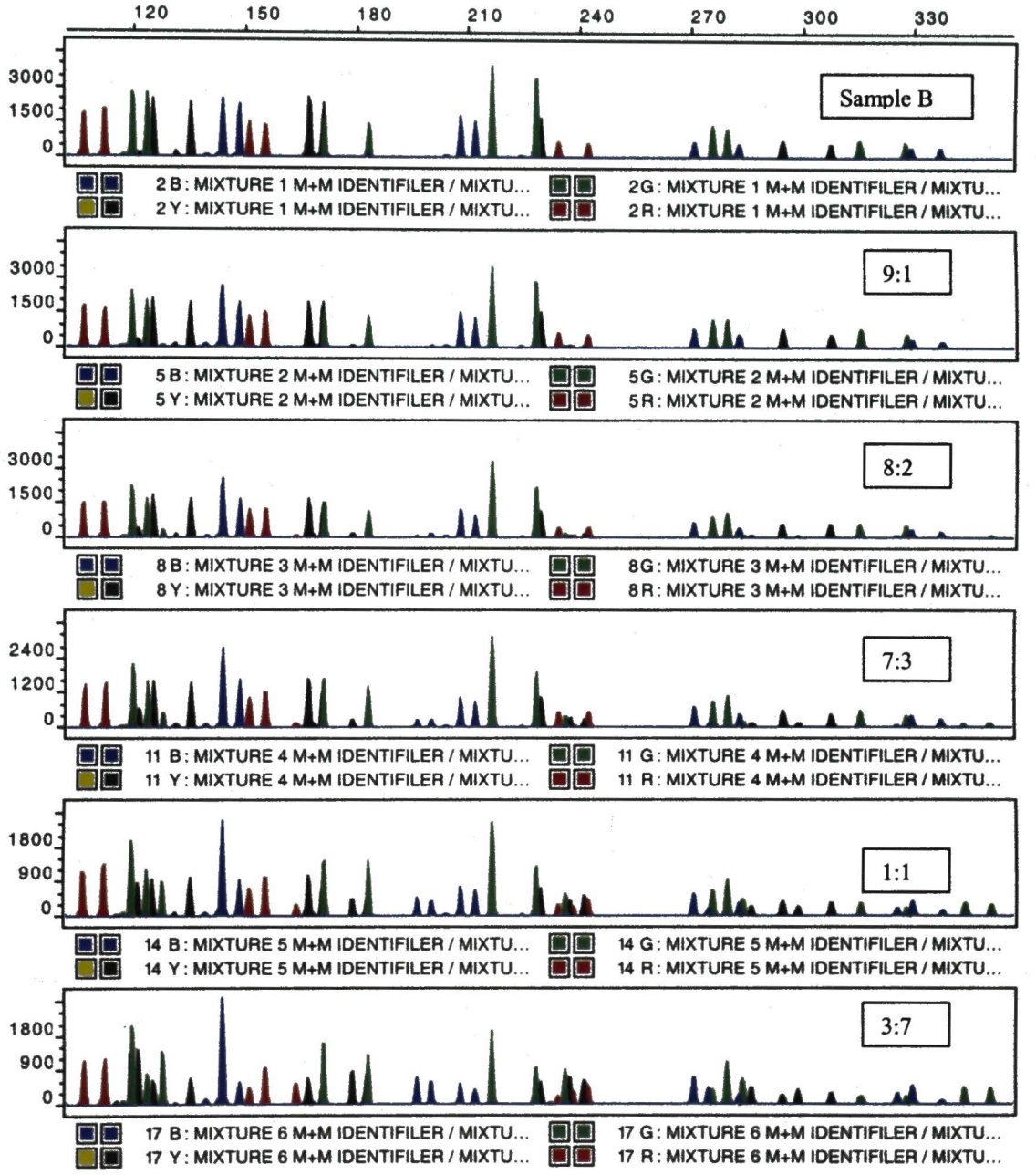
Initially, blood samples from three female and four males were extracted and quantitated. For this study, two male and one female sample (1 ng of total template DNA) was mixed at ratios of 1:0, 9:1, 8:2, 7:3, 1:1, 3:7, 2:8, 1:9 and 0:1.

Table 13- STR DNA profiles of the female and two male reference blood samples used in the mixture experiment. The STR DNA profiles were analyzed at 1.25 ng of input genomic DNA. AME = Amelogenin

	D3S1358	D5S818	D13S317	D7S820	D16S539	CSF1PO	THO1	TPOX
Sample A FEMALE	15,15	11,12	11,12	10,14	11,12	10,12	7,9	8,9
Sample B MALE 1	14,15	11,12	8,11	10,13	11,12	12,14	6,9	8,8
Sample C MALE 2	14,16	12,14	8,13	10,11	12,13	11,12	6,9	10,11

D18S51	D21S11	vWA	FGA	D8S1179	AME	Penta D	Penta E	D2S1338	D19S433
16,18	31.2,32.2	15,16	22,24	12,15	X X	9,10	8,9	17,25	11,15.2
15,18	30,31	14,15	22,24	13,14	X Y	9,12	10,12	12,14	15,17.2
13,16	27,28	17,18	23,24	13,13	X Y	12,13	16,19	24,26	13.2,14

Figure 20. The DNA samples from two male individuals are shown at defined mixture ratios. Samples were amplified using the AmpFℓSTR® Identifiler™ PCR Amplification Kit. The minor contribution was resolved at <10% of the total DNA. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (bp).



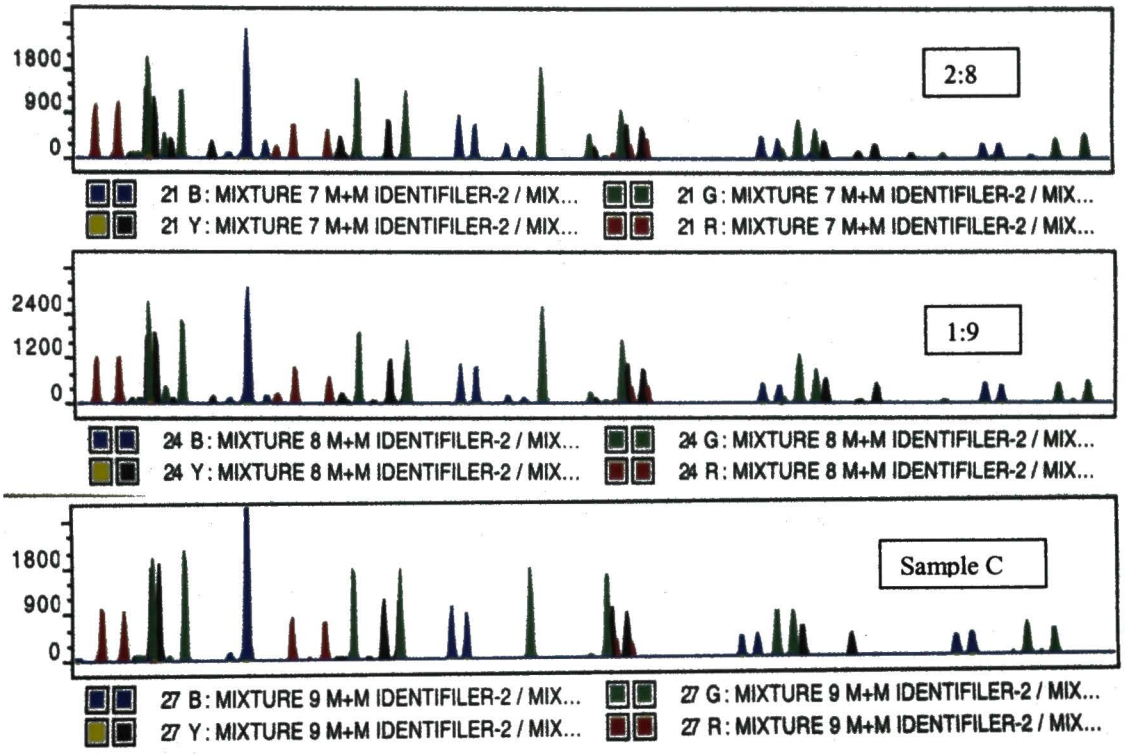
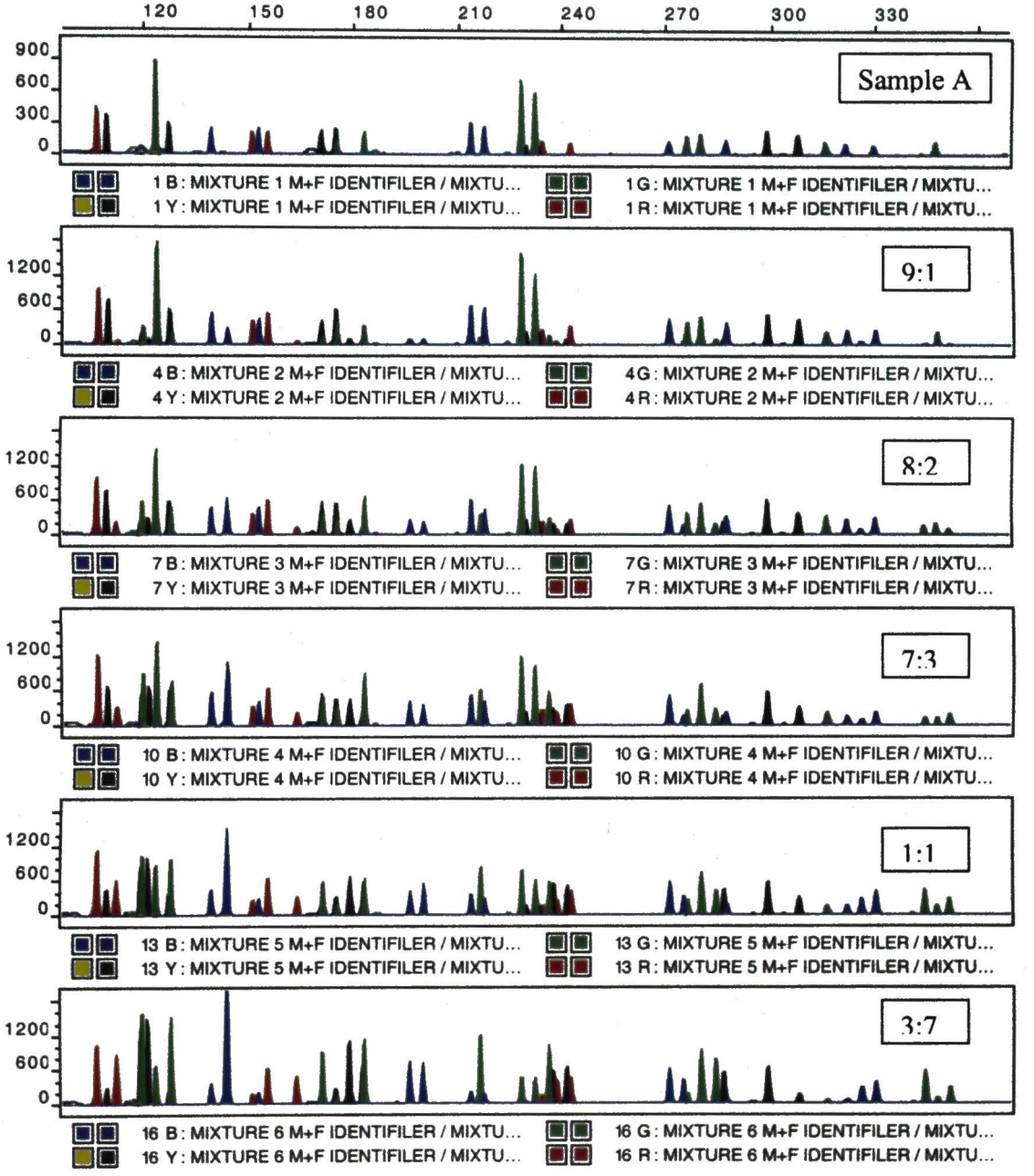


Figure 20. continued

Figure 21. The DNA samples from one male and female individual are shown at defined mixture ratios. Samples were amplified using the AmpFℓSTR® Identifiler™ PCR Amplification Kit. The minor contribution was resolved at <10% of the total DNA. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (bp).



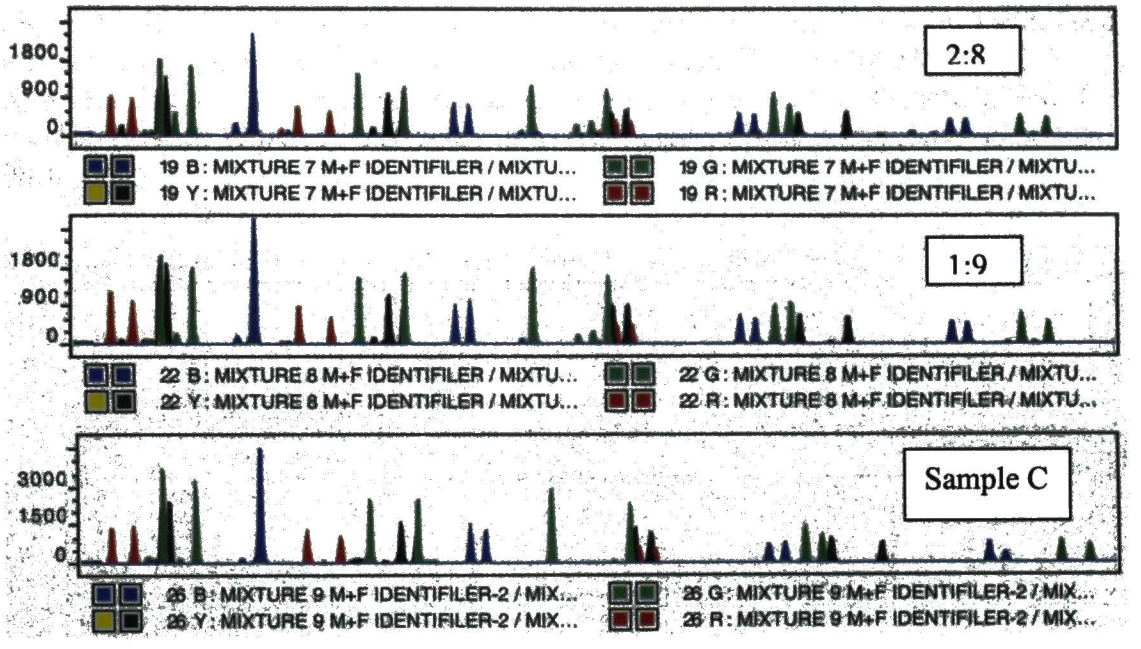
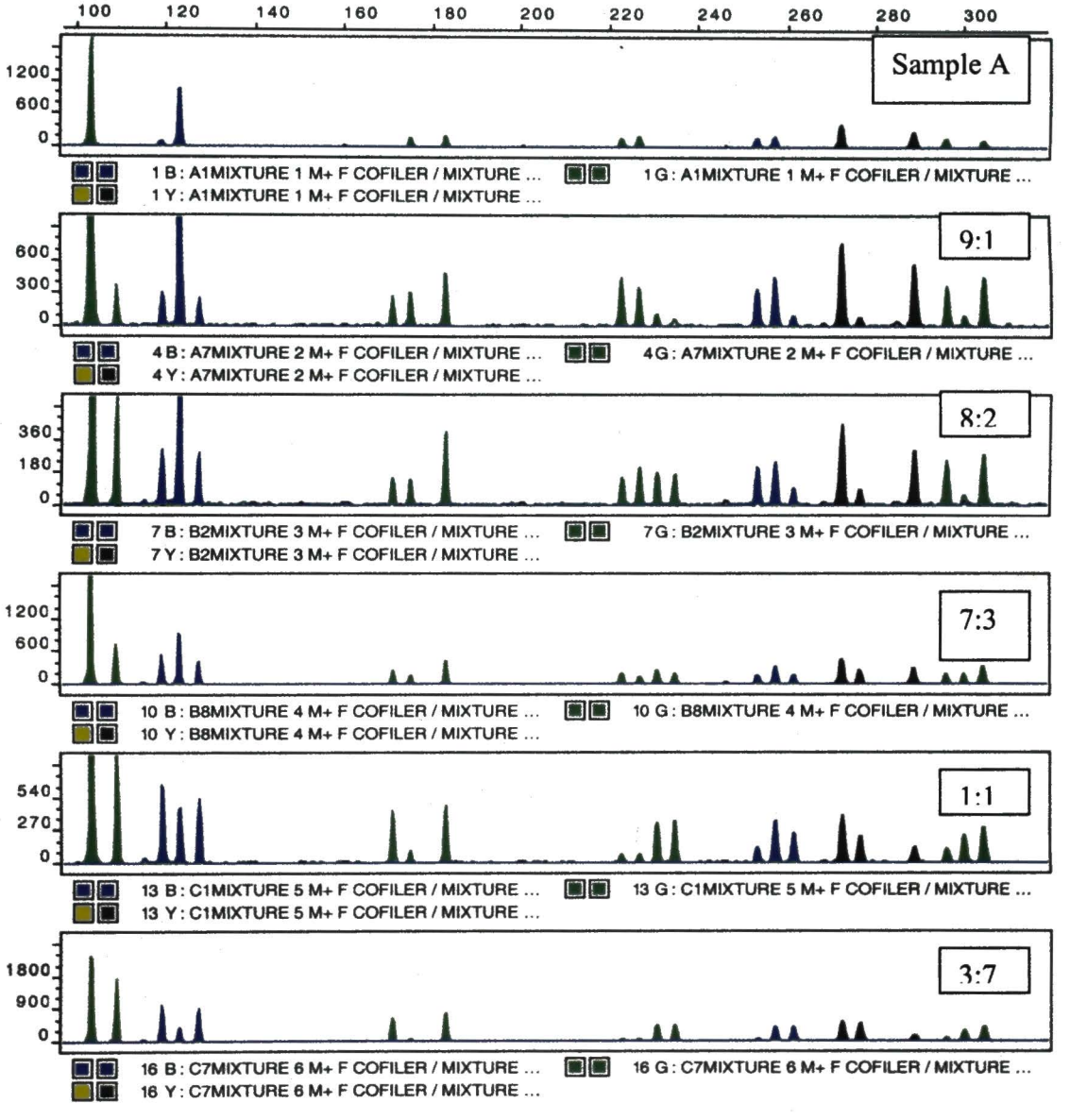


Figure 21. continued

Figure 22. The DNA samples from one male and female individual are shown at defined mixture ratios. Samples were amplified using the AmpFℓSTR® COfiler™ PCR Amplification Kit. The minor contribution was resolved at <10% of the total DNA. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (bp).



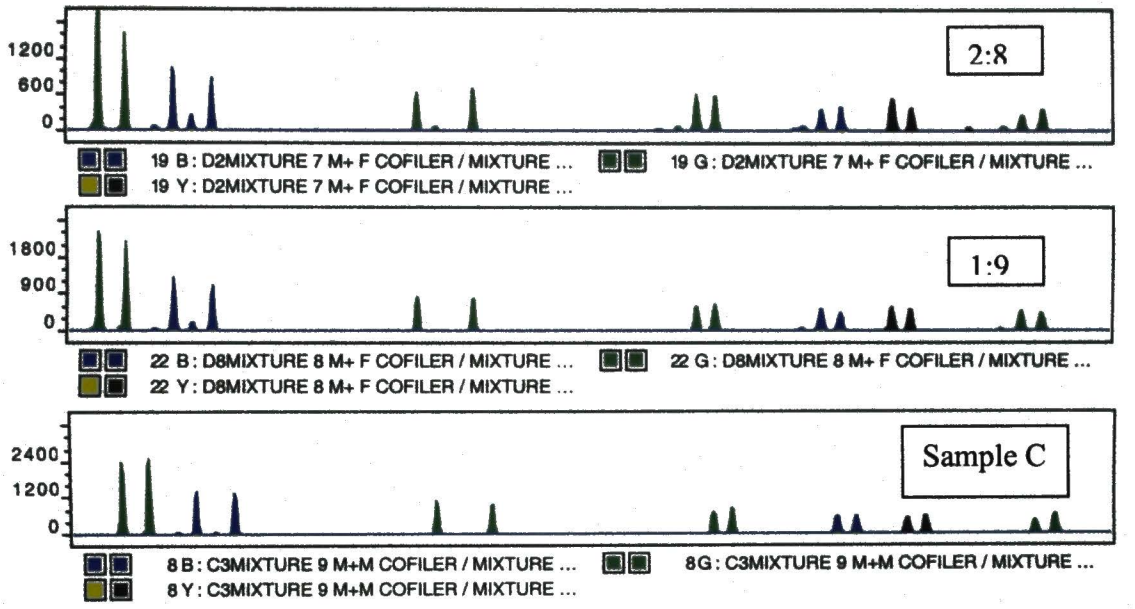
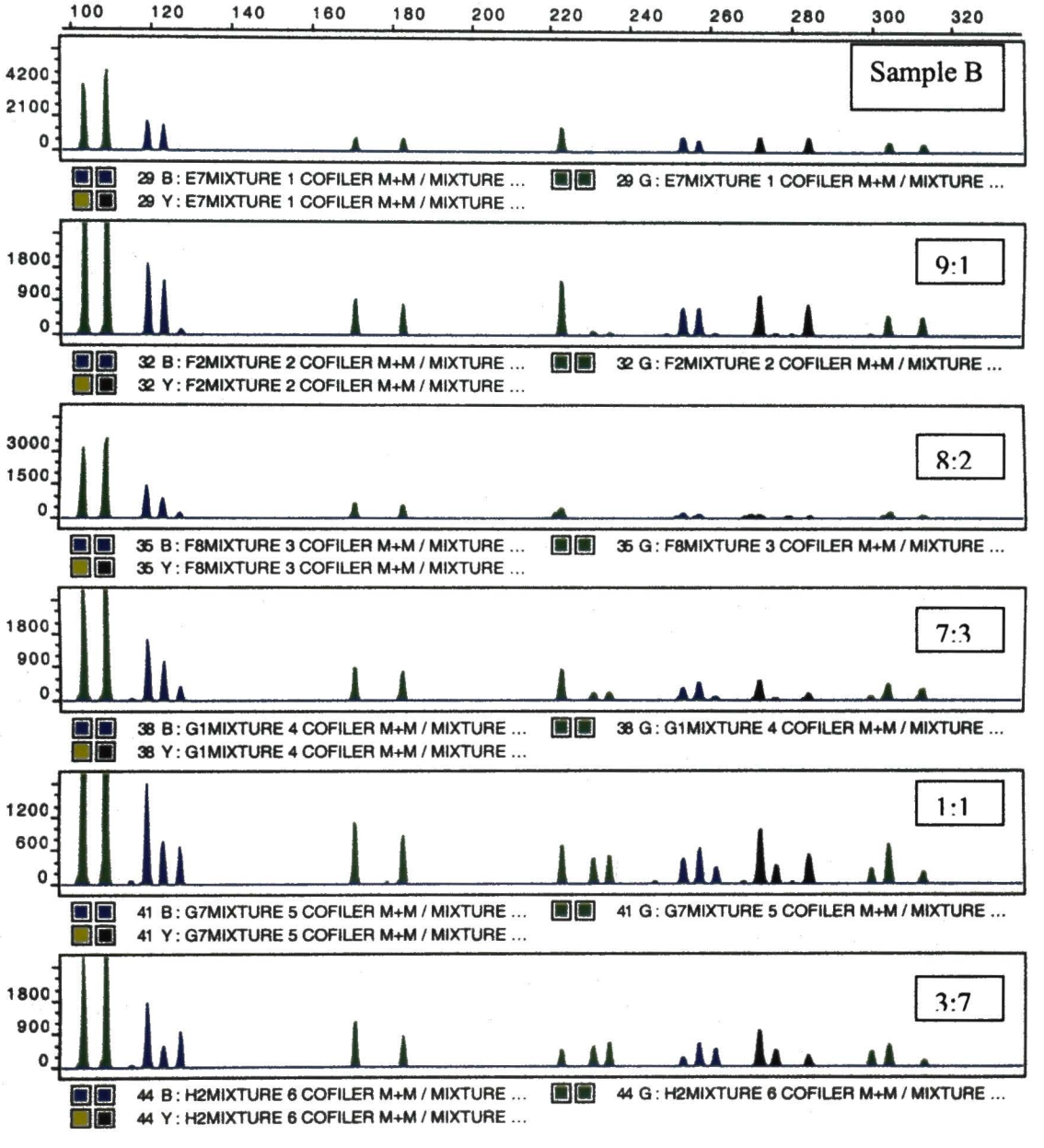


Figure 22. continued

Samples A, B and C share numerous alleles at various loci. Shared peaks RFU values, present in the stutter positions, were generally greater than that expected in a single source sample (≤10%). Peak heights ranged from 17% to 60%. Table 12, shows that the mean peak height ratio for most STR loci is generally greater than 85%. Some loci exhibit mean peak height ratios less than 85%. In the mixtures between the two male individuals, the amelogenin peak heights remained balanced (>88%) as each component was decreased and increased. In the mixtures between the female and male pair, the Y-allele was detected at a mixture ratio of 9:1 and the peak height ratio between the X/Y pair increased progressively as the female component decreased. Minor alleles were detected at the mixture ratio of 1:9 and 9:1. However, many of these alleles were less than 100 RFU.

Figure 23. The DNA samples from two male individuals are shown at defined mixture ratios. Samples were amplified using the AmpFℓSTR® COfiler™ PCR Amplification Kit. The minor contribution was resolved at <10% of the total DNA. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (bp).



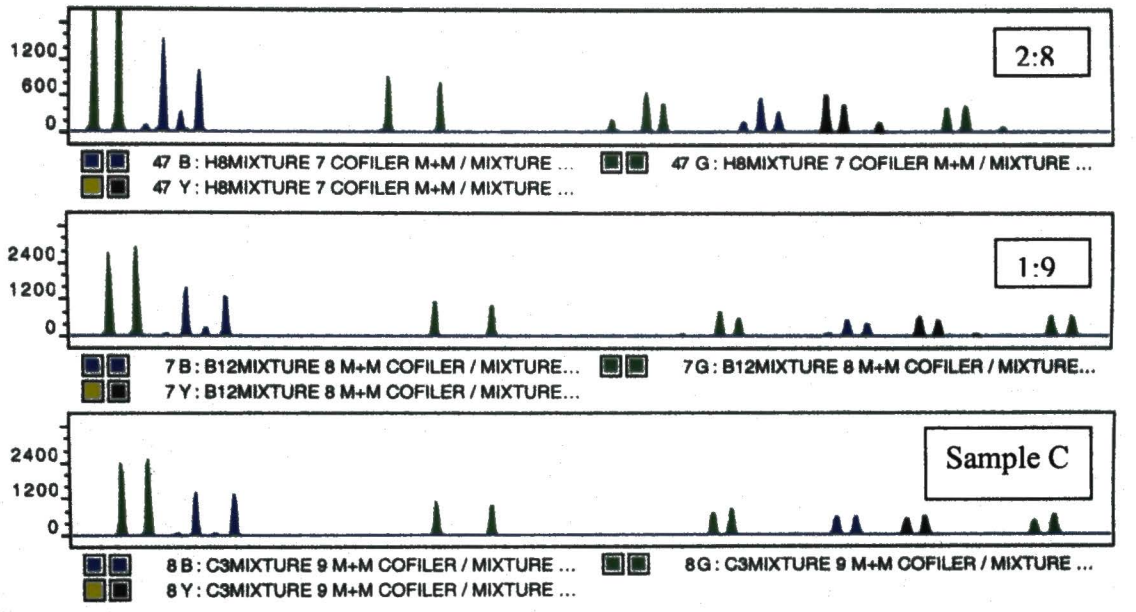
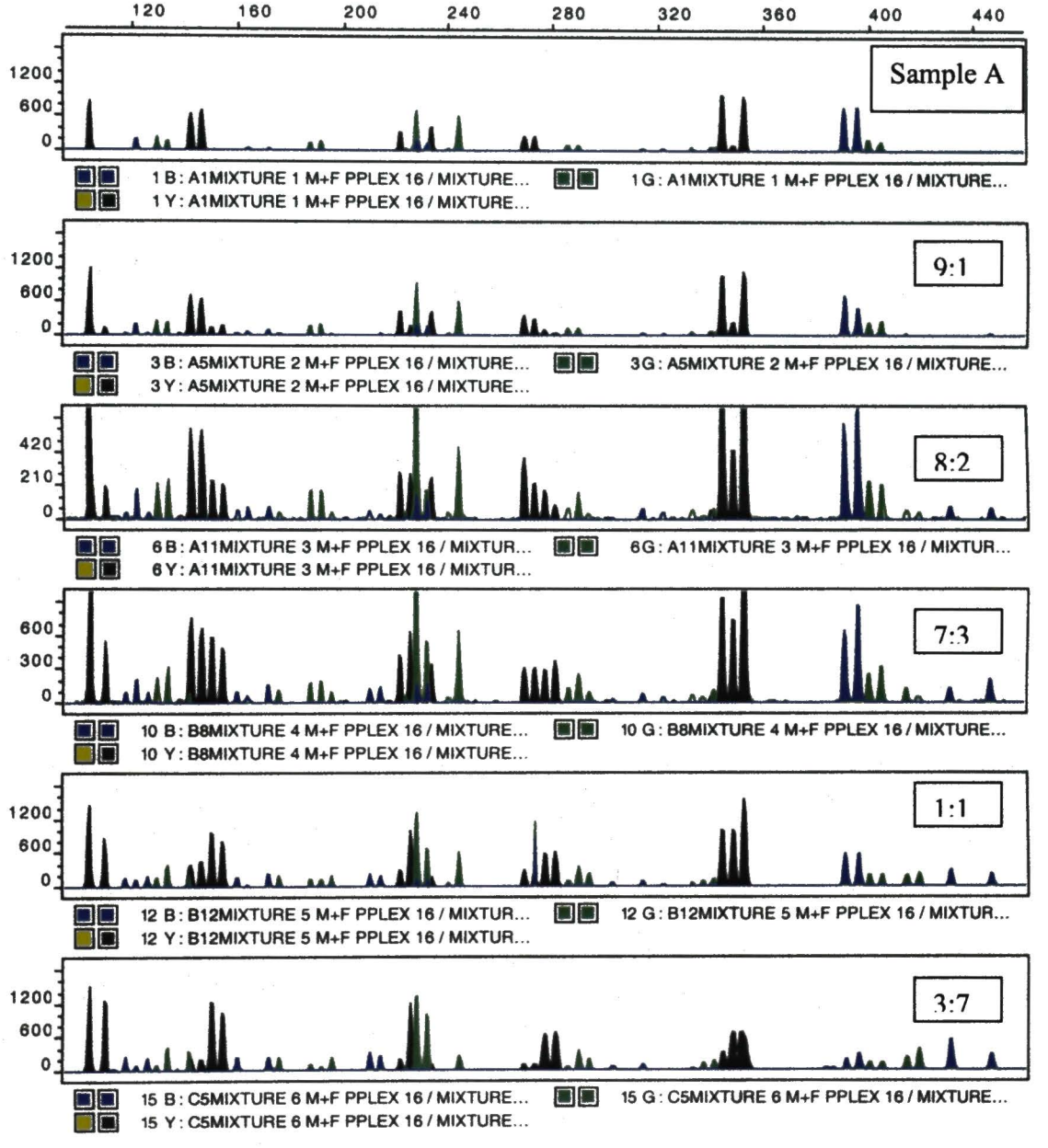


Figure 23 continued

As mixture ratios progress from 1:0 to 1:1 (and at the reverse ratios), stutter decreased in non-overlapping areas. Stutter, not previously seen, appeared as one component was increased relative to the other component. At the 2:8, 8:2, 3:7 and 7:3 ratios minor and major components in the DNA profiles were able to, generally, be resolved by examining peak height ratios. Also at these ratios, the generation of combinations of possible alleles pairs and homozygote peaks was possible. When alleles were shared between individuals, these peak height were much greater than peak heights of the other alleles at that locus. As one component of the mixtures was decreased, previously minor alleles became the dominant, major, component. At a 1:1 mixture ratio, it was difficult, at times, to discern major and minor contributions. However, no more than 4 alleles were present at any locus, suggesting a mixture between two individuals.

Figure 24. The DNA samples from one male and female individual are shown at defined mixture ratios. Samples were amplified using the PowerPlex™ 16 PCR Amplification Kit. The minor contribution was resolved at <10% of the total DNA. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (bp).



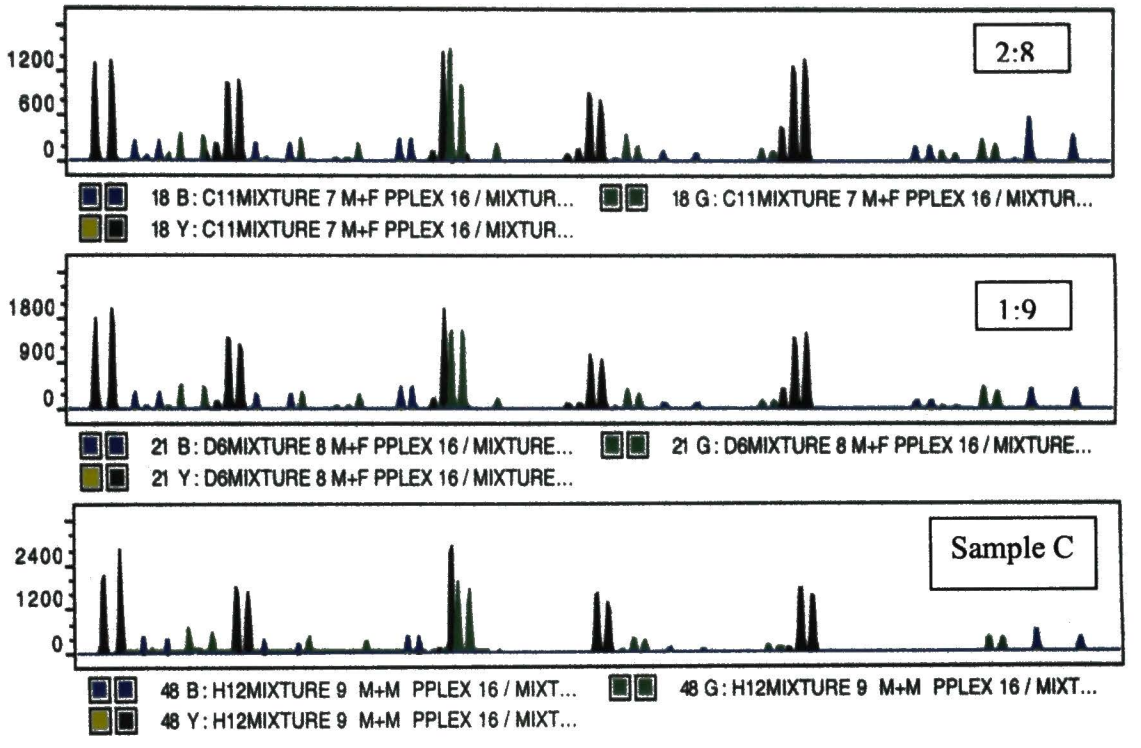
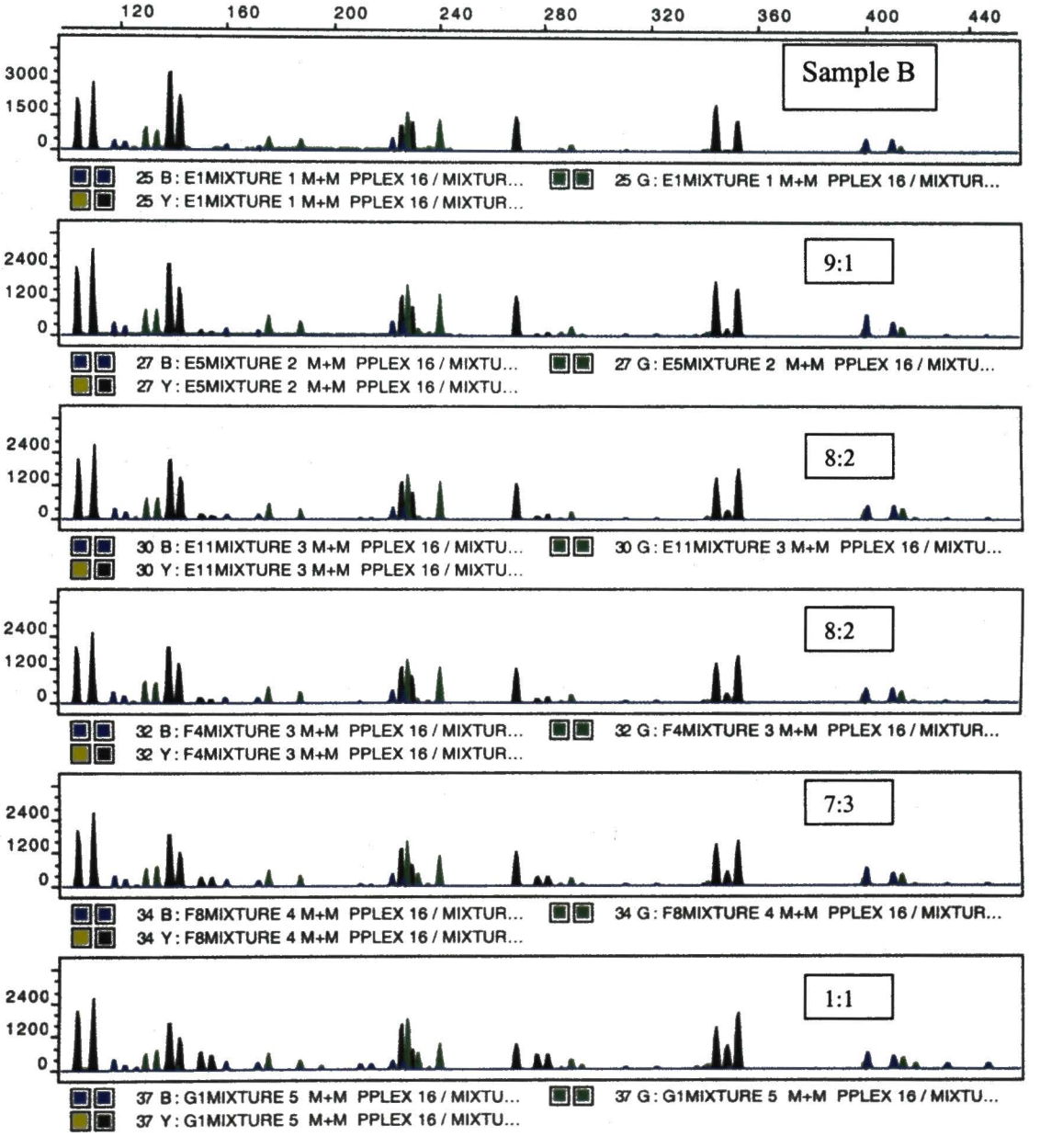


Figure 24 continued

Figure 25. The DNA samples from two male individuals are shown at defined mixture ratios. Samples were amplified using the PowerPlex™ 16 PCR Amplification Kit. The minor contribution was resolved at <10% of the total DNA. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (bp).



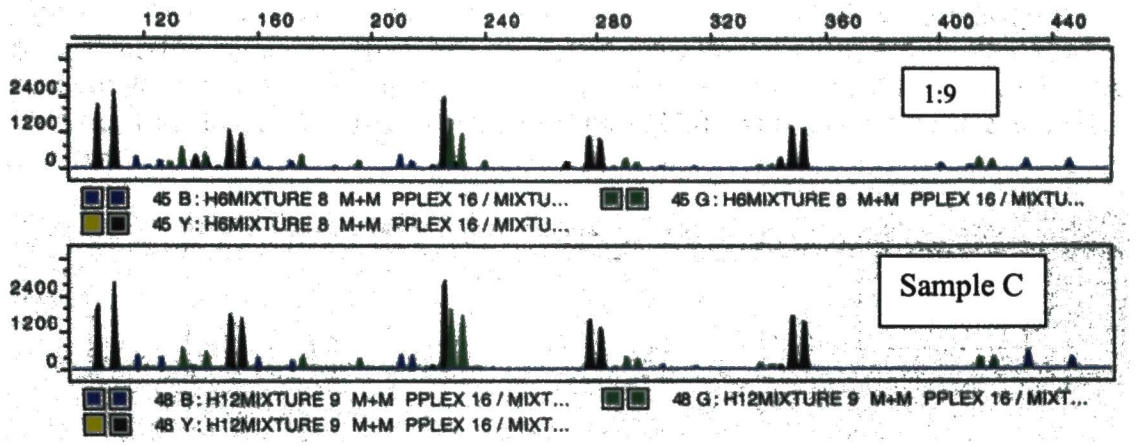
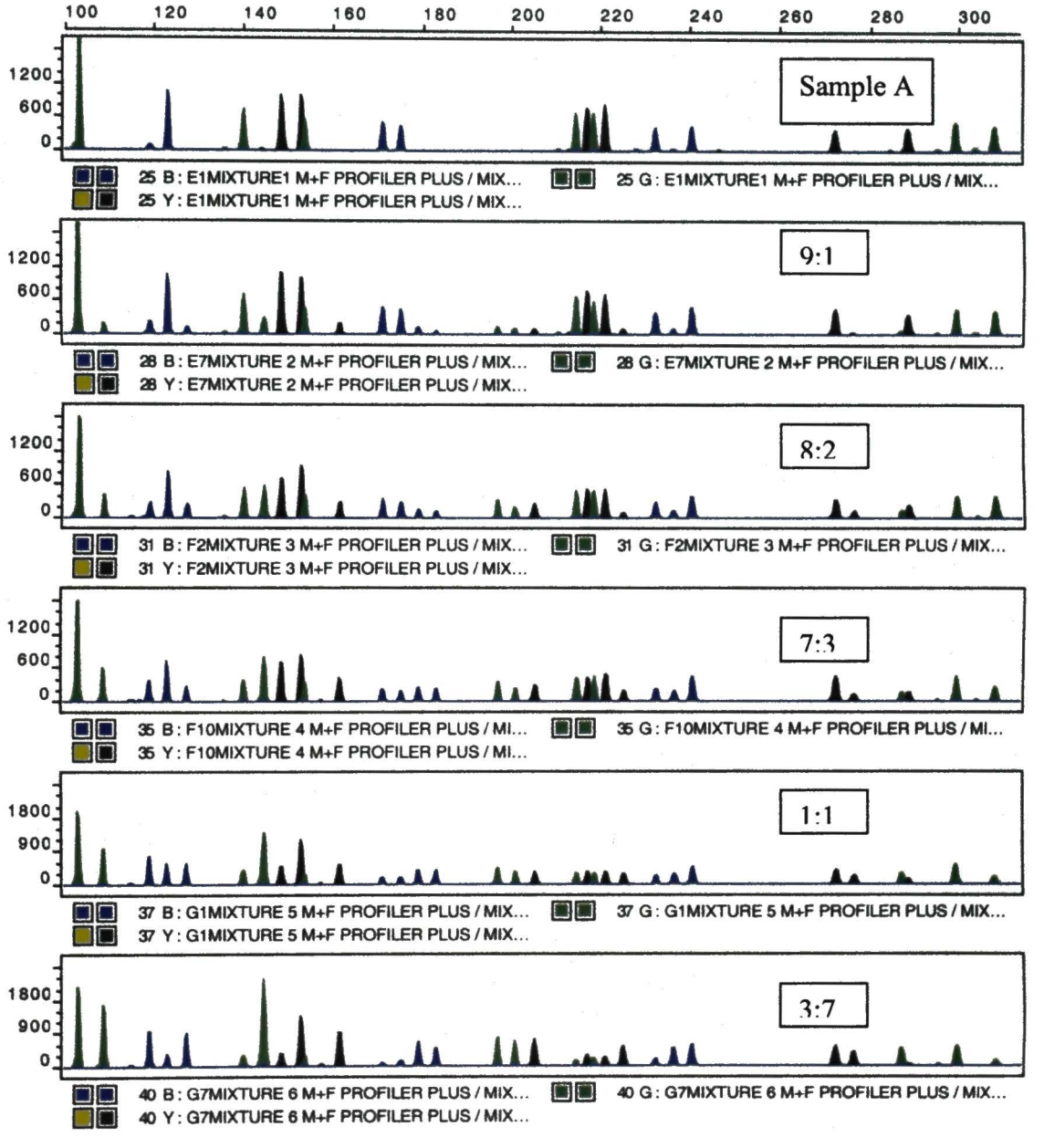


Figure 25 continued

Figure 26. The DNA samples from one female and one male individual are shown at defined mixture ratios. Samples were amplified using the AmpFlSTR® Profiler Plus™ PCR Amplification Kit. The minor contribution was resolved at <10% of the total DNA. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (bp).



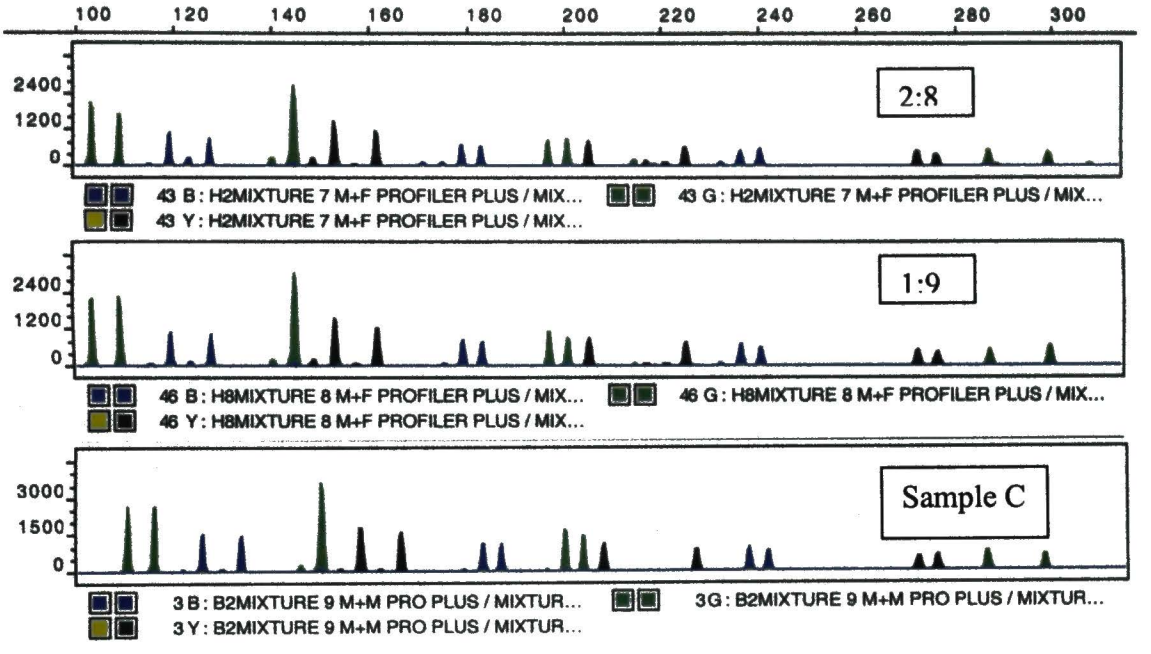
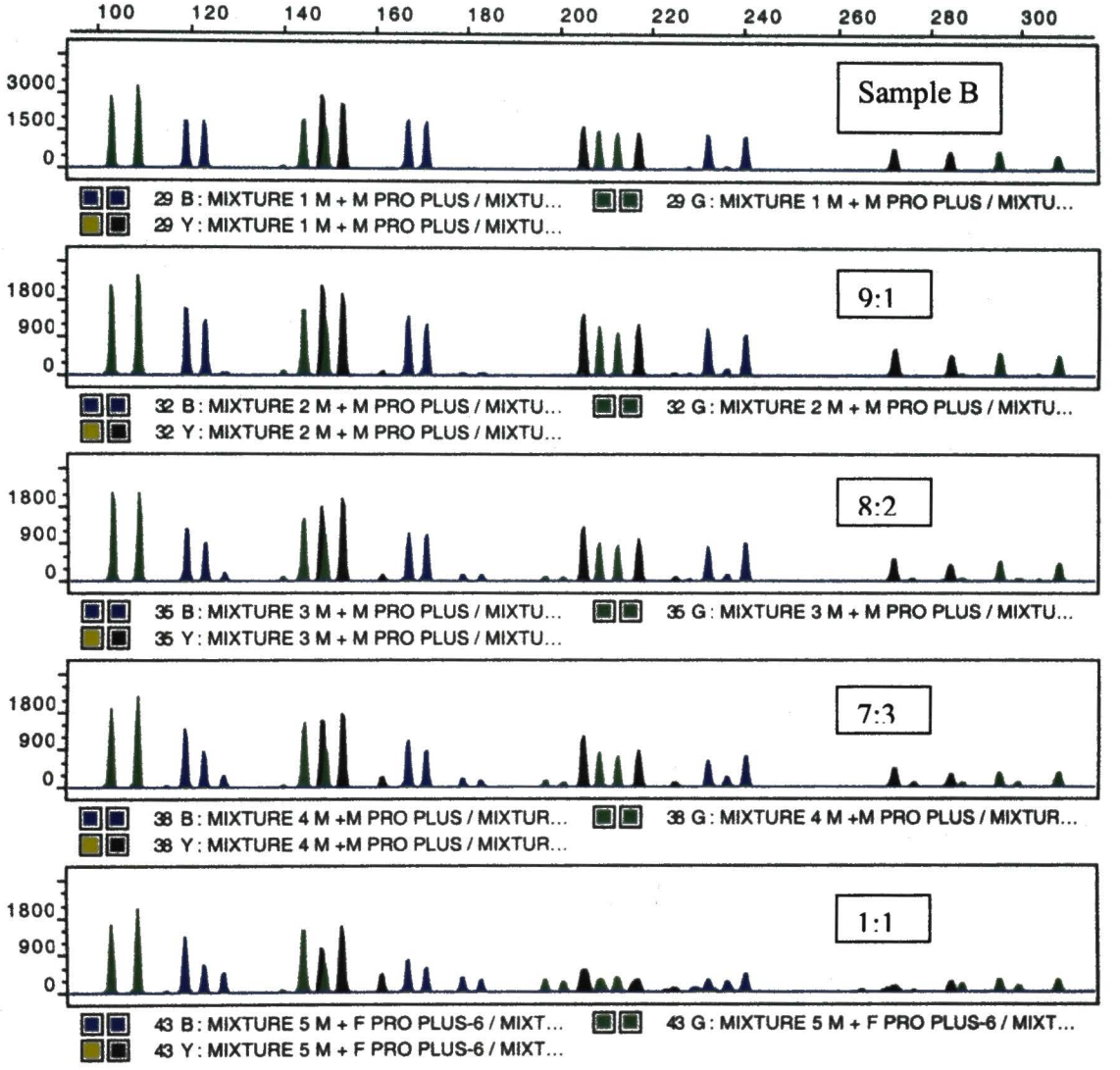


Figure 26 continued

Figure 27. The DNA samples from two male individuals are shown at defined mixture ratios. Samples were amplified using the AmpFlSTR® Profiler Plus™ PCR Amplification Kit. The minor contribution was resolved at <10% of the total DNA. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (bp).



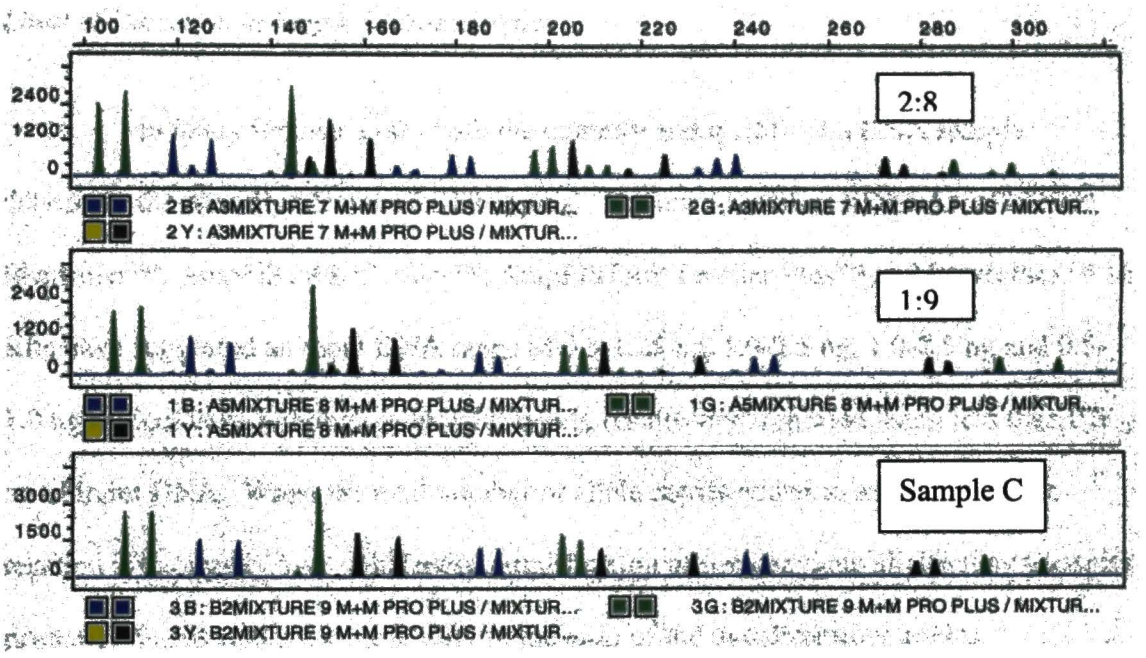


Figure 27 continued

Limit of Detection in Single Source samples

In many forensic cases both the quantity and quality of a DNA sample determine the overall outcome of STR typing. The manufacturers of the AmpFlSTR® Identifiler™, AmpFlSTR® COfiler™, AmpFlSTR® Profiler Plus™ and PowerPlex™ 16 Kits have suggested an input DNA range of 0.5-1.25 ng, 1.0-2.5 ng, 1.0-2.5 ng and 0.5-1.0 ng respectively, although successful typing results were reported using less than 0.5 ng of input DNA. When the total number of allele copies added to an amplification reaction is extremely low, unbalanced amplification of heterozygote alleles may transpire primarily due to stochastic variability in the ratio of the two dissimilar alleles.

In this experiment, 5.0, 1.25, 1.0, 0.75, 0.50, 0.25, 0.125, 0.10, 0.05 and 0.01 ng of extracted DNA taken from two buccal swab samples, one from a female individual (Sample A) and one from a male individual (Sample B), were amplified using the AmpFlSTR® Identifiler™, AmpFlSTR® COfiler™, AmpFlSTR® Profiler Plus™ and PowerPlex™ 16 Amplification Kits.

Table 14- STR DNA typing results taken from one female and one male reference blood samples used in the mixture experiment. Sample A and Sample B were typed using 1.25 ng of input genomic DNA.

	D3S1358	D5S818	D13S317	D7S820	D16S539	CSF1PO	THO1	TPOX
Sample A FEMALE	15,15	11,12	11,12	10,14	11,12	10,12	7,9	8,9
Sample B MALE	14,15	11,12	8,11	10,13	11,12	12,14	6,9	8,8

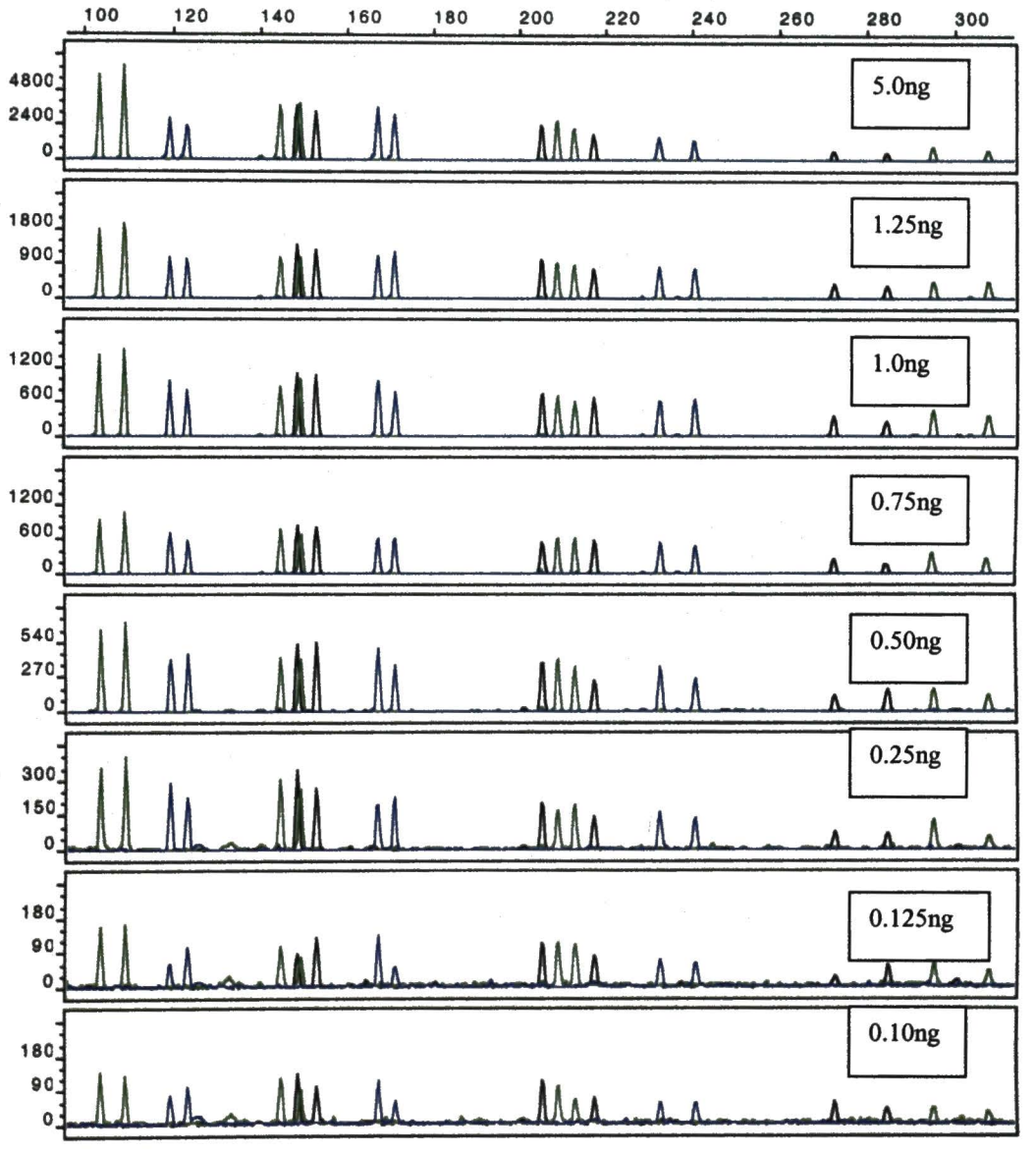
D18S51	D21S11	vWA	FGA	D8S1179	AME	Penta D	Penta E	D2S1338	D19S433
16,18	31.2,32.2	15,16	22,24	12,15	X X	9,10	8,9	17,25	11,15.2
15,18	30,31	14,15	22,24	13,14	X Y	9,12	10,12	12,14	15,17.2

Using the AmpFlSTR® Profiler Plus™, COfiler™ and Identifiler™ Kits, samples amplified at 5.0 ng displayed off-scale peaks. Amplifications at 5.0 ng using the PowerPlex™ 16 Kit did not display any off-scale peaks. Off-scale data results when too much sample DNA is added to a PCR reaction mixture and the fluorescence intensity from the PCR products have exceed the linear dynamic range for detection by the ABI Prism® 310 Genetic Analyzer. Off-scale data is represented on an electropherogram as a vertical red line through the peaks, or alleles, which have exceeded the liner detection range of the ABI Prism® 310 Genetic Analyzer (5).

AmpFlSTR® Profiler Plus™

In Sample A, all alleles were present between 5.0 to 0.25 ng and no allelic dropout was observed. Allelic dropout can be explained as occurring when one or both alleles at a locus fall below the set minimum RFU threshold (Figure 26). Usually, the smaller alleles are balanced and the larger loci show a significant decrease in peak height. Loci were balanced between 1.0 to 0.50 ng. Differential amplification, or locus imbalance, was observed at 5.0 to 1.25 ng. Off-scale peaks were observed at the D8S1179 and D5S818 loci, at 5.0 ng of input DNA. Minimal stutter was observed at 0.50ng and no stutter was observed at 0.25 ng of input DNA. Allelic dropout was observed at 0.125ng. The following loci displayed either one or no alleles at 0.125 ng: vWA, FGA, D8S1179, D21S11, D18S51, D13S317 and D7S820. No alleles were detected at 0.01 ng. In Sample B, all homozygote and heterozygote alleles were present between 5.0 to 0.50 ng (Figure 27). All loci and alleles were balanced between 1.25 and 0.50 ng of input DNA. Off-scale data was only observed at the amelogenin locus (5.0 ng). Allelic dropout occurred at 0.25 ng but only at the D18S1179 locus. No stutter was detected at 0.50 ng of input DNA. The X-Y gender pair was present down to 0.10ng and only the x-allele was present at 0.05 ng. No alleles were detected at 0.01 ng.

Figure 28- Samples were amplified using the AmpFℓSTR® Profiler Plus™ Amplification Kit at varying amounts of template DNA ranging from 5.0 ng to 0.01 ng. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (base pairs-bp). Notice the decline in RFU value as the amount of template DNA decreases. Samples were obtained from a male individual.



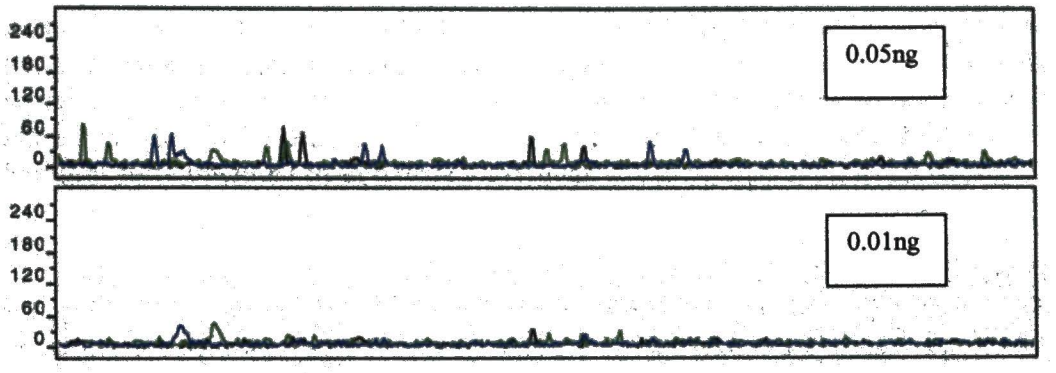
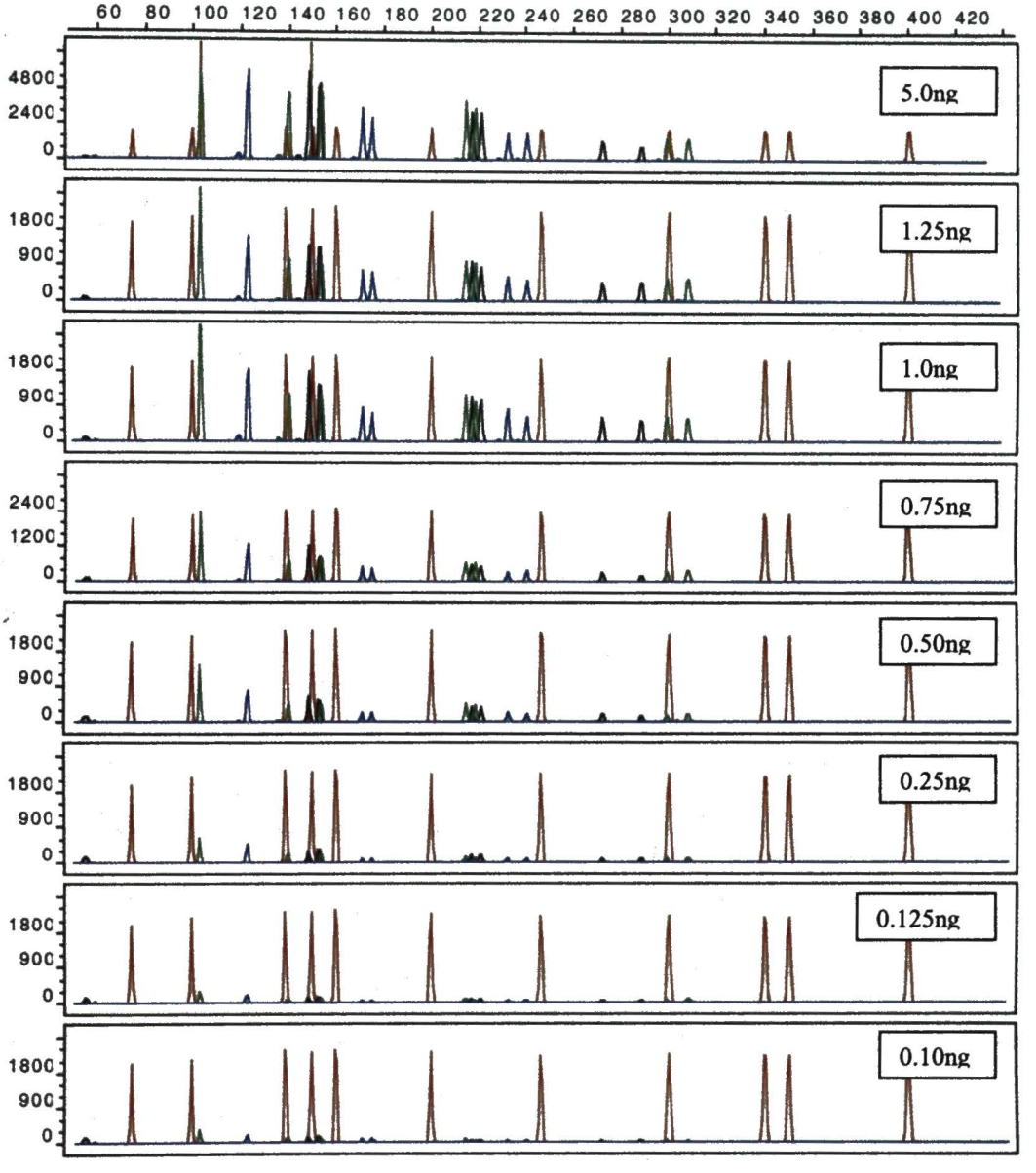


Figure 28 continued – Alleles are visualized at 0.01 and 0.05ng of input DNA. However, RFU values fall below the 75 RFU cutt-off.

Figure 29- Samples were amplified using the AmpFℓSTR® Profiler Plus™ Amplification Kit at varying amounts of template DNA ranging from 5.0 ng to 0.01 ng. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (base pairs-bp). Notice the decline in RFU value as the amount of template DNA decreases. Samples were obtained from a male individual.



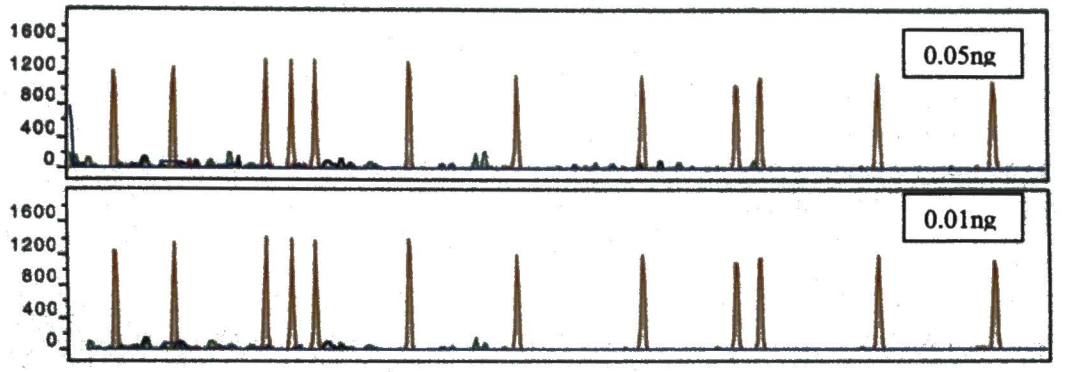
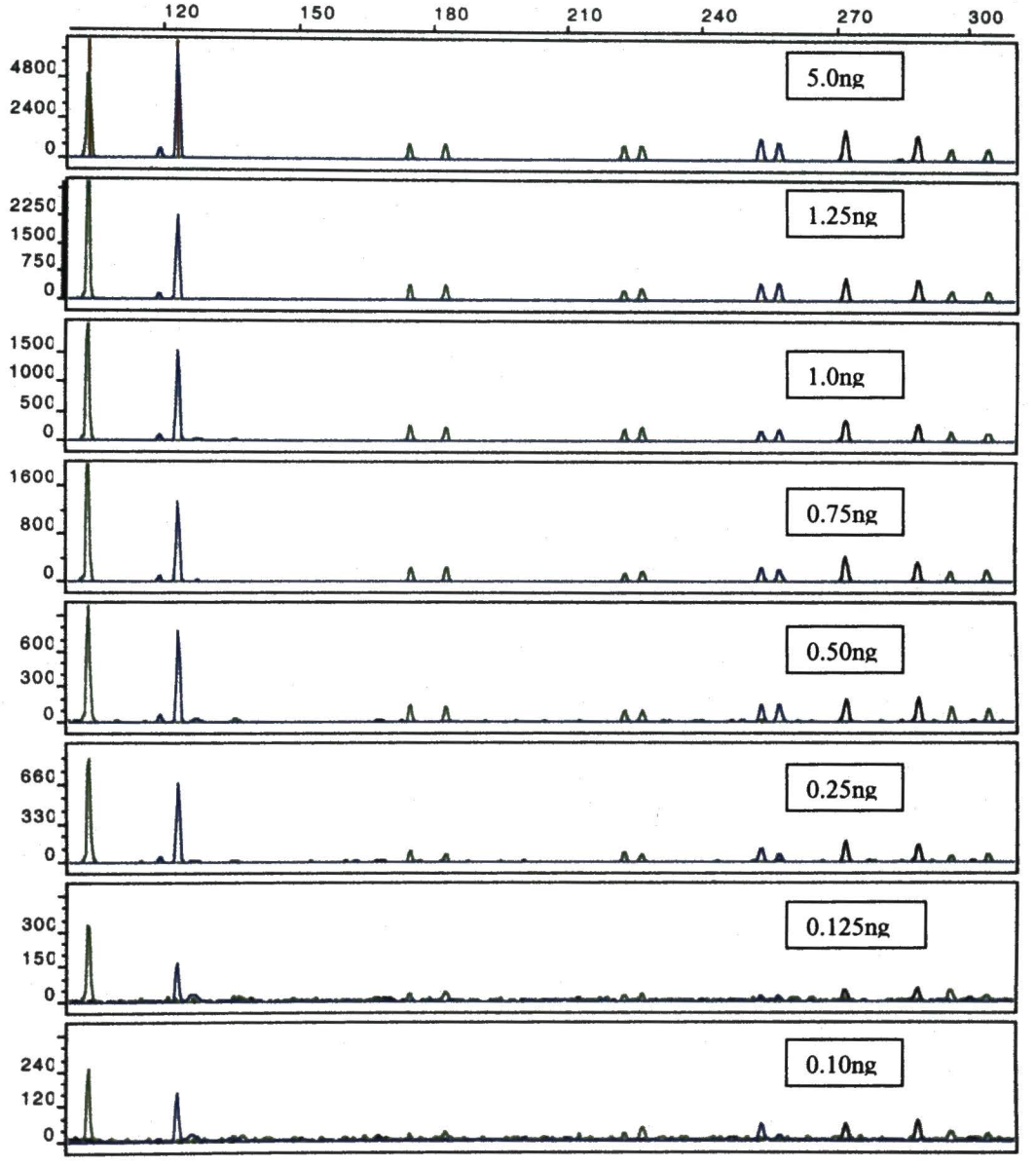


Figure 29 continued

AmpFlSTR® COfiler Plus™

In Sample A, all alleles were present between 5.0 to 0.50 ng and no allelic dropout was observed. Loci were balanced between 1.0 to 0.50 ng (Figure 28). Off-scale peaks were observed at the amelogenin locus, at 5.0 ng of input DNA. Stutter was observed between 5.0 to 0.75 ng of input DNA. Allelic dropout was observed at 0.25 ng but only at the CSF1PO locus. Only the 15-allele at the D3S1358 and the X-locus were detected at 0.125 ng, 0.10 ng and 0.05 ng. No alleles were detected at 0.01 ng. In Sample B (Figure 29), all homozygote and heterozygote alleles were present between 5.0 to 0.25 ng. Off-scale data was only observed at the amelogenin locus (5.0 ng). Allelic dropout occurred at 0.125 ng but only at the CSF1PO locus. Locus imbalance was observed between 1.25 to 0.25ng of input DNA. No stutter was detected at 0.50 ng of input DNA. The X-Y gender pair was present down to 0.10ng and only the x-allele was present at 0.05 ng. No alleles were detected at 0.01 ng.

Figure 30- Samples were amplified using the AmpFℓSTR® COfiler™ Amplification Kit at varying amounts of template DNA ranging from 5.0 ng to 0.01 ng. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (base pairs-bp). Notice the decline in RFU value as the amount of template DNA decreases. Samples were obtained from a female individual.



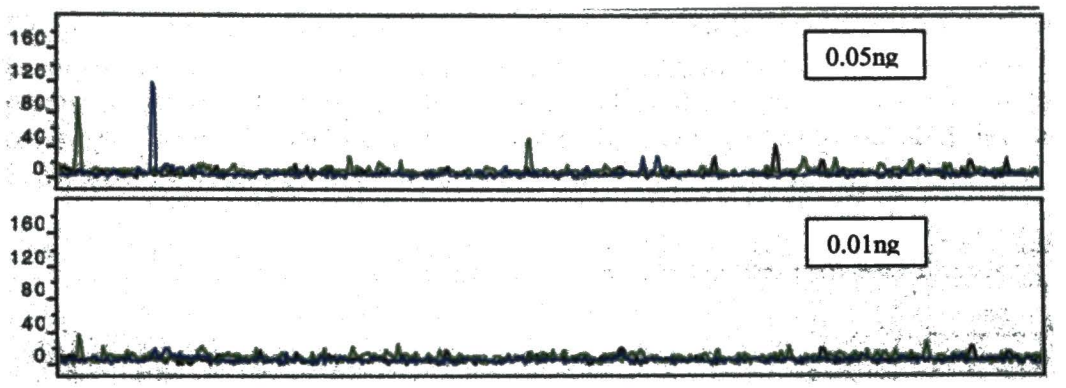
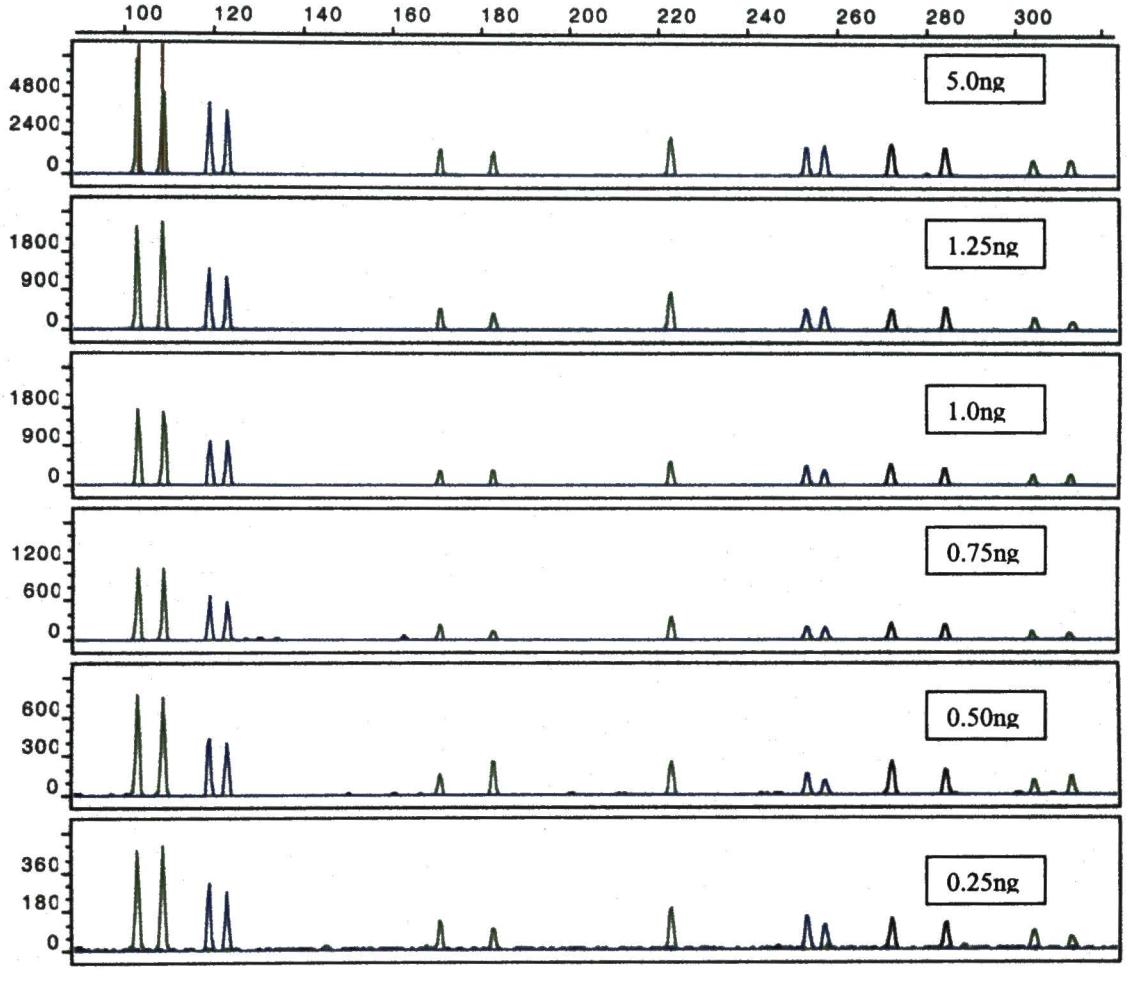


Figure 30 continued

Figure 31- Samples were amplified using the AmpFℓSTR® COfiler™ Amplification Kit at varying amounts of template DNA ranging from 5.0 ng to 0.01 ng. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (base pairs-bp). Notice the decline in RFU value as the amount of template DNA decreases. Samples were obtained from a male individual.



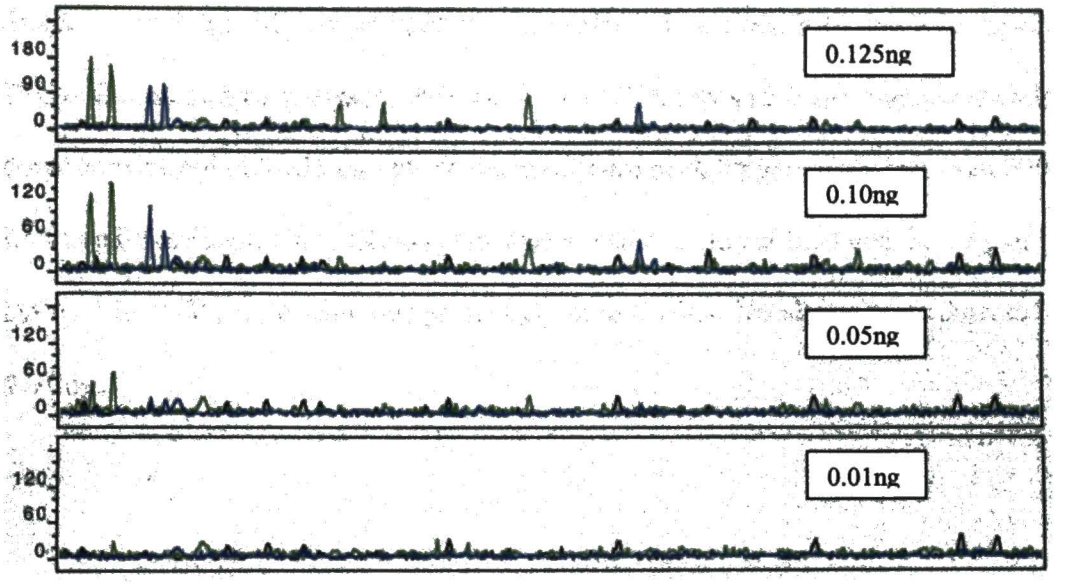


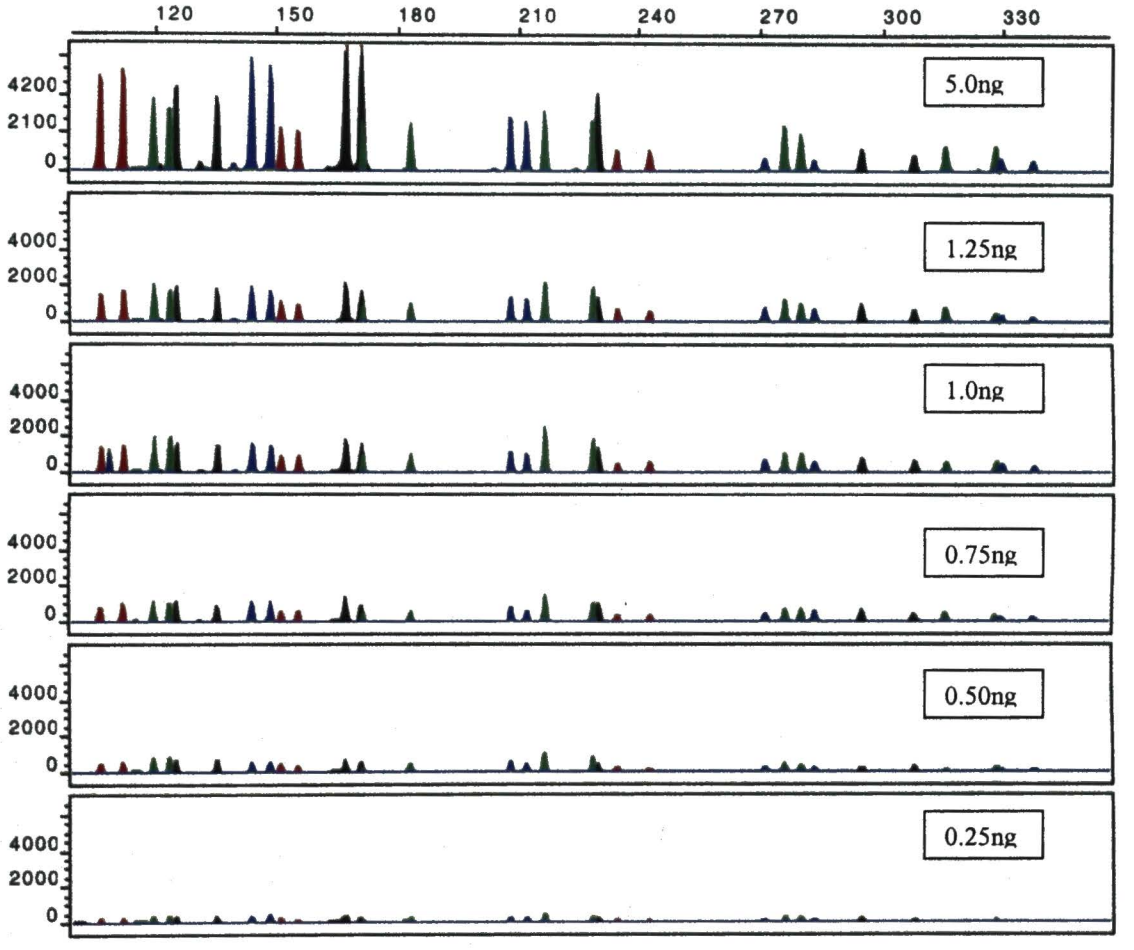
Figure 31 continued

AmpFlSTR® Identifiler™ Kit

In Sample A (Figure 30), all alleles were present between 5.0 to 0.25 ng and no allelic dropout was observed. Loci were balanced between 1.0 to 0.50 ng. Differential amplification, or locus imbalance, and off-scale peaks (D3S1358 and Amelogenin) were observed at 5.0 ng. Loci become balanced and differential amplification is not observed between 1.25 ng to 0.50 ng. Allelic dropout is observed at 0.125 ng. The noise to signal ratio increased at 0.25 ng and the baseline appeared bumpy. Minimal stutter was observed at 0.50ng (D3S1358 and D13S317) and no stutter was observed at 0.25 ng of input DNA. Allelic dropout was observed at 0.125ng. The following loci displayed either one or no alleles at 0.125 ng: FGA, TPOX, D2S1338, D5S818, CSF1PO and D16S539. No alleles were detected at 0.01 ng. In Sample B (Figure 31), all homozygote and heterozygote alleles were present between 5.0 to 0.25 ng. Locus imbalance was

detected at 5.0 ng. All loci and alleles were balanced between 1.25 and 0.25 ng of input DNA. Off-scale data was only observed at the vWA locus (5.0 ng). Significant allelic dropout occurred at 0.125 ng, where the maximum peak heights were less than 200 Relative Fluorescent Units (RFU). No stutter was detected at 0.50 and 0.25 ng of input DNA. The X-Y gender pair was present down to 0.10ng. No alleles were detected at 0.01 ng.

Figure 32- Samples were amplified using the AmpFℓSTR® Identifiler™ Amplification Kit at varying amounts of template DNA ranging from 5.0 ng to 0.01 ng. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (base pairs-bp). Notice the decline in RFU value as the amount of template DNA decreases. Samples were obtained from a male individual.



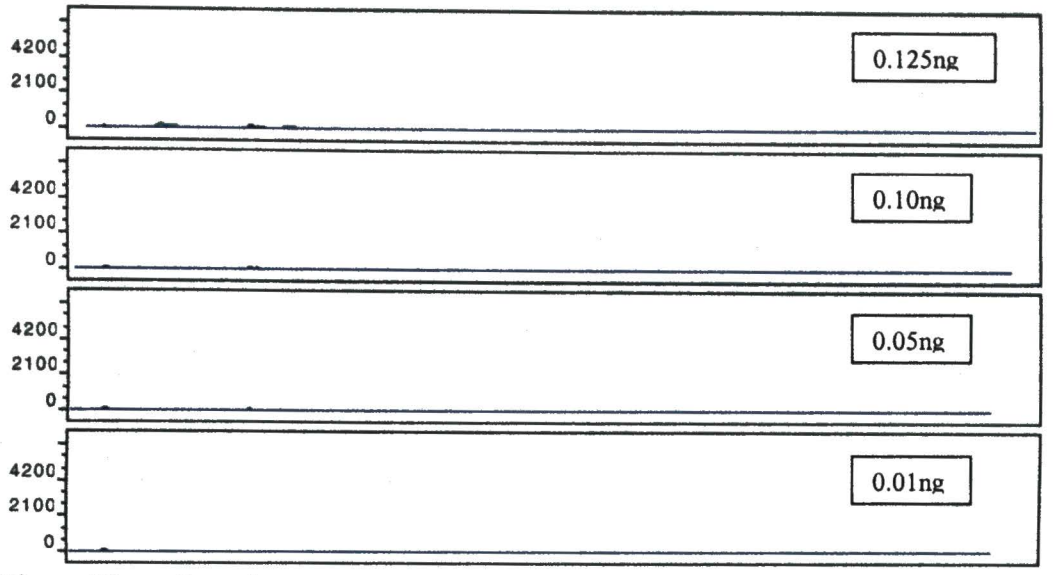
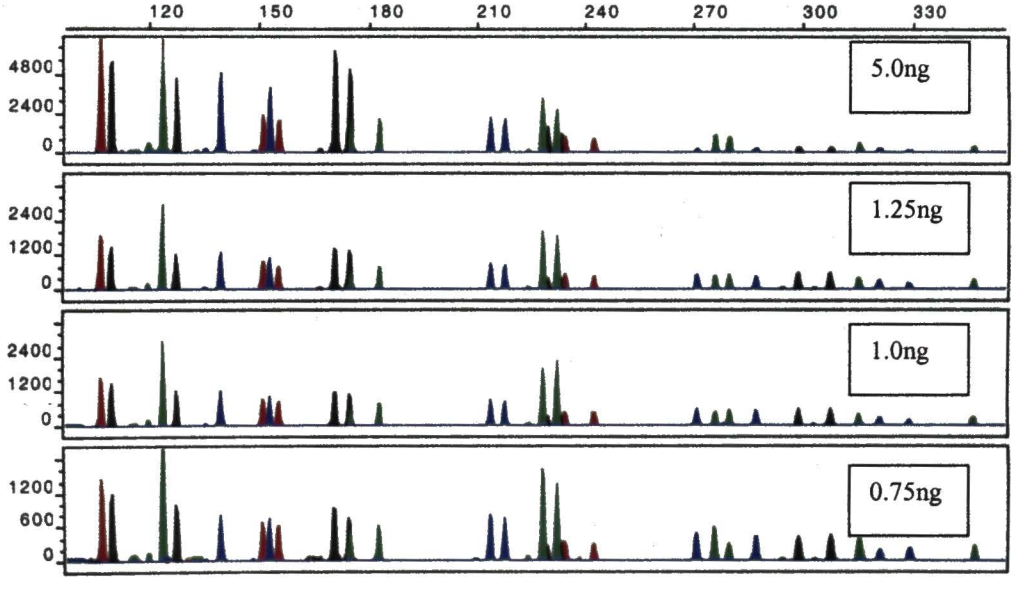


Figure 32 continued

Figure 33- Samples were amplified using the AmpFℓSTR® Identifiler™ Amplification Kit at varying amounts of template DNA ranging from 5.0 ng to 0.01 ng. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (base pairs-bp). Notice the decline in RFU values as the amount of template DNA decreases. Samples were obtained from a female individual.



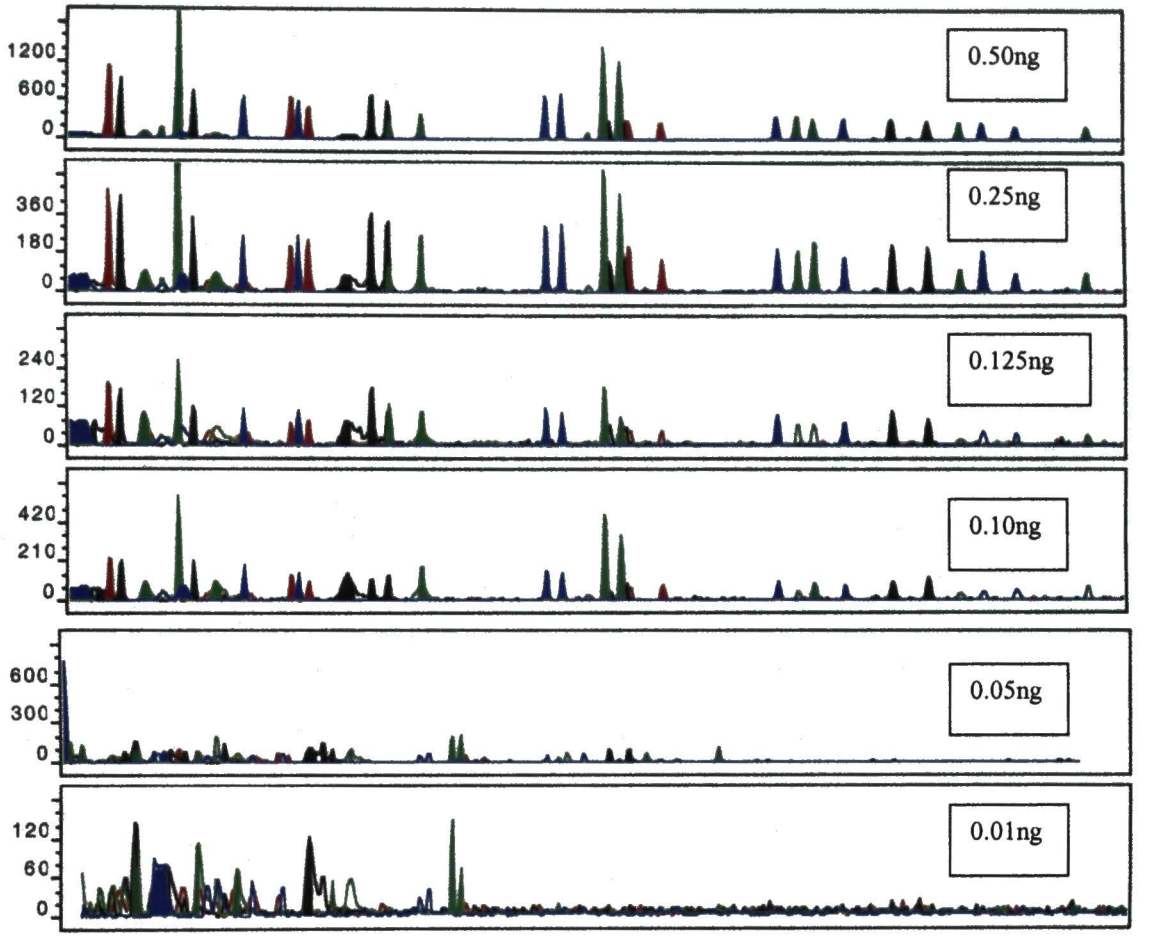
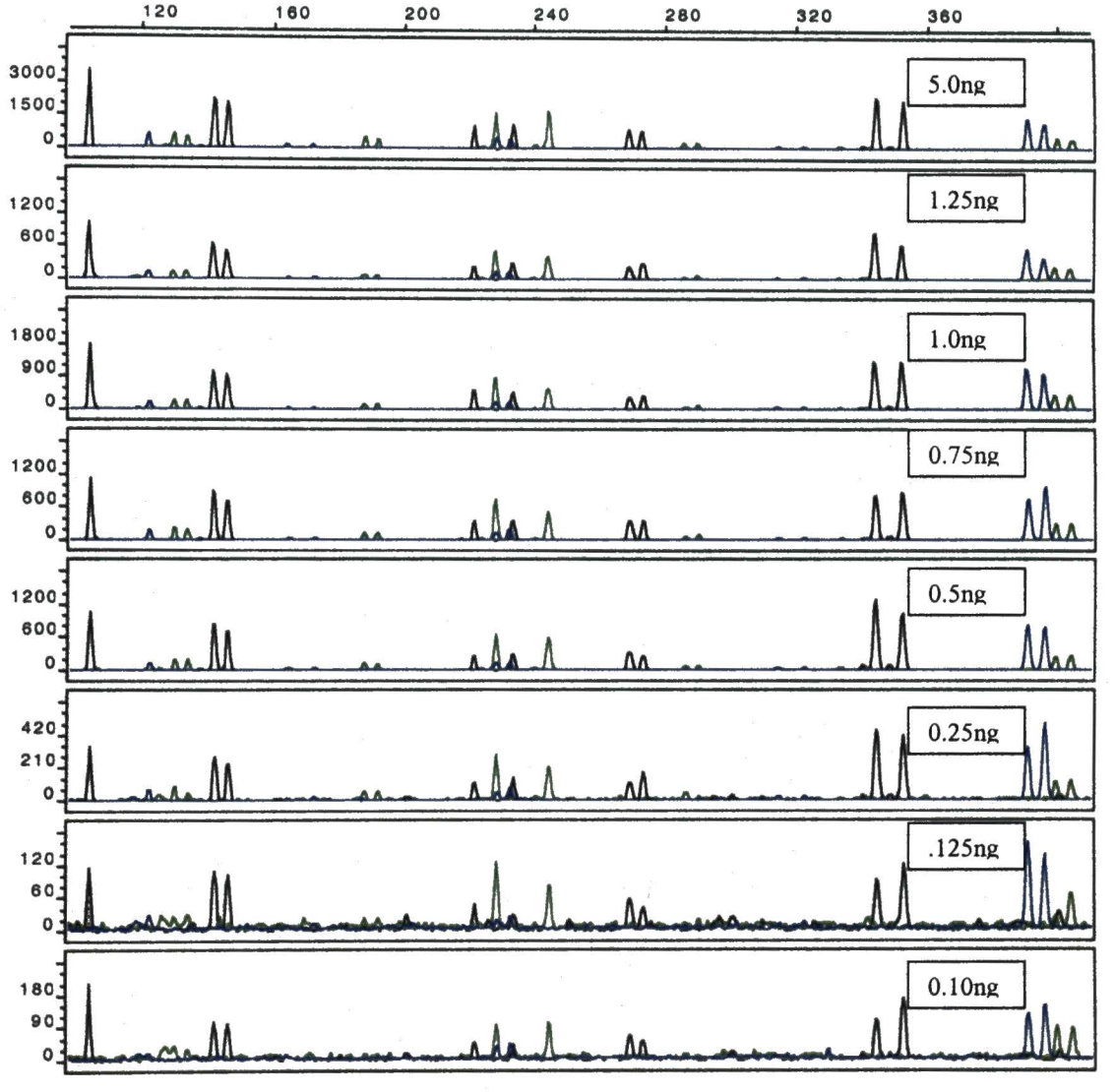


Figure 33 continued

PowerPlex™16 Kit

In Sample A (Figure 32), all alleles were present at 5.0 ng. Off-scale peaks were not observed at 5.0 ng. Alleles began dropping-out at 1.25 ng and continued down to 0.01 ng. Several THO1, CSF1PO, D16S539 and D18S51 alleles were not present at 1.25 ng. Extreme differential amplification was observed across all loci at any given input DNA amount. Several loci consistently exhibited higher peak heights between 5.0 to 0.125 ng: Penta E, Penta D, vWA, FGA and D7S820. The amelogenin gender marker was not detected 0.01 ng. Stutter was not observed between 0.50 to 0.05 ng. No alleles were detected at 0.01 ng. In Sample B (Figure 33), all homozygote and heterozygote alleles were present between 5.0 and 1.25 ng. Extreme locus imbalance was detected at any given input DNA amount. All loci and alleles were balanced between 1.25 and 0.25 ng of input DNA. Several loci consistently exhibited higher peak heights between 5.0 to 0.125 ng: Penta E, TPOX, vWA, FGA and D7S820. No stutter was detected at 0.50 and 0.25 ng of input DNA. The X-Y gender pair was present down to 0.10ng. No alleles were detected at 0.10 ng and 0.05 ng of input DNA, excluding vWA, FGA and Amelogenin. Alleles were not detected at 0.01 ng.

Figure 34- Samples were amplified using the PowerPlex™ 16 Amplification Kit at varying amounts of template DNA ranging from 5.0 ng to 0.01 ng. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (base pairs-bp). Notice the decline in RFU value as the amount of template DNA decreases. Samples were obtained from a female individual.



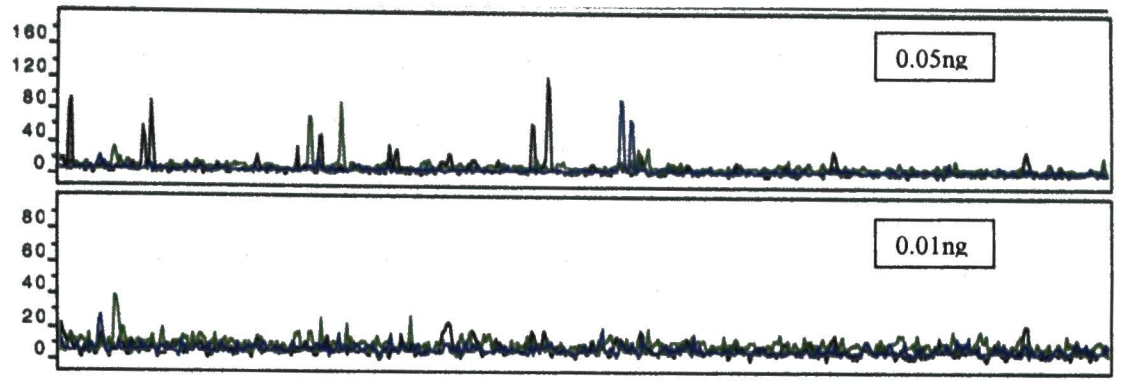
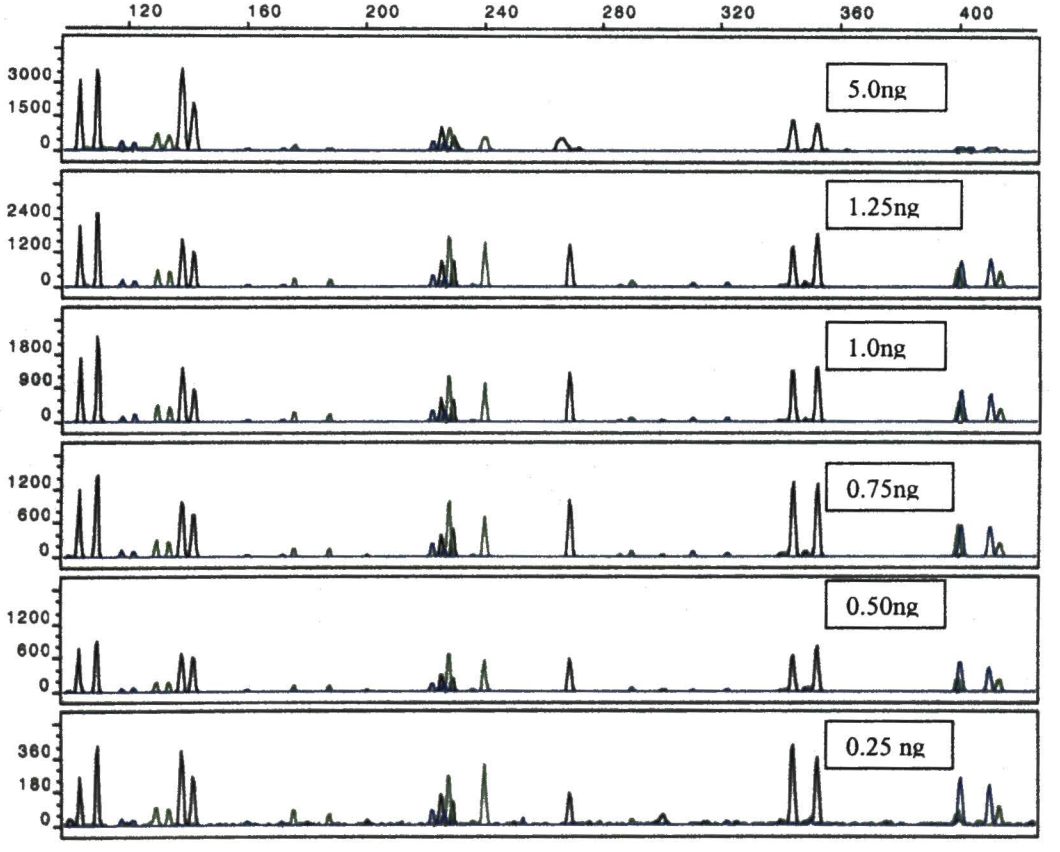


Figure 34 continued

Figure 35- Samples were amplified using the PowerPlex™ 16 Amplification Kit at varying amounts of template DNA ranging from 5.0 ng to 0.01 ng. The y-axis represents Relative Fluorescent Units (RFU) and the x-axis represents allele size (base pairs-bp). Notice the decline in RFU value as the amount of template DNA decreases. Samples were obtained from a male individual.



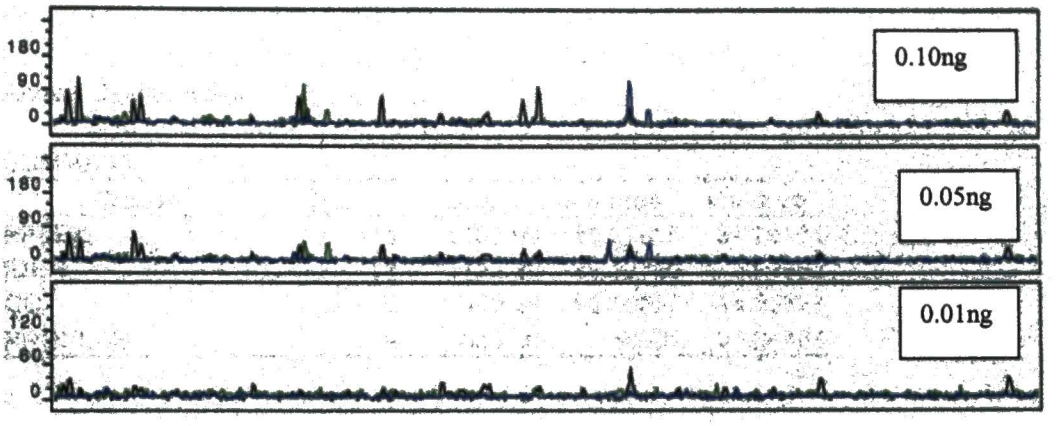


Figure 35 continued

Statistical Analysis

The mean and standard deviation values, for each STR locus, were calculated for heterozygote peak heights. Samples were analyzed at 1.25ng of template DNA due to the allelic dropout observed in the PowerPlex™ 16 system below 1.25ng. The ANOVA test revealed that significant variation ($p=0.0103$) in heterozygote peak height ratios between the AmpFℓSTR® Identifiler™, AmpFℓSTR® Profiler Plus™, AmpFℓSTR® COfiler™ and PowerPlex™ 16 Kits was detected. The most variation was observed within the PowerPlex™ 16 system followed by the AmpFℓSTR® Identifiler™ System. The single factor ANOVA also revealed that more variation was found within each kit as opposed to between each kit.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
PPLEX	13	1108.59	85.27615	37.76161
IDEN	13	1122.39	86.33769	33.2983
CO	6	540.64	90.10667	14.59327
PROPLUS	9	833.49	92.61	21.65468

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	350.0673	3	116.6891	3.928845	0.015702	2.858798
Within Groups	1098.923	37	29.70061			
Total	1448.99	40				

Table 15- Single Factor ANOVA: Average peak height ratios ranged between 85 to 92 RFU, within a system. Significant variations in mean peak height ratios were observed within amplification kits. The most variation was observed within the PowerPlex™16 Kit. (α=0.05, N=9)

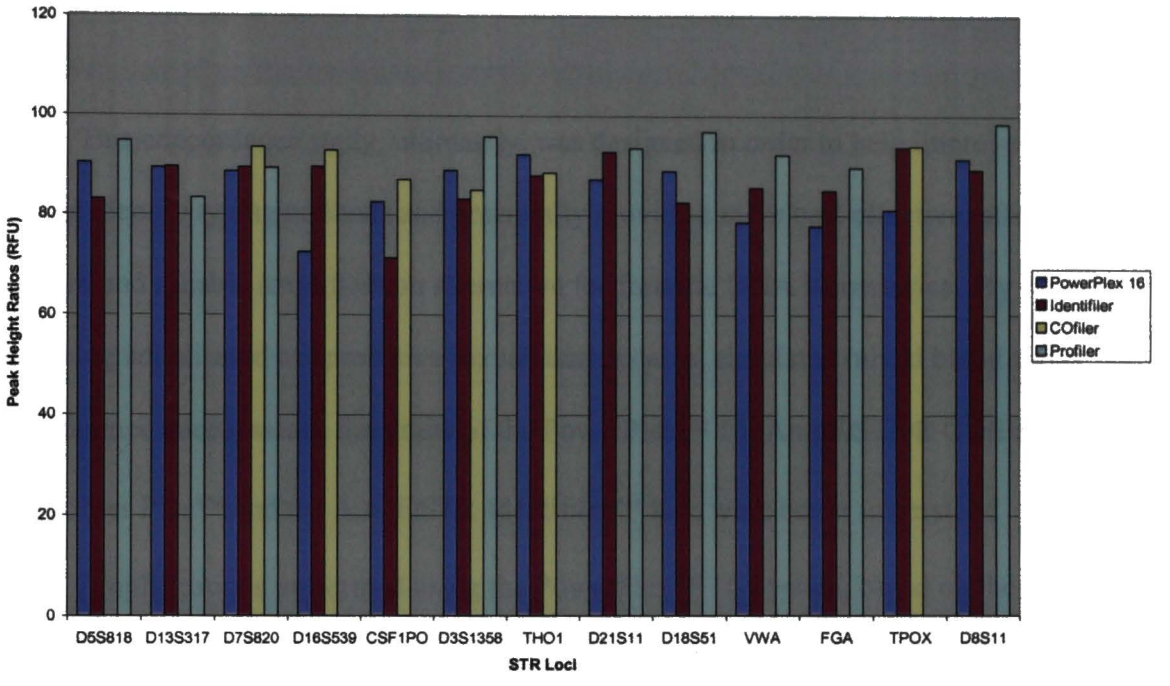


Figure 36- Mean Heterozygote Peak Height Ratios of the AmpFlSTR® and PowerPlex™16 Systems. The x-axis represents the 15 STR loci contained within the AmpFlSTR® and PowerPlex™16 Systems. The y-axis represents the average mean peak heights of heterozygote alleles. NOTE: No system contains all 15 STR loci. All peak height ratios are, in general, balanced between 80 and 100.

CONCLUSIONS

This concordance study, ultimately, was designed in order to help improve current forensic identity typing methods and potentially provide a more cost effective, efficient, sensitive and reliable amplification alternative for forensic DNA laboratories. By examining adjudicated nonprobative sexual assault cases, simulated mixed blood stains and reference buccal swabs the limits of the PowerPlex™ 16, AmpFℓSTR® COfiler™ and Profiler Plus™ and the AmpFℓSTR Identifiler™ Kits were better understood

Amplifications performed using the PowerPlex™ 16 System, based on the results from this study, are not reliable or suitable for forensic DNA casework. The PowerPlex™ 16 System continuously and repeatedly exhibited allelic dropout, preferential and differential amplification and locus and allele imbalance. This system was also unable to yield complete typing results below 1.25ng of input DNA. As typically expected, homozygote peaks should be twice the height of heterozygote peaks. However this was not observed using the PowerPlex™ 16 Kit. Generally, loci between 160-225 and 280-340 basepairs were practically uninterpretable and fell below the 100 RFU cutt-off.

It is my conclusion that the AmpFℓSTR® Profiler Plus™, COfiler™ and Identifiler® STR amplification systems are extremely sensitive, reliable and displayed little to no allelic dropout and preferential and differential amplification between 1.25 and 0.25 ng of template DNA. This allelic dropout can be attributed to a very subjective quantitation system, which utilizes a visual inspection and comparison of sample

intensities to the intensity of DNA Standards. The AmpFℓSTR® Systems were capable of yielding complete DNA profiles at 0.25ng of template DNA and displayed concordant typing results and balanced loci. The AmpFℓSTR® systems were able to practically resolve mixtures at approximately <10% of the total DNA and also successfully type sexual assault evidence. The AmpFℓSTR® Identifiler™ system is a suitable, reliable and cost effective amplification alternative to the AmpFℓSTR® COfiler and Profiler Plus Systems.

At low levels of input DNA, care should be taken when examining homozygote alleles and the amelogenin gender marker. Stochastic affects and allelic dropout can make truly heterozygote alleles appear homozygote. Also, it has been shown in this study that as the concentration of template DNA decreases, the Y-allele at the amelogenin locus drops out, making a male DNA sample appear as if it originated from a female individual.

DISCUSSION

Although the following factors do not directly affect the total cost effectiveness of each amplification kit, they do however affect the total fragment analysis processing time and thus requires some discussion. The average time it takes an analyst to process and analyze data would have to be taken into account in order to convert time saved into a monetary value. Cost effectiveness is also dependent on the complexity of a case, analyst's knowledge and ability to interpret data, the sample volume, analyst's salary and other ancillary budgeting expenses required to perform STR DNA typing. However, the AmpFℓSTR® Identifiler™ System is the least expensive system per reaction. Prior to adopting a new amplification platform, a laboratory director or DNA technical leader might take these factors into account.

Amplifications using either the PowerPlex™ 16 and AmpFℓSTR® Identifiler™ kits are performed simultaneously in a single tube and analyzed in a single injection. Single tube analysis allows for the preservation of challenged or limited samples. The AmpFℓSTR® COfiler™ and Profiler Plus™ systems use twice the sample amount, must be analyzed in two injections and are each amplified simultaneously in two separate tubes. However, the AmpFℓSTR® COfiler™ and Profiler Plus™ amplification kits have a "built-in" internal proofreader in that the two systems share the amelogenin sex marker and two STR loci, D3S1358 and D7S820. If the amelogenin sex marker and the two STR loci are not concordant between the two amplification systems, then the testing must either be ruled inconclusive or invalid, or repeated. Neither the PowerPlex™ 16 kit nor

the AmpFℓSTR® Identifiler™ kit provide this type of internal checks-and-balance, however samples could be run in duplicate in order to provide some degree of proofreading, but this duplication would negate the time, sample volume and money saved.

The PowerPlex™ 16 system was specifically designed for use with the ABI Prism® 310 Genetic Analyzer and the ABI Prism® 377 DNA Sequencer. This design prevents its use with gel-based systems, such as the Hitachi FMBIO® I and II.

The AmpFℓSTR® Identifiler™ kit, unlike the PowerPlex™ 16 kit, AmpFℓSTR® COfiler™ and Profiler Plus™ kits, now includes a degenerate unlabeled primer for the D8S1179 locus. This primer set addressed a mutation observed within a specific population located in Guam without modifying the overall performance of the AmpFℓSTR® Identifiler™ kit.

The AmpFℓSTR® Identifiler™ PCR Amplification kit has been designed to perform exclusively using either the Perkin-Elmer GeneAmp® PCR System 9700 Thermal Cycler or the Perkin-Elmer GeneAmp® PCR System 9600 Thermal Cycler. Many forensic DNA laboratories do not utilize the 9600 and 9700 systems. Other older models such as the Perkin-Elmer 480 and 2400 Thermal Cycler are still being used on a daily basis. In order to utilize the AmpFℓSTR® Identifiler™ system either the Perkin-Elmer GeneAmp® PCR System 9700 Thermal Cycler or the Perkin-Elmer GeneAmp® PCR System 9600 Thermal Cycler would have to be purchased.

Currently, CODIS will only allow the upload or input of casework STR DNA profiles from the following amplification systems: PowerPlex ™ 1.1 and 2.1,

PowerPlex™ 16 (offender samples only) and AmpFℓSTR® COfiler™ and Profiler Plus™. Casework samples amplified using either the AmpFℓSTR® Identifiler™ and PowerPlex™ 16 PCR systems can not be imported into CODIS, where they would be searched against DNA profiles from crime scenes and convicted offenders across the country. This situation, I believe, will be resolved very soon due to the demand by many forensic DNA testing laboratories

More casework validation studies will have to be performed on the AmpFℓSTR® Identifiler™ and PowerPlex™ 16 PCR systems in order for their acceptance and utilization in forensic DNA casework. Other suggested validation studies could include, but are not limited to, increasing template input pre-amplification for degraded or low quantity DNA samples, increasing DNA input and injection time for capillary electrophoresis, increasing PCR cycle numbers for degraded or low quantity DNA samples and performing temperature and humidity studies.

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