
UNIVERSITY OF NORTH TEXAS
HEALTH SCIENCE CENTER AT FORT WORTH
1970

Moore, Melody Ann. Evaluation of the Gold-plated Silver Sample Block on the GeneAmp® PCR System 9700 Thermal Cycler. Master of Science (Forensic Genetics), May 2003, 49 pages, 9 figures, 3 tables, 2 appendices, 24 references.

The GeneAmp® PCR System 9700 Thermal Cycler has been introduced with interchangeable silver and gold-plated silver sample blocks. To validate the new gold-plated silver sample block on the System 9700, amplifications were performed on both the gold-plated silver and the previously validated silver sample blocks. PCR amplifications using the AmpFℓSTR® Profiler Plus *ID*™, COfiler™, Identifiler™ and SGM Plus™ typing kits (Applied Biosystems, Foster City, CA) were performed. Electrophoretic characteristics such as allele concordance, peak heights, and peak height ratios were used to discern any differences in the amplification capabilities of the two sample blocks. The results demonstrate that the PCR reactions on both silver and gold-plated silver blocks are equivalent. In addition, the data obtained (allele calls, peak heights, peak height ratios) from both Macintosh™ and Windows NT™ platforms were identical for each amplification kit, indicating concordance between the software packages of each operating system. The results of this study demonstrate the interchangeability of the silver and gold-plated silver sample blocks.

EVALUATION OF THE GOLD-PLATED SILVER SAMPLE BLOCK ON THE GENEAMP® PCR SYSTEM 9700 THERMAL CYCLER

Melody Ann Moore, B.S.

APPROVED:

Major Professor

Committee Member

Committee Member

University Member

Chair, Department of Cell Biology and Genetics

Dean, Graduate School of Biomedical Sciences

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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

Melody Ann Moore, B.S.

Fort Worth, Texas

May 2003

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to Rhonda Roby, MPH, for all her help in preparing this paper. I couldn't have finished this without her. I also thank Applied Biosystems for providing the many reagents and extensive background materials for this study. Finally, my sincerest appreciation to Art Eisenberg, PhD, and John Planz, PhD, for their guidance and input into the preparation of this manuscript.

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ABBREVIATIONS

COfiler™, AmpFℓSTR® COfiler™ typing kit

DNA, deoxyribonucleic acid

DAB, DNA Advisory Board

dNTP, deoxyribonucleoside triphosphate

G-C, guanine-cytosine base pair

HRP:SA, horseradish peroxidase: streptavidin

Identifiler™, AmpFℓSTR® Identifiler™ typing kit

ng, nanogram

PCR, polymerase chain reaction

Profiler Plus *ID*™, AmpFℓSTR® Profiler Plus *ID*™ typing kit

QuantiBlot®, QuantiBlot® Human DNA Quantitation Kit

RFLP, restriction fragment length polymorphism

RFU, relative fluorescent unit

SGM Plus™, AmpFℓSTR® SGM Plus™ typing kit

STR, short tandem repeat

System 9600, GeneAmp® PCR System 9600 Thermal Cycler

System 9700, GeneAmp® PCR System 9700 Thermal Cycler

TE-4, tris EDTA pH 8.0 -> 10mM Tris and 0.1mM EDTA (ethylenediaminetetraacetic disodium salt)

µL, microliter

CHAPTER 1

INTRODUCTION

The validation of new equipment and reagents used in forensic laboratories is required for compliance with national standards set forth by the DNA Advisory Board (DAB). These quality assurance standards, set up in 1998, state that "Internal validation shall be performed and documented by the laboratory" (standard 8.1.3), and that "Material modifications made to analytical procedures shall be documented and subject to validation testing" (standard 8.1.3.4)(1). Because of these requirements, the introduction of new technologies and instrumentation into the forensic laboratory must first be preceded by validation studies. These validation studies should be designed to address the reproducibility and consistency of the results obtained in comparison with previously established protocols and equipment.

The polymerase chain reaction (PCR) is a process for the amplification of DNA. The use of PCR enables a laboratory to amplify a genetically informative sequence of DNA, usually from 100 to about 2,000 base pairs in length. The reaction itself is analogous to normal DNA replication (2,3,4). Very small amounts of DNA can be used in the PCR process. Theoretically even a single nucleated cell contains enough DNA to serve as a template for amplification (5,6). In the PCR two short oligonucleotide primers are designed to hybridize to a region on opposite strands of template DNA.

These primers are oriented such that their 3' ends "face" each other, targeting the sequence in between them for replication. These primers serve as a starting point for the enzyme-mediated synthesis of new DNA strands. Components of the polymerase chain reaction are buffers to maintain a normal environment for replication; dNTP's to provide the building blocks for the newly synthesized DNA strands, template DNA to be copied, oligonucleotide primers, and thermal stable DNA polymerase to synthesize the new strand of DNA. The three steps of the PCR are: 1) denaturation of the template DNA, 2) hybridization (annealing) of the primers to the template DNA strands, and 3) synthesis of the new DNA strands.

Denaturation, the first step in a PCR, is the dissociation of the template DNA into its two complementary strands. This is accomplished by incubating the DNA at high temperatures, usually around 94°C. The hybridization of the primers to these separate complementary strands occurs at lower temperatures. This annealing temperature depends on the nucleotide composition of the primer pair(s) being used. For example, a primer pair with a larger number of G-C pairings will have a higher annealing temperature than one with lower G-C content. Usually annealing of the primers occurs around 50°C-60°C. Once the primers have hybridized to the template DNA the enzyme DNA polymerase attaches to the 3'-OH (hydroxyl) end of the primer and begins synthesizing a new DNA strand complementary to the template sequence. This results in new double stranded DNA identical in sequence to the original template DNA. The temperature at which the DNA polymerase is most active determines the temperature of the extension step. The DNA polymerase must also be able to withstand the high

temperatures of denaturation without falling apart (7). The DNA polymerase used in earlier PCR procedures originated from the Klenow fragment in *E. coli*, but its lack of thermal stability necessitated addition after every denaturation cycle. The most commonly used form of thermal stable DNA polymerase is *Taq* polymerase. This form is found in *Thermus aquaticus*, a bacterium native to hot springs, and is more active at higher temperatures than some other forms of DNA polymerase while withstanding the extremes of heat associated with denaturing DNA. AmpliTaq® Gold DNA Polymerase (Applied Biosystems, Foster City, CA) (used in this study) is a form of hot-start polymerase. This type of polymerase is bound to a chemical moiety that inactivates the enzyme until incubated at 95°C for 5-10 minutes. This prevents mispriming events from occurring during the initial temperature ramping, which could result in non-specific PCR products. Other forms of DNA polymerase have a proofreading ability that can lower the inherent error rate of *Taq* polymerase. The error rate of most forms of *Taq* polymerase has been shown to be 1.1×10^{-4} errors/bp (8). This low error rate insures that even with a small sample size there will be little or no effect when amplifying shorter (100-400 bp) fragments of DNA. If the efficiency of the PCR were 100% the amount of target sequence would double with each cycle. However, a typical 30 cycle PCR will yield 10^6-10^7 copies of the target sequence.

The impact of the discovery of PCR has been felt in many areas including biomedical and academic research, pharmaceutical research, and in forensics. Its inventor, Kary B. Mullis, received the Nobel Prize in 1993 – less than 10 years after PCR was first described (9). In forensics the impact has been enormous. The DNA obtained

from crime scenes and from unidentified remains is often limited in quantity. It is often degraded due to aging, exposure to chemicals and/or exposure to the elements. PCR is sensitive, rapid, and not affected by the quantity or quality of template DNA like other identification methodologies (e.g. RFLP). Degraded samples of DNA can be used in the PCR to obtain profiles, or partial profiles, from evidentiary samples. This sensitivity has brought the field of forensic genetics to the forefront of forensic investigation.

The instrument that performs the heating and cooling of the DNA samples during PCR is called a thermal cycler. Accurate, rapid and consistent sample heating and cooling is crucial to PCR, guaranteeing reproducible results. Prior to the development of the thermal cycler, the various components of the PCR had to be added by hand at different stages of the cycle. The addition of various components during cycling increased the problems of pipetting errors and contamination between tubes. The reaction tubes themselves would have to be manually transferred between blocks of differing temperatures. This increased handling of the tubes generated the potential for tube mix-ups. Several generations of thermal cyclers have been manufactured, each one improving upon the last in the areas of heating and cooling, and in software development.

Two of the most commonly used thermal cyclers are the GeneAmp® PCR System 9600 and System 9700. The System 9600 was released in 1990 and utilized a compressor to regulate sample temperature by pumping a fluid composed of an ethylene glycol and water mixture around the sample block wells. The normal ramp profile of a System 9600 thermal cycler is approximately 1°C per second. Compressors have been used in many models of thermal cyclers and have the advantage of being powerful and having long-

term reliability. Many compressors, such as those in refrigerators and freezers, last for years or decades. The compressor cools a reservoir tank of fluid, which is then pumped around the sample wells to cool them. This coolant is maintained at around –20°C. However, this coolant will warm when pumped around the sample block, and this can change the cooling profile of a cycle. This is called cycle-to-cycle variation. The compressor must be powerful enough to maintain the coolant at a low temperature during the cooling process in order to minimize cycle-to-cycle variation. The disadvantages to having a compressor driven thermal cycler are the size of the equipment and the cost of the compressor. The reservoir tanks and pumps are bulky, requiring more countertop space in the laboratory. The cost of building compressors is expensive and increases the cost of the thermal cycler.

The GeneAmp® PCR System 9700 Thermal Cycler (Applied Biosystems, Foster City, CA) is a common piece of equipment in many forensic laboratories today (Figure 1). The System 9700 is available with several different sample blocks and block formats. The GeneAmp® PCR System 9700 was released in 1997 and uses a Peltier heating and cooling system. This system takes advantage of the thermal differential that develops when a current is passed through the Peltier element. Invented by Jean Peltier in 1834 (10,11) the Peltier modules in the System 9700 thermal cycler are composed of many stacks of bismuth telluride arranged in columns. When electrical currents are passed through the Peltier element in one direction, a thermal differential occurs across the layers (the top heats and the bottom cools). By reversing the current the thermal differential can be reversed (the bottom heats and the top cools) (Figure 2). These Peltier

Figure 1 – The GeneAmp® PCR System 9700 thermal cycler. Picture shows the gold-plated silver block in place. Courtesy Applied Biosystems, Foster City, CA. http://www.appliedbiosystems.com

units are very efficient and heat rapidly, but are less efficient at cooling compared to a compressor based system. Due to the different coefficients of expansion in the elements of a Peltier unit, long-term reliability is compromised as well. Relative to the temperature, the sub-components of the Peltier system can expand and contract at different rates at each temperature the unit reaches, causing stress. Over time, this stress can cause fractures that disrupt interconnecting components and lead to failure of the unit.

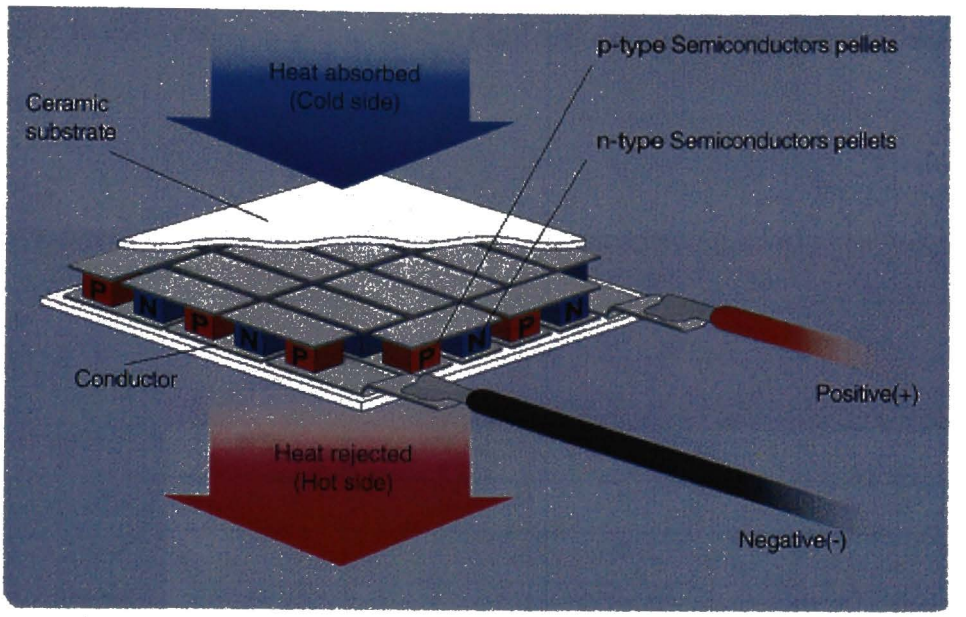


Figure 2 – Peltier thermoelectric module. http://www.inbthermoelectric.com

The System 9700 has software that allows the unit to be run in either the Max mode or in the 9600 emulation mode. This feature came about because many of the protocols and commercially available PCR kits had previously been validated on the System 9600 (12-20) (Table 1). The Max mode of the System 9700 has a slightly faster cycling profile than the System 9600 (a difference of about 12 minutes per run). However, running the System 9700 in the 9600 emulation mode keeps the ramp profile to 1°C per minute, and therefore matches the cycling parameters of the System 9600. This allows for the direct transfer of protocols from the System 9600 to the System 9700 thermal cycler. The System 9700 thermal cycler has a heated lid like the System 9600. This eliminates the need for the oil overlay of the PCR components and reduces condensation on the top of the PCR tube. The heated lid minimizes the loss due to

evaporation in the standard reaction volumes utilized with most commercially available PCR kits.

AmpF1STR Typing Kit	Thermal Cycler				
	480	2400	9600	silver 9700	gold-plated silver 9700
Profiler Plus *ID*™	NT	NT	+	+	-
Profiler Plus™	+	+	+	+	-
COfiler™	+	+	+	+	-
SGM Plus™	+	+	+	+	-
Identifiler™	NT	NT	+	+	-

Table 1 – STR typing kits and the thermal cyclers on which they have been validated. (+ = validated previously; NT = not tested; - = not previously validated)

The interchangeable sample blocks available on the System 9700 provide a laboratory with flexibility in their choice of PCR format. The sample blocks are designed to accept PCR amplification plates in either a 96-well, 384-well, or 768 well format. Blocks can also accommodate micro-amp tubes, either 48 0.5 ml tubes or 96 0.2 ml tubes. The blocks have been constructed of different metals. The aluminum sample block was the first one introduced, and is marketed for standard PCR usage. It was not tested in this study. The silver sample block was introduced shortly after the aluminum block and provided improved thermal transfer capabilities. The gold-plated silver sample block is the latest of the three. It retains the thermal transfer capabilities of the silver block (Figure 3) while adding the durability of gold to the components of the block.

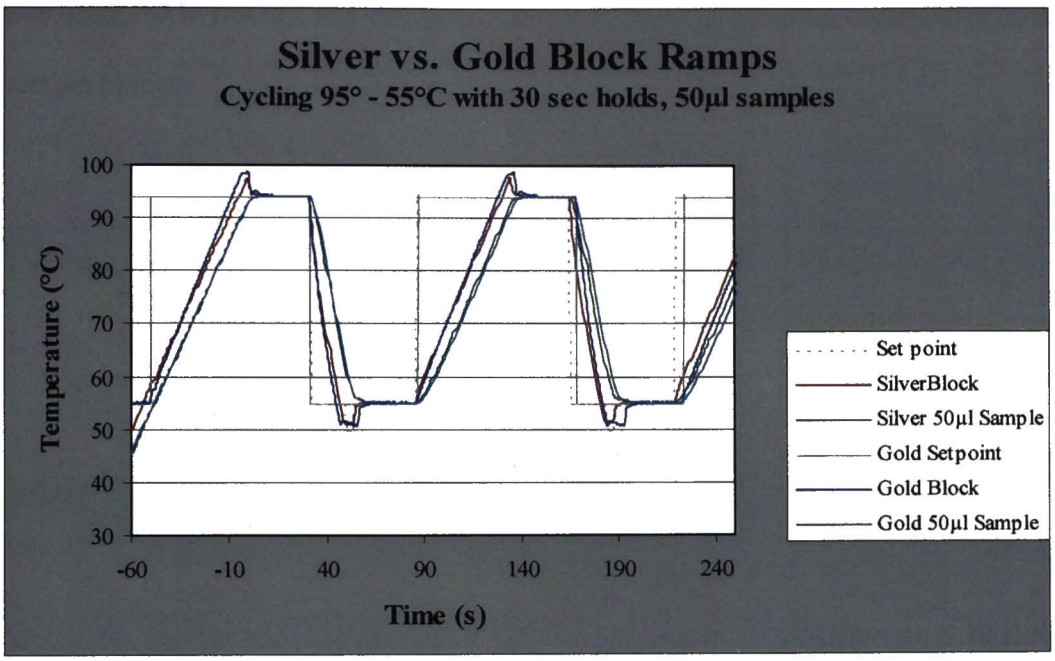


Figure 3 – A comparison of the silver and gold-plated silver block ramping profiles. Courtesy Applied Biosystems, Foster City, CA.

It was the purpose of this study to validate the use of the gold-plated silver sample block for PCR in the forensic laboratory. For this study a comparison was made between the silver sample block and the newer gold-plated silver sample block. This was accomplished by analyzing amplicons generated on both silver and gold-plated silver sample blocks and by comparing their electrophoretic characteristics, including allele calls, peak heights and peak height ratios. The allele calls refer to the DNA profile of the sample. A comparison was made of the accuracy and concordance of the allele calls of profiles generated from both sample blocks. Profiles from the same sample should have allele concordance at each locus. The peak heights are given in relative fluorescent units (RFUs), and relate to the amount of PCR product generated from a sample. The presence

was designed to discern any differences between the amplification capabilities of the two sample blocks.

CHAPTER 2

MATERIALS AND METHODS

Samples

For this study four male DNA samples, as well as the control DNA that was provided in the typing kits (9947A, female), were used. The male DNA was extracted from whole blood on filter paper using a standard organic method (21). A cutting of the stain was placed into 300 µLof stain extraction buffer (10 mM Tris, 100 mM NaCl, 39 mM DTT, 10 mM EDTA, 2% SDS) and incubated overnight at 56° C. After removal of the cuttings, 300 µL of phenol: chloroform: isoamyl alcohol (25: 24: 1, v/v) (Invitrogen, Carlsbad, CA) was added and the samples were vortexed well. Following centrifugation for 3 minutes at high speed, the aqueous layer was transferred into clean microcentrifuge tubes. The DNA was ethanol precipitated, the pellet was dried and then resuspended in 100 µL of TE^{-4} buffer. Samples were stored at 4°C until quantitation.

DNA Quantification

DNA was quantified using the QuantiBlot® Human DNA Quantitation Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's directions for colorimetric detection of DNA (22). The QuantiBlot® kit is a membrane hybridization assay specific for the detection of higher primate DNA. For this study 5 µL of sample DNA was spotted onto a Biodyne® B nylon membrane (Invitrogen, Carlsbad, CA) along

with a set of known DNA standards ranging from 0 to 2 ng DNA. Calibrators of known DNA concentration were also spotted onto the membrane to serve as positive controls and to determine the accuracy of the standards. The membrane was hybridized with a biotinylated oligonucleotide probe complementary to a primate specific DNA sequence. The probe was detected through the binding of Enzyme Conjugate: HRP-SA (horseradish peroxidase: streptavidin). When exposed to Chromogen: TMB (Applied Biosystems, Foster City, CA), the horseradish peroxidase activity causes the deposition of a blue-colored precipitate directly onto the membrane when primate DNA is present. By visually comparing the intensity of the blue colored precipitate from the DNA sample spots to the known standards, the amount of DNA can be subjectively determined.

Temperature Verification of Thermal Cyclers

Each GeneAmp® PCR System 9700 thermal cycler and sample block was temperature checked to verify that it was operating within the manufacturer's specified operating range. A temperature probe (PE Temperature Verification System, Applied Biosystems, Foster City, CA) was placed into 16 specified wells while the thermal cycler cycled between 95°C and 40°C. Temperatures in those wells were recorded at both set points. For each set point the lowest measurement was subtracted from the highest measurement. Neither value exceeded the 1°C maximum differential. This verified that both thermal cyclers and sample blocks were operating within normal limits.

Amplification

PCR was performed using AmpFℓSTR® Profiler Plus *ID*™, AmpFℓSTR® COfiler™, AmpFℓSTR® SGM Plus™ and AmpFℓSTR® Identifiler™ typing kits

(Applied Biosystems, Foster City, CA). These kits are commonly used in forensic laboratories and have previously been validated for use on both the System 9600 and 9700 (silver sample block only) thermal cyclers (12-20). The AmpFℓSTR® Profiler Plus *ID*™ kit in conjunction with the AmpFℓSTR® COfiler™ kit amplifies all thirteen of the core CODIS loci. CODIS, the combined DNA index system, is a nationwide database containing profiles from convicted offenders, missing persons cases, and evidentiary samples from unsolved cases. These profiles consist of thirteen loci established by the FBI in 1998 as those to be included in the CODIS database (23). These loci are D3S1358, vWA, FGA, THO1, TPOX, CSF1PO, D5S818, D13S317, D7S820, D8S1179, D21S11, D18S52, and D16S539. The AmpFℓSTR® Identifiler™ kit amplifies all thirteen CODIS loci, the sex typing marker amelogenin, and the two additional loci D2S1338 and D19S433. The AmpFℓSTR® SGM Plus™ is a kit used mostly in the U.K. and Europe, and contains eight of the core thirteen loci along with amelogenin, D2S1338 and D19S433. See Table 2 for a summary of the loci amplified by each kit and their associated fluors.

AmpFℓSTR® Profiler Plus *ID*™ and COfiler™

Amplification parameters were those specified in the AmpFℓSTR® Profiler Plus™ Users manual, the AmpFℓSTR® Profiler Plus *ID*™ bulletin, and/or the AmpFℓSTR® COfiler™ Users Manual (16, 17, 18). Each PCR tube contained a PCR master mix composed of 1X AmpFℓSTR® PCR Reaction Mix, primers specific for the loci amplified by the AmpFℓSTR® Profiler Plus *ID*™ kit, and AmpliTaq Gold® DNA Polymerase. The PCR Reaction mix consists of $MgCl_2$, BSA, dNTP's, Tris-HCL (pH

8.3), KCl, and sodium azide combined in one tube for ease of use (exact concentrations of each component are proprietary and belong to Applied Biosystems). For 50 µL reactions 20 µl of sample DNA in either 0.5 ng or 2 ng quantities was added to

Locus	AmpFℓSTR Profiler Plus ID	AmpFℓSTR Cofiler	AmpFℓSTR Identifiler	AmpFℓSTR SGM Plus
D3S1358				
VWA				
FGA				
THO1				
TPOX				
CSF1PO				
D5S818				
D13S317				
D7S820				
D8S1179				
D21S11				
D18S52				
D16S539				
AMELOGENIN				
D2S1338				
D19S433				

Table 2 – Loci amplified by each kit and their associated fluor color; CODIS loci are in Italics.

tubes containing 30 µl of the PCR master mix. For 25 µl reactions 10 µL sample DNA in 0.5 ng or 1 ng quantities was added to tubes containing 15 µL of the PCR master mix. Thermal cycling parameters were: initial incubation (95°C for 11 minutes), followed by 28 cycles of denaturation (94°C for one minute), primer annealing (59°C for one minute), and extension (72°C for one minute). A final extension was then performed at 60°C for 45 minutes, followed by a 4°C hold. All samples were amplified in duplicate. The

duplicates were spread across the sample block in order to determine evenness in the sample block performance. The amplifications were carried out on two GeneAmp® PCR System 9700s using the silver and gold-plated silver sample blocks on each thermal cycler. A total of four amplifications was done for each sample. All amplifications on the System 9700 thermal cycler were done in 9600 emulation mode.

AmpFℓSTR® Identifiler™

Amplification parameters were those specified in the AmpFℓSTR® Identifiler™ User's Manual (19). Each PCR tube contained a PCR master mix composed of 1X AmpFℓSTR® PCR Reaction Mix, primers specific for the loci amplified by the AmpFℓSTR® Identifiler™ kit, and AmpliTaq Gold® DNA Polymerase. The PCR Reaction mix consists of $MgCl_2$, BSA, dNTP's, Tris-HCL (pH 8.3), KCl, and sodium azide combined in one tube for ease of use (exact concentrations of each component are proprietary and belong to Applied Biosystems). For 25 µL reactions 10 µL sample DNA in 0.5 ng or 1 ng quantities was added to tubes containing 15 µL of the PCR master mix. Thermal cycling parameters were: initial incubation (95°C for 11 minutes), followed by 28 cycles of denaturation (94°C for one minute), primer annealing (59°C for one minute), and extension (72°C for one minute). A final extension was then performed at 60°C for 60 minutes, followed by a 4°C hold. All samples were amplified in duplicate. The duplicates were spread across the sample block in order to determine evenness in the sample block performance. The amplifications were carried out on two GeneAmp® PCR System 9700s using the silver and gold-plated silver sample blocks on each thermal

cycler. A total of four amplifications was done for each sample. All amplifications on the System 9700 thermal cycler were done in 9600 emulation mode.

.AmpFℓSTR® SGM Plus™

Amplification parameters were those specified in the AmpFℓSTR® SGM Plus™ User's Manual (20). Each PCR tube contained a PCR master mix composed of 1X AmpFℓSTR® PCR Reaction Mix, primers specific for the loci amplified by the AmpFℓSTR® SGM Plus™ kit, and AmpliTaq Gold® DNA Polymerase. The PCR Reaction mix consists of $MgCl_2$, BSA, dNTP's, Tris-HCL (pH 8.3), KCl, and sodium azide combined in one tube for ease of use. (The exact concentrations of each component are proprietary and belong to Applied Biosystems.) For 50 µL reactions, 20 µL of sample DNA in either 0.5 ng or 2 ng quantities was added to tubes containing 30 µL of the PCR master mix. Thermal cycling parameters were: initial incubation (95°C for 11 minutes), followed by 28 cycles of denaturation (94°C for one minute), primer annealing (59°C for one minute), and extension (72°C for one minute). A final extension was then performed at 60°C for 45 minutes, followed by a 4°C hold. All samples were amplified in duplicate. The duplicates were spread across the sample block to determine evenness in the sample block performance. The amplifications were carried out on two GeneAmp® PCR System 9700s using the silver and gold-plated silver sample blocks on each thermal cycler. A total of four amplifications was done for each sample. All amplifications on the System 9700 thermal cycler were done in 9600 emulation mode.

Genetic Analysis

All amplified products were analyzed using the ABI PRISM™ 310 Genetic Analyzer (Figure 4) and associated GeneScan® and Genotyper® Analysis software (Applied Biosystems , Foster City, CA). Two genetic analyzers were used. One 310

Figure 4 – ABI PRISM™ 310 Genetic Analyzer. Courtesy Applied Biosystems, Foster City, CA. http://www.appliedbiosystems.com

analyzer was connected to a computer with a Macintosh® OS operating system (Apple Computer Corp., Seattle, WA) with GeneScan® version 3.1.2 and Genotyper® version 2.5, and the other 310 was connected to a computer with Windows NT® operating system (Microsoft Corp., Seattle, WA) using GeneScan® version 3.7 and Genotyper® version 3.7- NT. GeneScan® software is used to generate electropherogram graphics, allele size, and peak height. Data was collected using the GS STR POP-4 (1ml) F module (Applied Biosystems, Foster City, CA) for AmpFℓSTR® Profiler Plus *ID*™, COfiler™, and SGM

Plus™ samples. This module sets the following parameters: 30cm capillary, POP-4 polymer (Applied Biosystems, Foster City, CA), injection time of five seconds, injection voltage of 15kV, electrophoresis voltage of 15kV, collection time 24 minutes, and temperature of the heat plate equal to 60°C. AmpFℓSTR® Identifiler™ sample data was collected utilizing the GS STR POP-4 (1ml) G5 module (Applied Biosystems, Foster City, CA). This module specifies the following parameters: 30cm capillary, POP-4 polymer (Applied Biosystems, Foster City, CA), injection time of 5 seconds, injection voltage of 15kV, electrophoresis voltage of 15kV, collection time of 28 minutes, and temperature of the heat plate equal to 60°C. Unlike the F module that is specific for 4-dye systems (Profiler Plus *ID*™, COfiler™ and SGM plus™), the G5 module collects data from 5-dye systems like Identifiler™. The Genotyper® software then translates the size of the fluorescently tagged fragment into loci and allele designations by comparing the fragment size and fluor to a kit-specific allelic ladder. Allelic ladders contain the most common alleles for each locus. These alleles are designated by their sizes. When interpreting results, allele designations are assigned to samples based on their size relative to this ladder. Alleles with peak heights above 50 RFUs were analyzed.

Sample and Allelic Ladder Preparation

Samples were prepared for analysis according to manufacturer's directions (16-20). One microliter of amplification product or kit-specific allelic ladder (AmpFℓSTR® Profiler Plus™, AmpFℓSTR® COfiler™, and AmpFℓSTR® SGM Plus™) (Figures 5-8) was added to a tube containing 10 µL of a Hi-Di Formamide/GeneScan™-500 ROX™ size standard (9.5 µL: 0.5 µL) (Applied Biosystems, Foster City, CA). The AmpFℓSTR®

Profiler Plus™ ladder is used in conjunction with the Profiler Plus *ID*™ typing kit (16). For AmpFℓSTR® Identifiler™ amplification products, 1.5 µL PCR product or allelic ladder was added to tubes containing 24 µL of Hi-Di Formamide/GeneScan™-500 LIZ™ size standard (24.5 µL: 0.5 µL) (Applied Biosystems, Foster City, CA). All samples were denatured for three minutes at 95°C and then snap cooled on ice for at least three minutes prior to loading on the genetic analyzer.

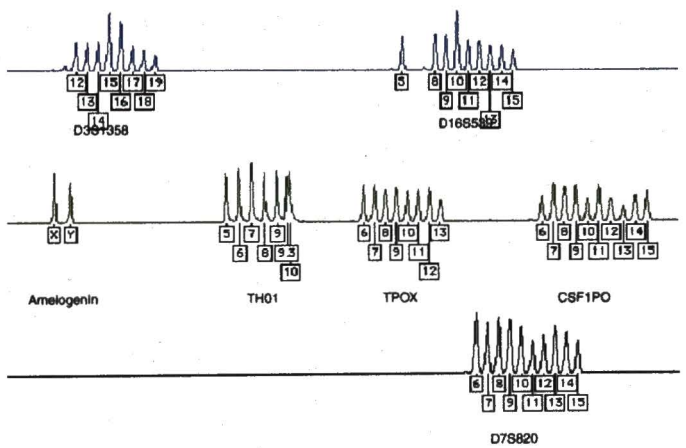


Figure 5 – AmpFℓSTR® COfiler™ allelic ladder (18)

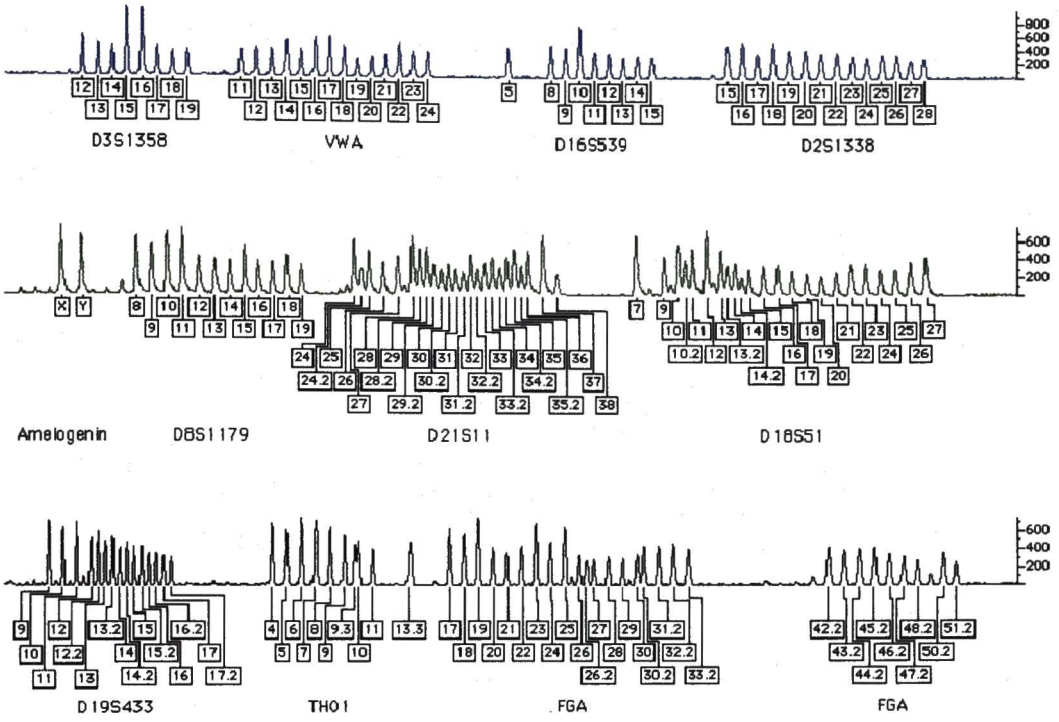


Figure 6 – AmpFℓSTR® SGM Plus™ allelic ladder (20)

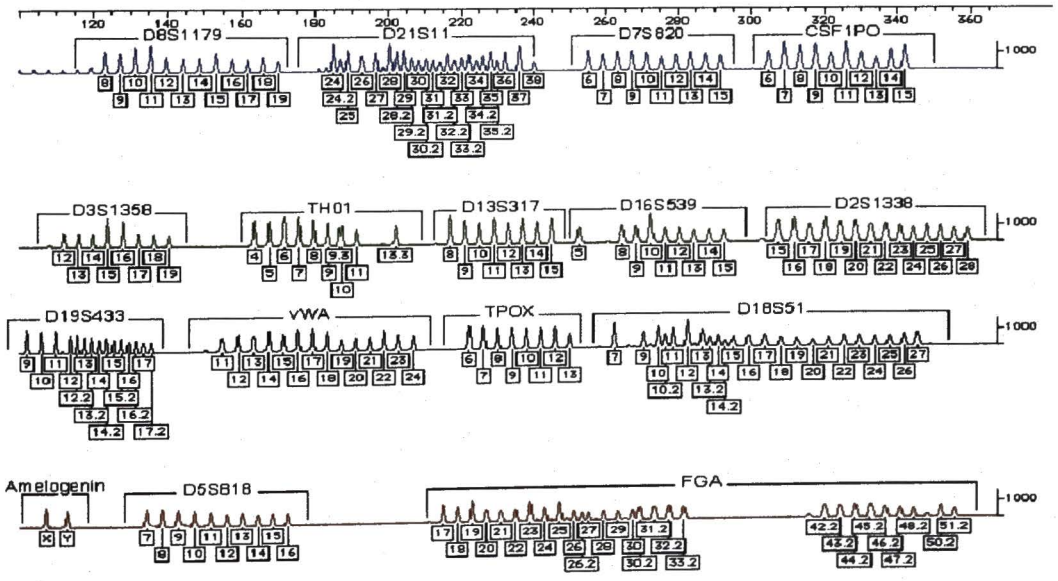


Figure 7 – AmpFℓSTR® Identifiler™ allelic ladder (19)

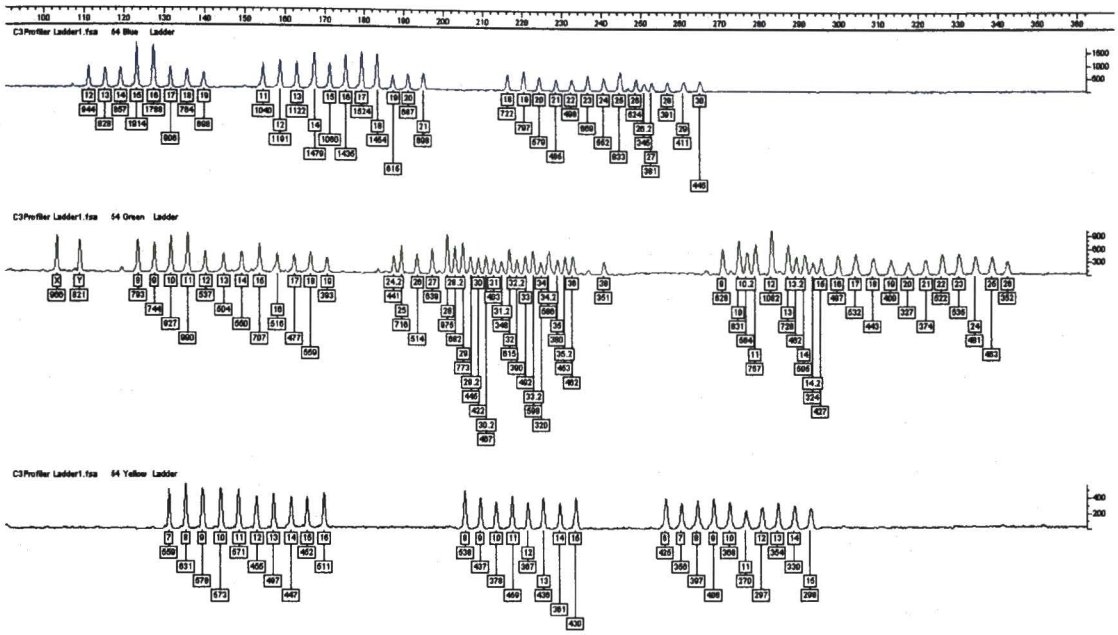


Figure 8 – AmpFℓSTR® Profiler Plus™ allelic ladder (16, 17)

Data Analysis

Results of the genetic analyses were tabulated into Microsoft® Excel™ spreadsheets (Microsoft Corp., Seattle, WA). Peak heights, expressed in relative fluorescent units (RFUs), were compared between samples amplified on each block. Peak height ratios were then calculated for each heterozygous locus. The peak height ratio is equal to the height of the smallest peak at a locus (in RFUs) divided by the height of the largest peak (in RFUs) at that same locus, and is expressed as a percentage. Applied Biosystems, the manufacturer of the AmpFℓSTR® typing kits, specifies a peak height ratio of >70% as acceptable. The peak height data generated on the two different ABI

PRISM™ 310 platforms were used to compare the analysis software. The Macintosh™ and Windows NT™ software packages utilize different algorithms for data analysis.

CHAPTER 3

RESULTS

Temperature Uniformity Validation

The temperature uniformity validation of both GeneAmp® PCR System 9700 Thermal Cyclers showed that both thermal cyclers and sample blocks were operating within normal limits. No wells tested in the sample blocks varied greater than 0.9°C from the set points when cycling between 95°C and 40°C. The same results were obtained on both thermal cycler bases with both silver and gold-plated silver blocks.

DNA Profile Comparison

A comparison of the DNA profiles obtained from each of the five samples utilizing the four AmpFℓSTR typing kits was done. The profiles were concordant for each of the five samples and all alleles were called identically regardless of which sample block was used in the amplification (Table 3). This was true for all four of the AmpFℓSTR typing kits used. The profiles generated using both the Macintosh® and the Windows NT® software packages were identical (Figures 9a, 9b, 9c, 9d).

Peak Height Ratio Comparison

Peak height ratio calculations showed no significant difference between the silver sample block and the gold-plated silver sample block. The peak height ratio is equal to the minimum peak height at a locus (in RFUs) divided by the maximum peak height at

that same locus (in RFUs), and is expressed as a percentage. All peak height ratios were within the desired range of 70-100% for each of the four AmpFℓSTR® typing kits (Appendix A, graphs 10-21). The results were consistent regardless of whether 0.5 ng, 1 ng, or 2 ng DNA was input. Congruity in the data was observed regardless of whether the reaction volume was 50 μL or 25 μL. These results were identical on both sample blocks and on both Macintosh™ and Windows NT™ software.

Peak Height Comparison

Peak heights relate to the amount of amplification product in a sample. Any differences or trends in data between the silver and the gold-plated silver sample block would be seen by consistently higher peak heights from amplifications performed on one type of sample block. When peak height data was compared from both sample blocks the results were congruent between the two blocks (Appendix B). For each locus and each concentration of DNA, the peak heights for all samples amplified on either the silver or gold-plated silver sample block were averaged. Although some amplification products seemed to show better PCR performance on one type of block, these preferences were not consistent (Appendix B, Graphs 22-33). Peak height variance could indicate pipetting errors. The apparent difference in block performance varied between days and between AmpFℓSTR® kits. Because data showing any trend towards better performance by one block over the other was inconsistent, pipetting errors may be responsible for these results.

Locus	DNA Sample Number									
	1		2		3		4		(+) Control	
	silver	gold-plated	silver	gold-plated	silver	gold-plated	silver	gold-plated	silver	gold-plated
D8S1179	14,15	14,15	10,14	10,14	14,15	14,15	13	13	13	13
D21S11	30,31.2	30,31.2	29,30	29,30	31.2,32.2	31.2,32.2	28,32.2	28,32.2	30	30
D7S820	9,12	9,12	8,9	8,9	10,12	10,12	10,12	10,12	10,11	10,11
CSF1PO	10,11	10,11	10,11	10,11	10,12	10,12	9,11	9,11	10,12	10,12
D3S1358	15,16	15,16	15,16	15,16	15	15	15,18	15,18	14,15	14,15
THO1	7	7	7,8	7,8	6	6	7	7	8,9.3	8,9.3
D13S317	11,12	11,12	12	12	8,12	8,12	11,14	11,14	11	11
D16S539	11,12	11,12	11,12	11,12	11,12	11,12	10,11	10,11	11,12	11,12
D2S1338	17	17	22	22	19,25	19,25	19	19	19,23	19,23
D19S433	16.2,17.2	16.2,17.2	15	15	14,16.2	14,16.2	13.2,14	13.2,14	14,15	14,15
vWA	17	17	19	19	15	15	14,17	14,17	17,18	17,18
TPOX	9,10	9,10	10,11	10,11	8,12	8,12	8	8	8	8
D18S51	13,18	13,18	16,19	16,19	14,17	14,17	13,17	13,17	15,19	15,19
D5S818	11,13	11,13	11,12	11,12	9,11	9,11	11	11	11	11
FGA	22,25	22,25	24,26	24,26	21,22	21,22	24,25	24,25	23,24	23,24

Table 3 – DNA profiles from all samples generated on the silver and gold-plated silver sample blocks using four AmpFℓSTR® typing kits

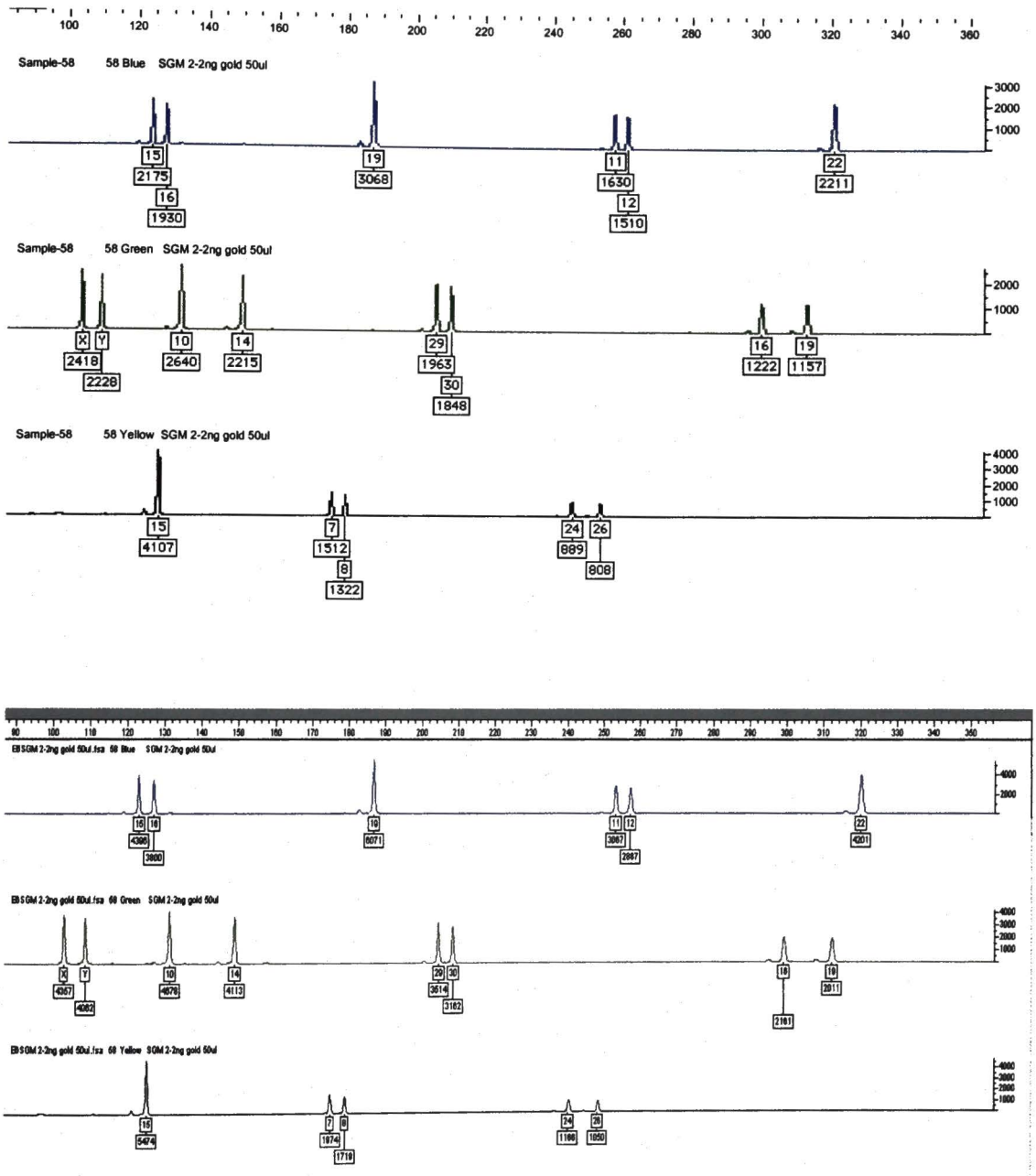


Figure 9a – DNA Profiles from the AmpFℓSTR® SGM Plus™ typing kit analyzed on the ABI PRISM™ Genetic Analyzer utilizing the Macintosh™ software (top) and Windows NT™ software (bottom).

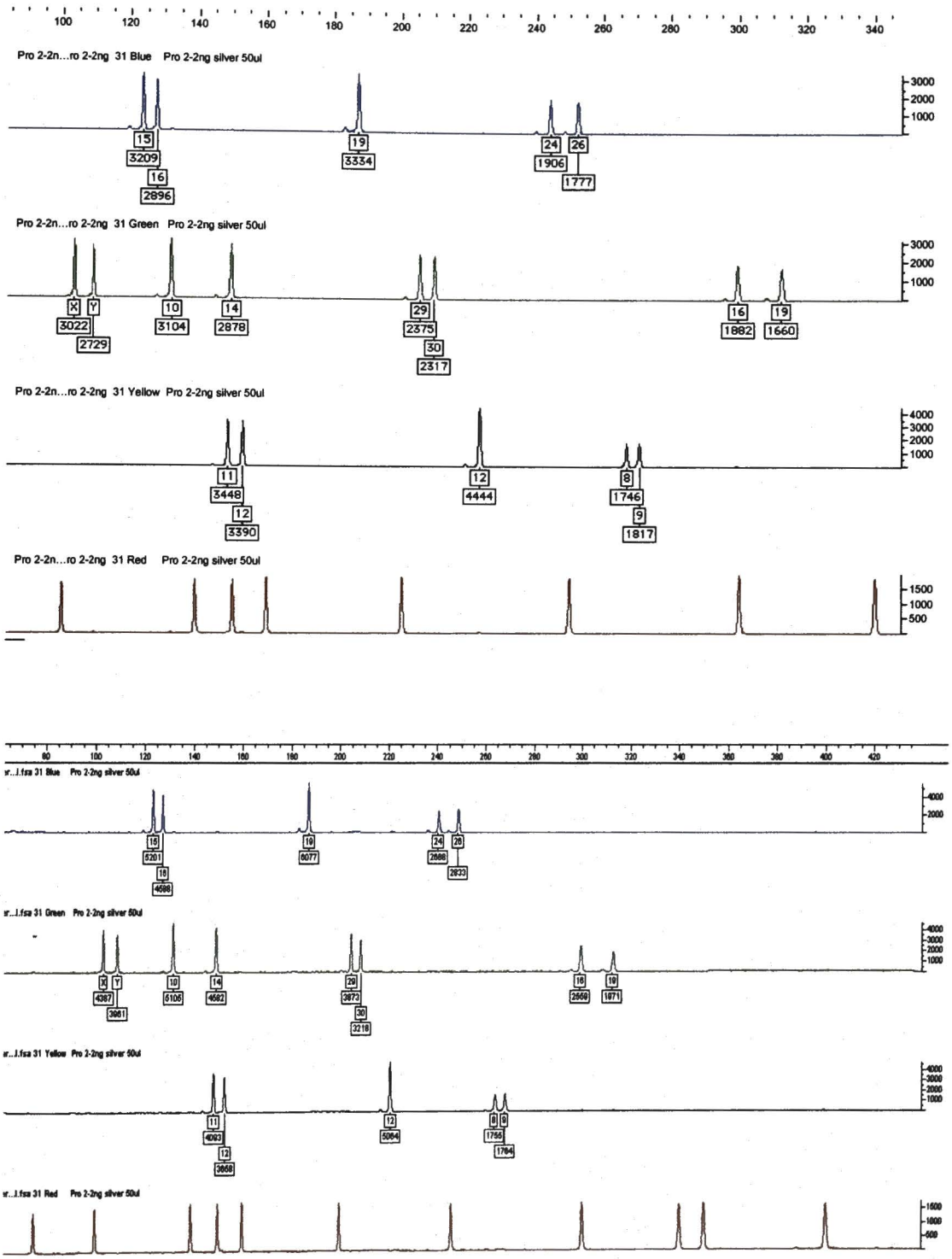


Figure 9b – DNA Profiles from the AmpFℓSTR® Profiler Plus *ID*™ typing kit analyzed on the ABI PRISM™ Genetic Analyzer utilizing the Macintosh™ software (top) and Windows NT™ software (bottom).

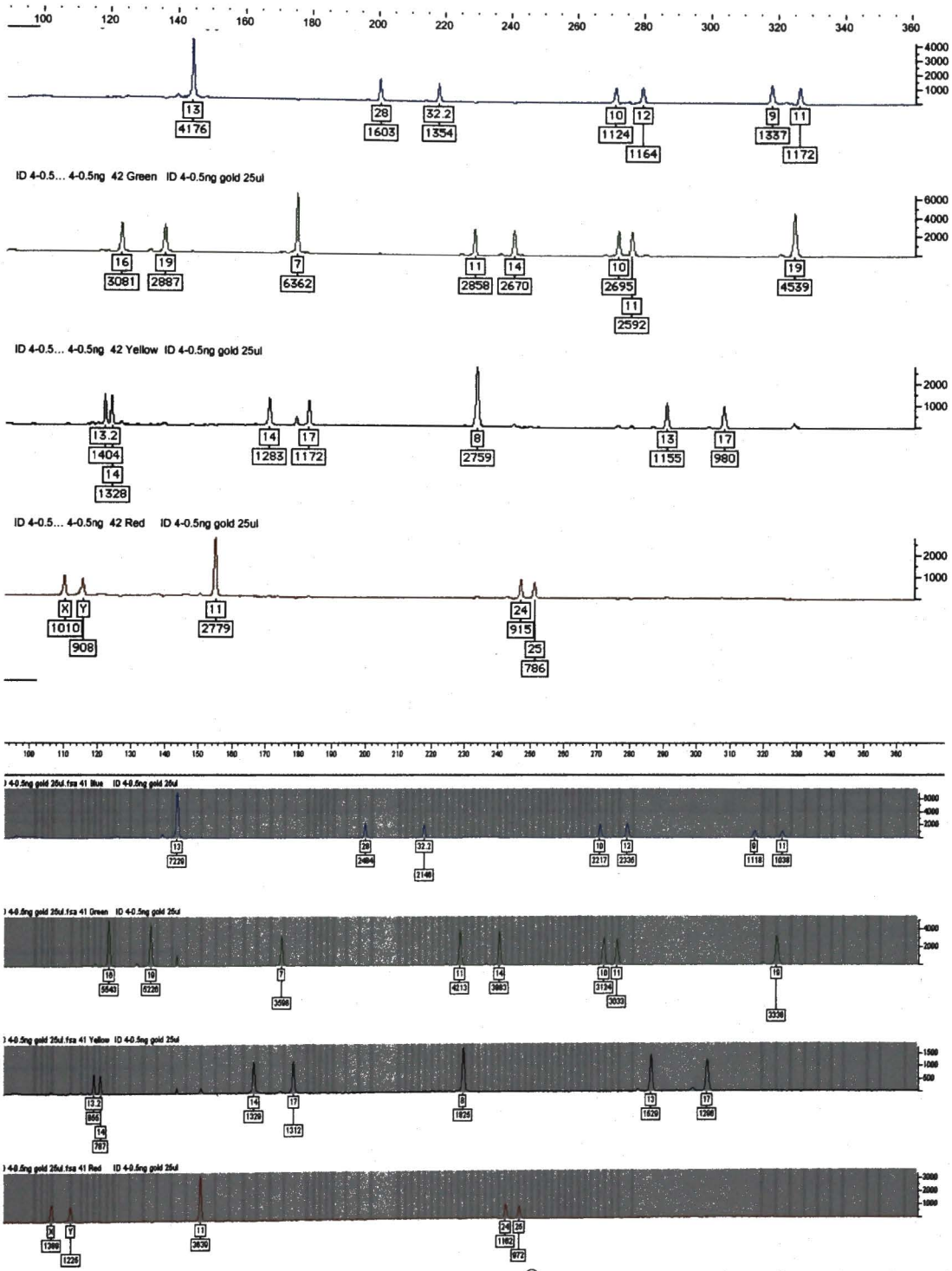


Figure 9c – DNA Profiles from the AmpFℓSTR® Identifiler™ typing kit analyzed on the ABI PRISM™ Genetic Analyzer utilizing the Macintosh™ software (top) and Windows NT™ software (bottom).

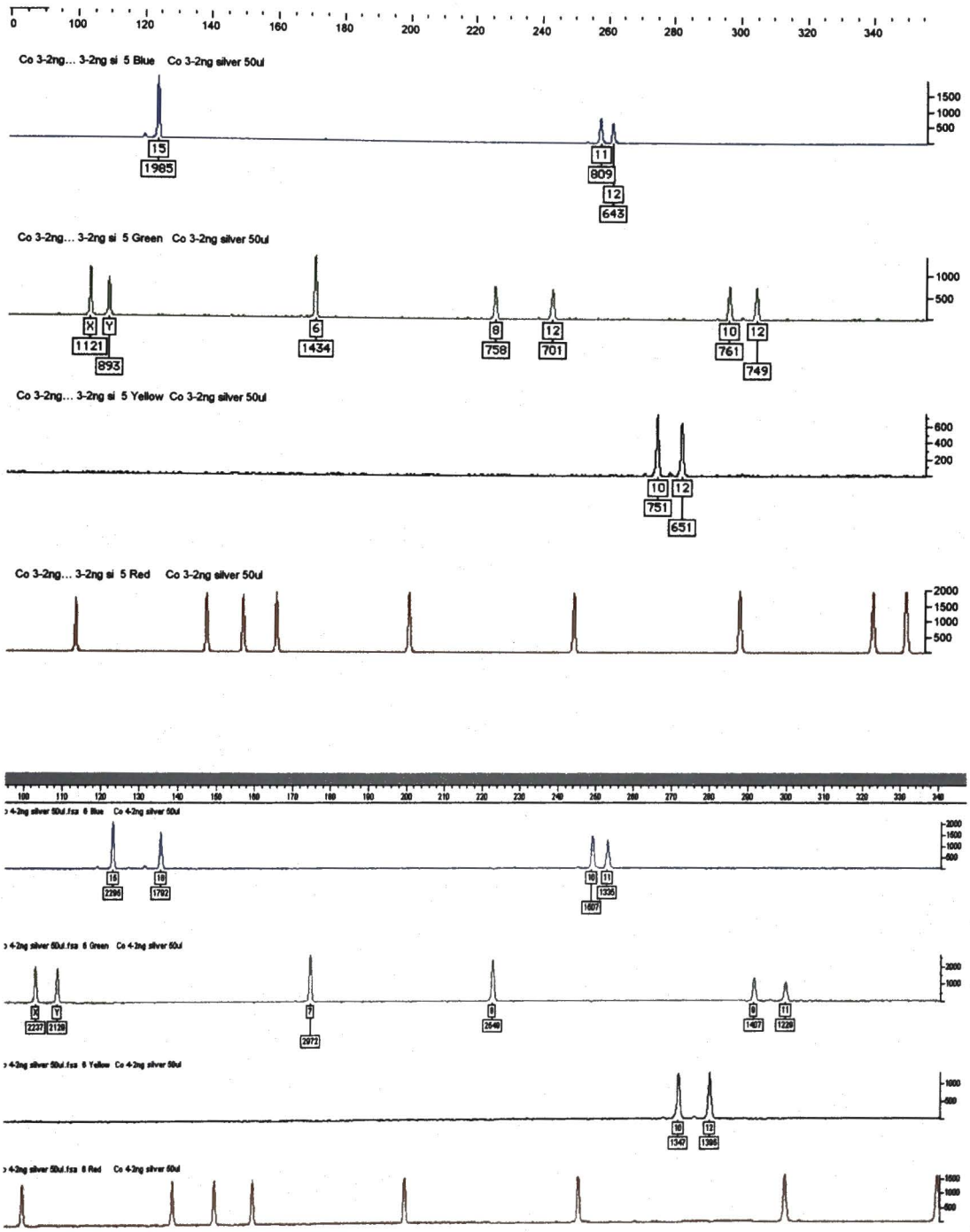


Figure 9d – DNA Profiles from the AmpFℓSTR® COfiler™ typing kit analyzed on the ABI PRISM™ Genetic Analyzer utilizing the Macintosh™ software (top) and Windows NT™ software (bottom).

CHAPTER 4

DISCUSSION

Validation of new technology in the forensic laboratory is required before its implementation in casework. Standards adopted by the DNA Advisory Board in 1998 (1) mandate that material changes in laboratory protocols must be validated, and the validation studies must be documented before any casework is performed utilizing the changes. This study was undertaken for the purposes of validating the gold-plated silver sample block on the GeneAmp® PCR System 9700 thermal cycler.

The concordance of the DNA profiles from one sample block to another demonstrates that results generated on either sample block will be identical to results generated on the other.

The quality and quantity of the PCR products from both silver and gold-plated sample blocks is also consistent, as revealed by the peak height and peak height ratio data. The peak height data indicate that there is no great difference between the amplification product quantities from the two sample blocks. Although some of the data from the SGM Plus™ and Identifiler™ amplifications look preferential (Appendix B, Graphs 21 and 33), it cannot be said that one block is better than the other. Instead this data may reflect that, because these samples were not amplified on the same day, pipetting error may be to blame. Because the samples were not all amplified on the same

day and because the PCR components were not all prepared at the same time this introduced variability into the study. Pipetting discrepancies in the preparation of the amplification components could account for some of the peak height differences that were seen. The method of quantitation of the DNA samples could also contribute to peak height variance. The method used to quantify the DNA is very subjective and the quantity of DNA in a sample can vary 10-fold from laboratory to laboratory and even between analysts in the same laboratory (24). When the positive control samples (known concentration 0.1 ng/uL) were evaluated, the peak heights were consistent from block to block between all four AmpFℓSTR® kits and between the concentrations of template DNA, further associating quantification error with these findings. Because average peak height variability cannot be attributed solely to differences in the sample blocks, and because all peaks were easily detected by the data collection software, both blocks could be said to perform equally well.

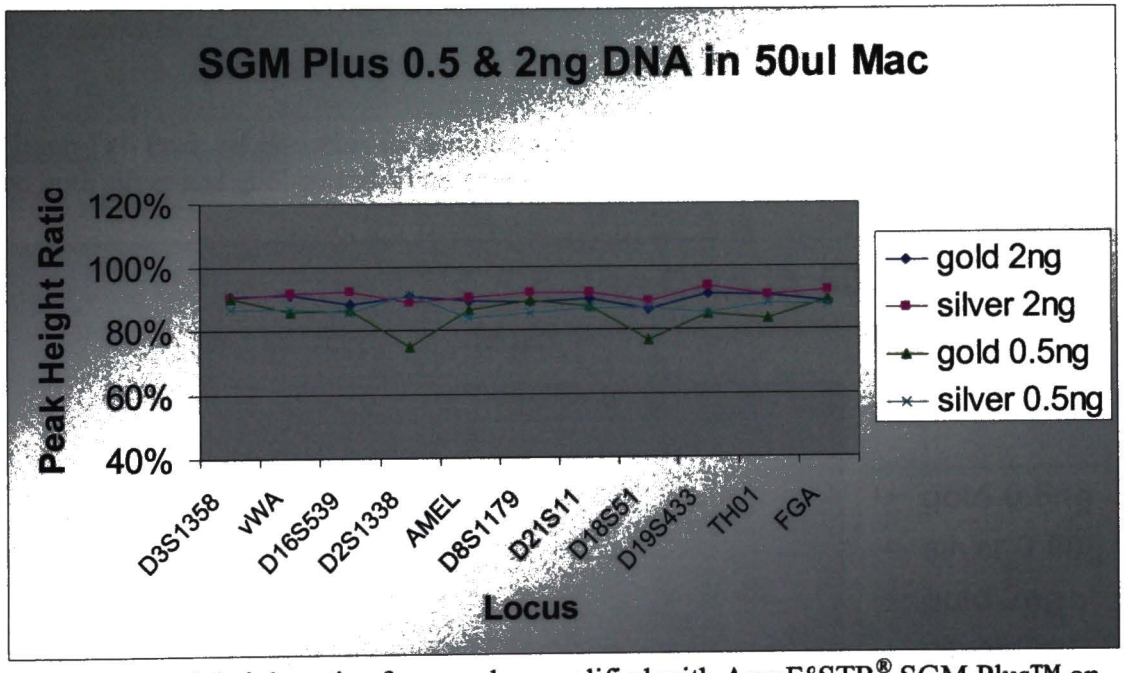
Because the peak height ratios from each block are remarkably similar the performance of these sample blocks can be said to be equivalent. Variability in the peak height ratios could have been attributed to one of many factors. Lower peak height ratios could be explained by the actual size of the alleles at a locus. If there is a large difference in the actual size of alleles at a heterozygous locus then the peak height ratio can be decreased. This is due to preferential amplification of the smaller allele at that locus. Also contributing to the variability of peak height ratio is the number of heterozygotes at each locus. For each locus in this study there was a possibility of having five heterozygotes (each sample could be heterozygous). With five samples run in duplicate,

there were possibly 10 peak height ratios to be averaged to determine the mean peak height ratio for that locus. Homozygosity at a locus eliminates two of the possible contributing peak height ratios. This could increase the variability at that locus simply because of the decreased sample size. In the data collected in this study there was no trend indicating that one sample block performs differently than the other. We would have expected to see consistently higher peak height ratios from one type of sample block across all loci if there was a difference in the sample block performance. Since we did not see this trend we are confident that the sample blocks are truly interchangeable.

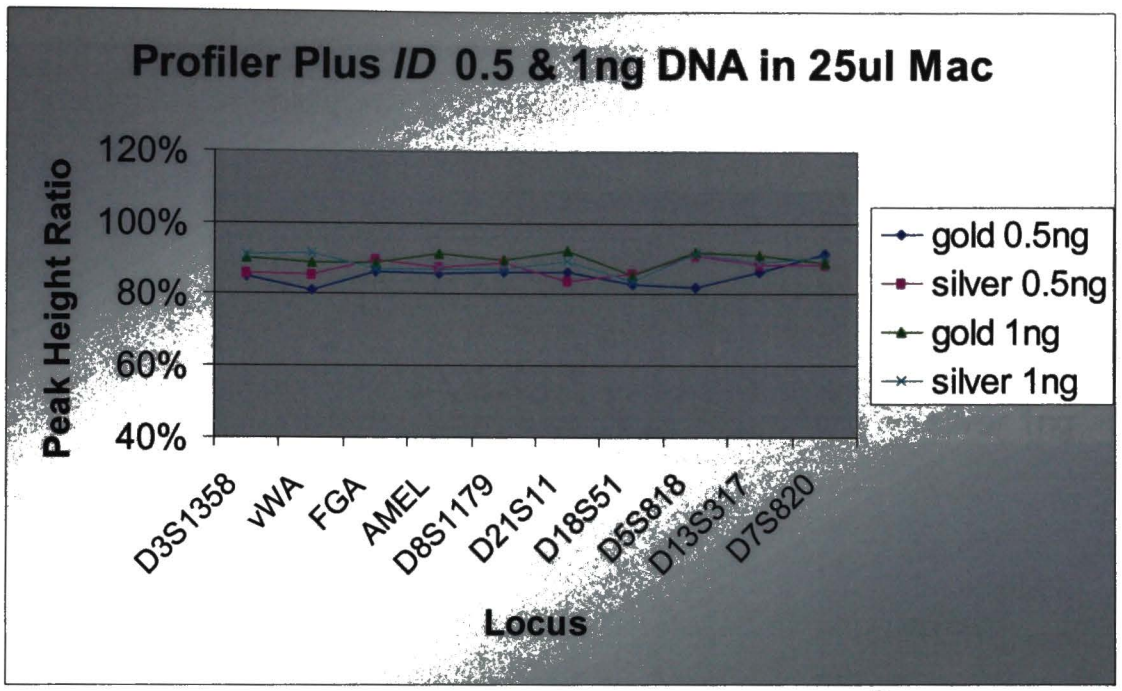
The results of this study demonstrate concordance between the silver sample block and the gold-plated silver sample block when used on the GeneAmp® PCR System 9700 thermal cycler. Therefore, the gold-plated silver sample block is validated for use in our laboratory.

APPENDIX A

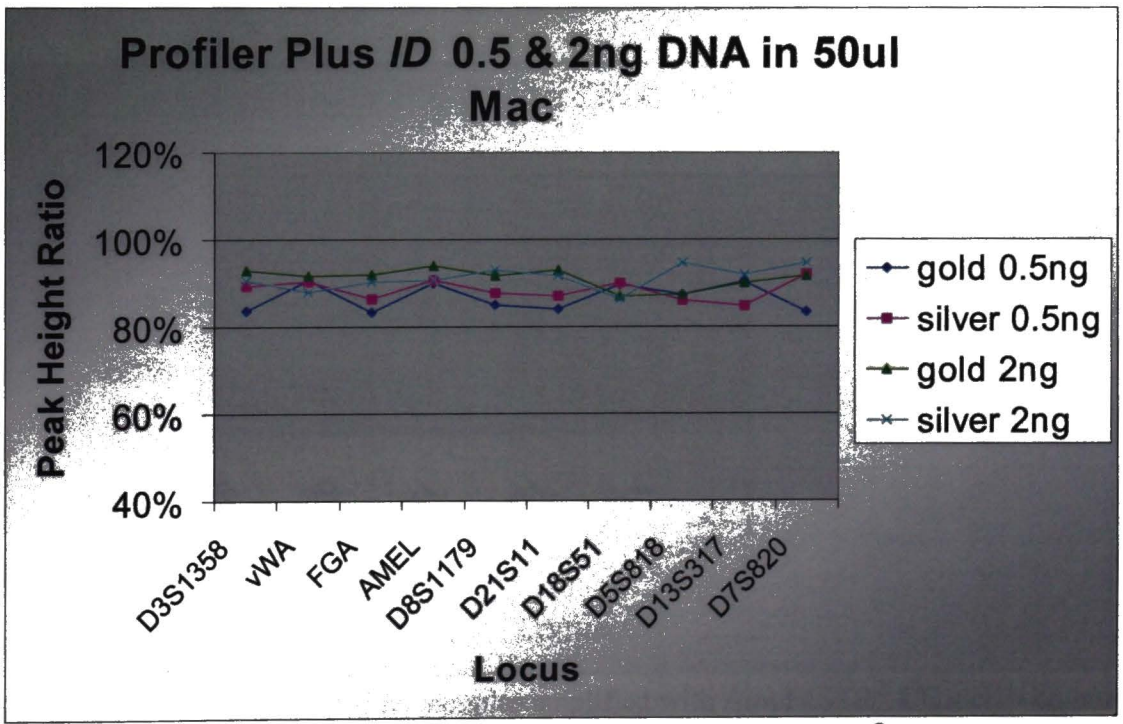
Graphs 10 through 21 show peak height ratios from samples amplified utilizing each AmpFℓSTR typing kit and analyzed using Macintosh™ and Windows NT™ software. Applied Biosystems, the manufacturer of the AmpFℓSTR typing kits specifies a range of 70-100% as acceptable.



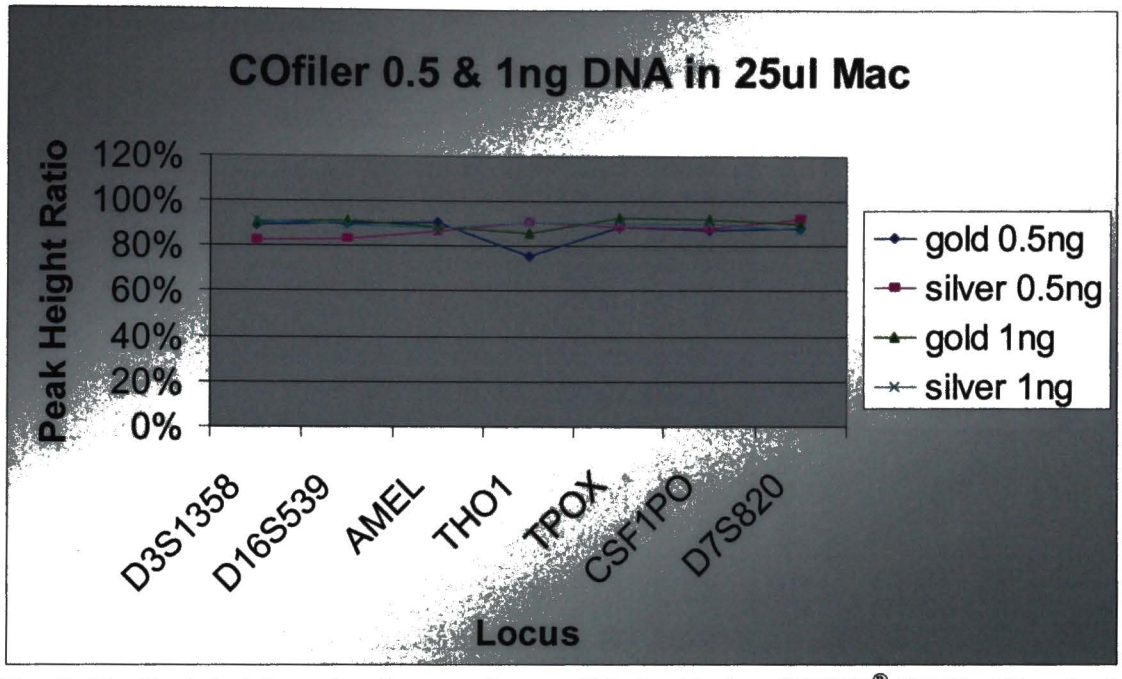
Graph 10 - Peak height ratios for samples amplified with AmpFℓSTR® SGM Plus™ on both silver and gold-plated silver sample blocks.



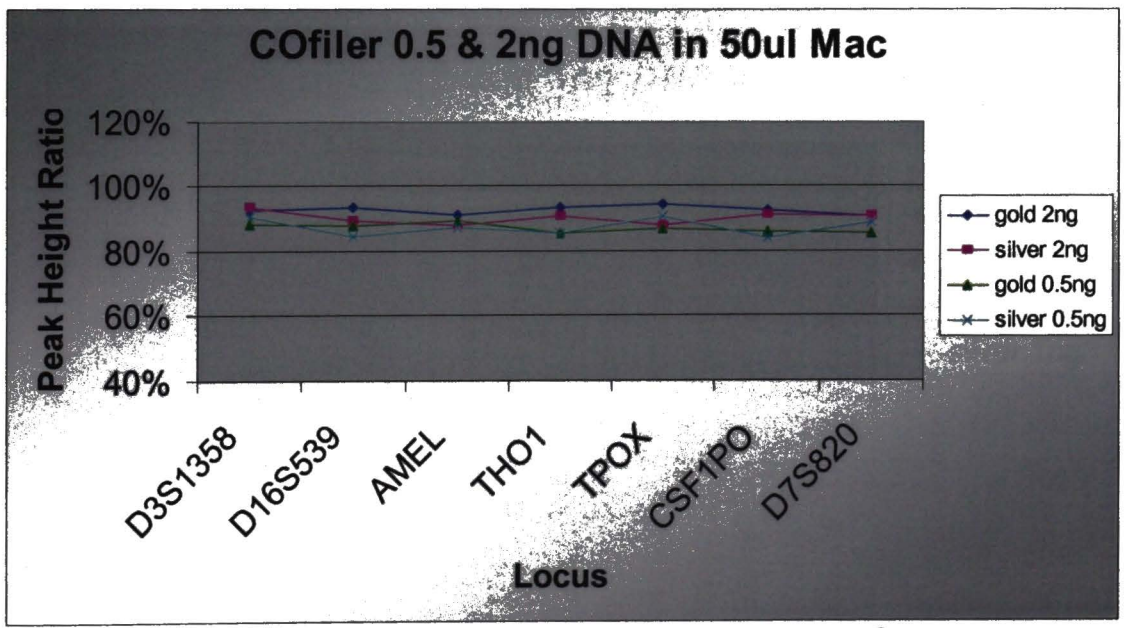
Graph 11 - Peak height ratios for samples amplified with AmpFℓSTR® Profiler Plus *ID*™ on both silver and gold-plated silver sample blocks.



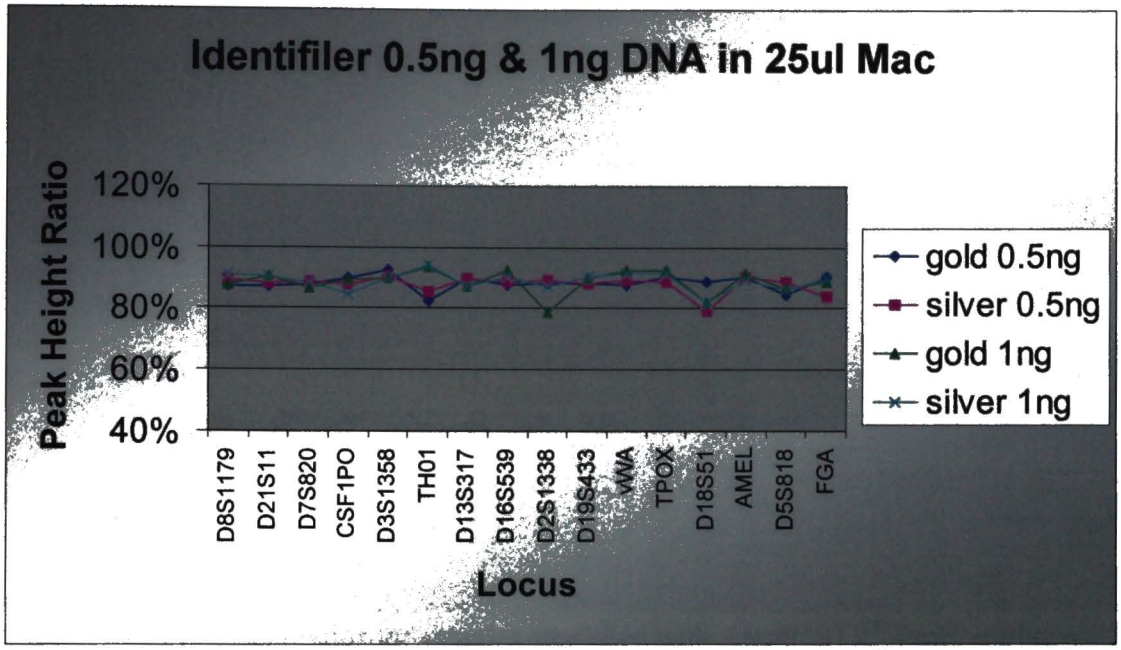
Graph 12 - Peak height ratios for samples amplified with AmpFℓSTR® Profiler Plus *ID*™ on both silver and gold-plated silver sample blocks.



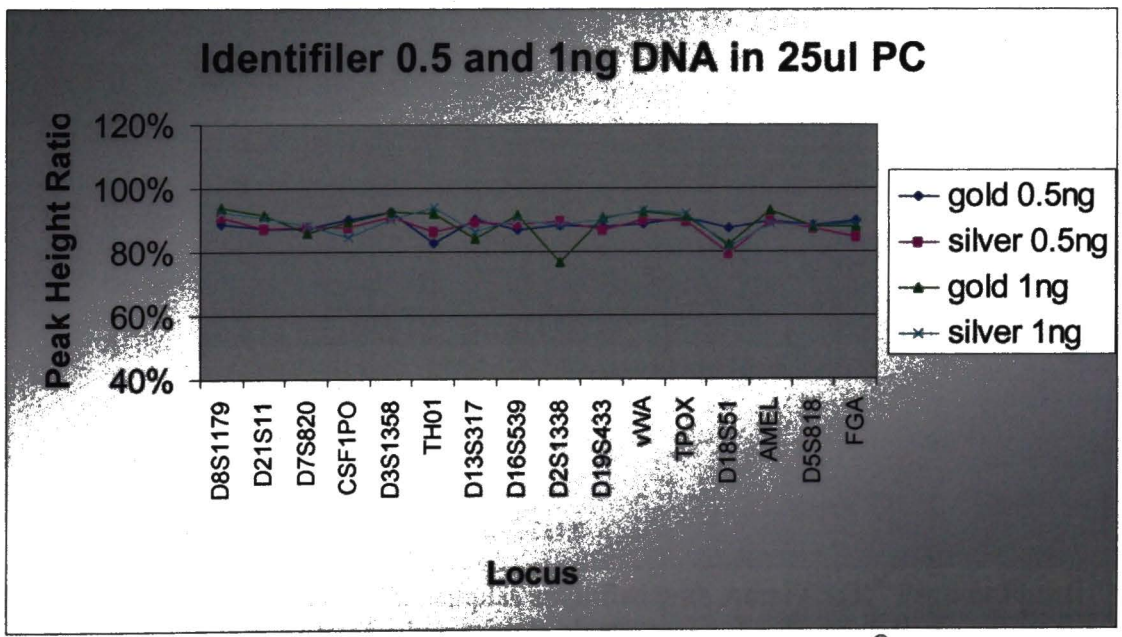
Graph 13 - Peak height ratios for samples amplified with AmpFℓSTR® COfiler™ on both silver and gold-plated silver sample blocks.



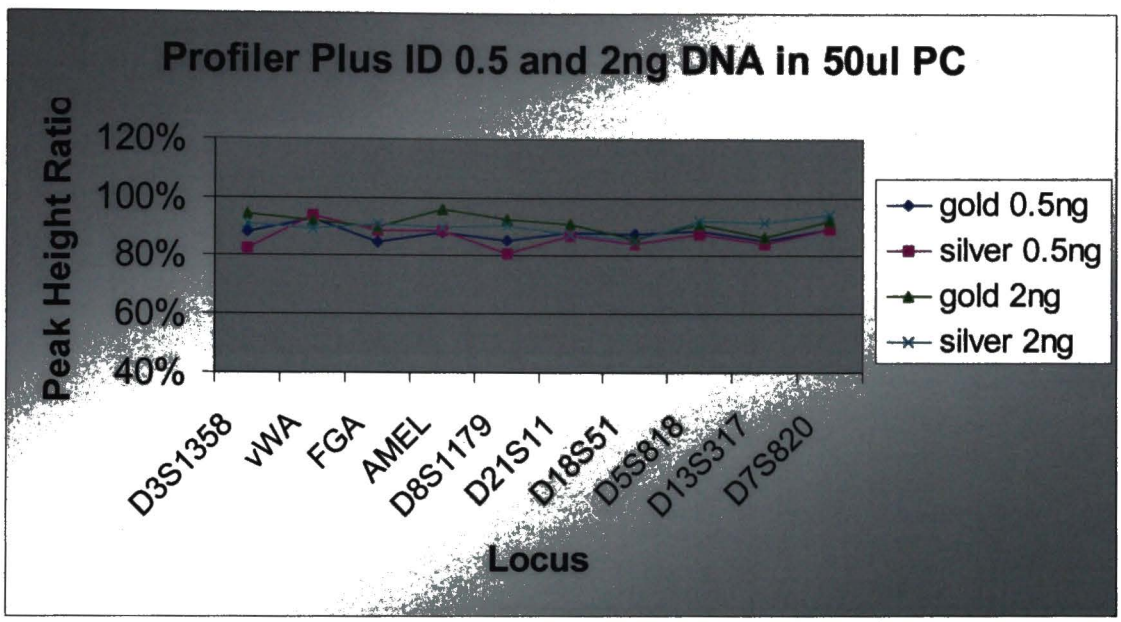
Graph 14 - Peak height ratios for samples amplified with AmpFℓSTR® COfiler™ on both silver and gold-plated silver sample blocks.



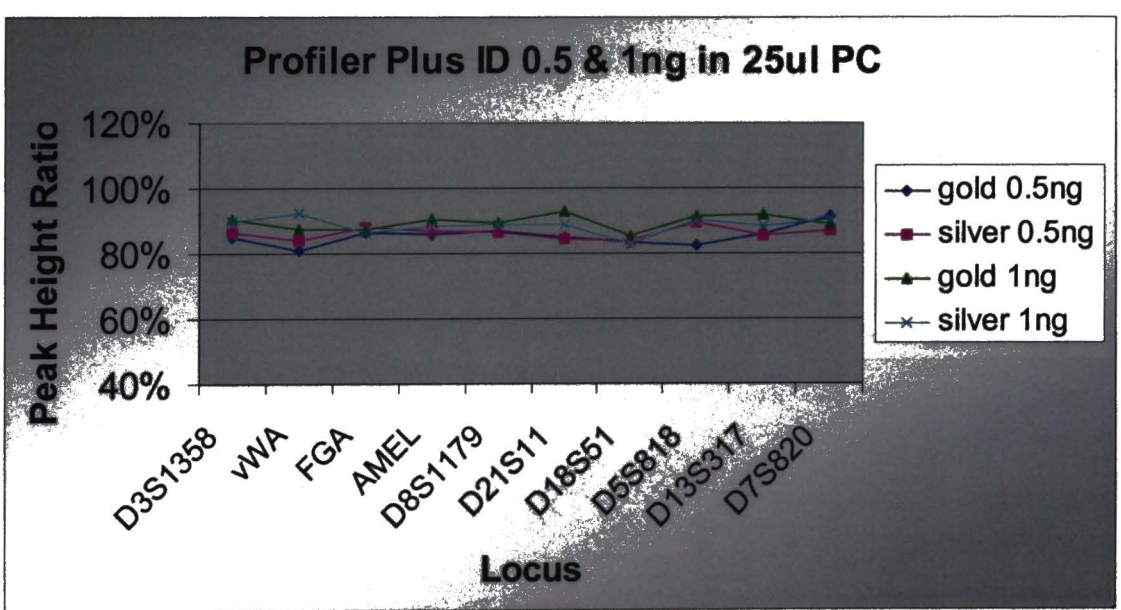
Graph 15 - Peak height ratios for samples amplified with AmpFℓSTR® Identifiler™ on both silver and gold-plated silver sample blocks.



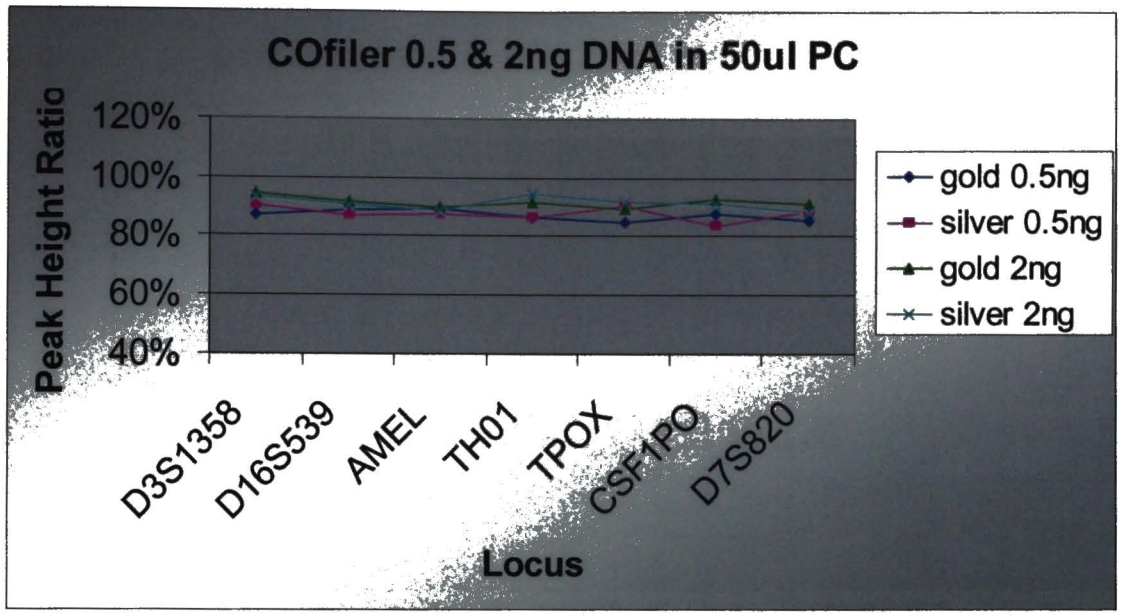
Graph 16 - Peak height ratios for samples amplified with AmpFℓSTR® Identifiler™ on both silver and gold-plated silver sample blocks.



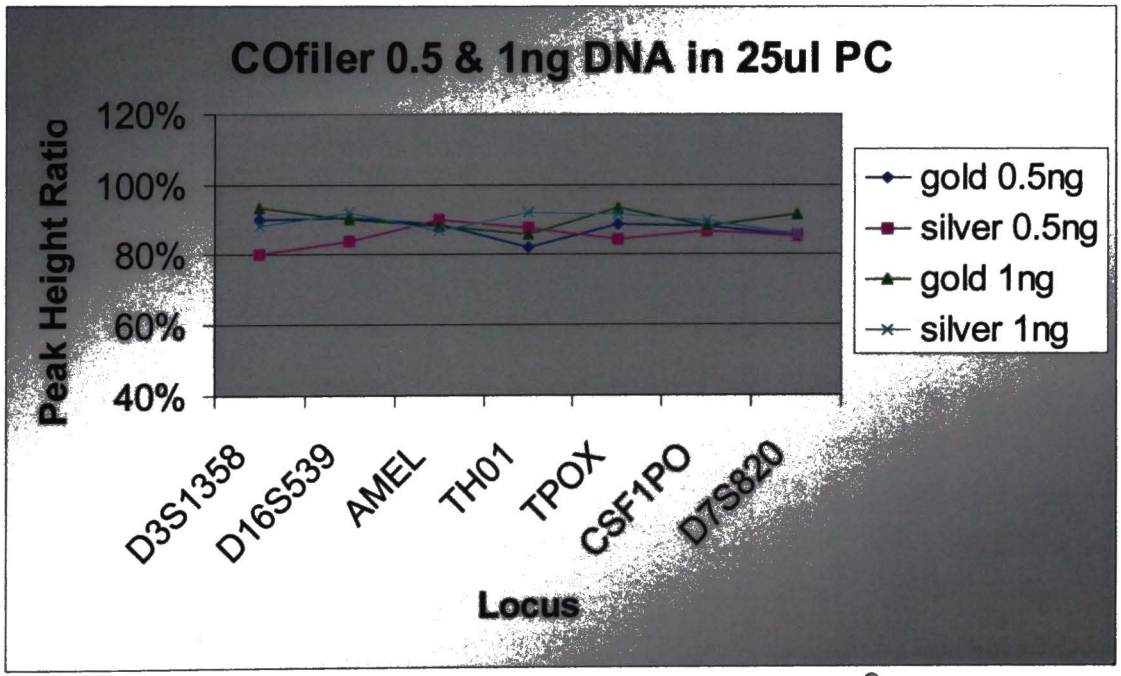
Graph 17 - Peak height ratios for samples amplified with AmpFℓSTR® Profiler Plus *ID*™ on both silver and gold-plated silver sample blocks.



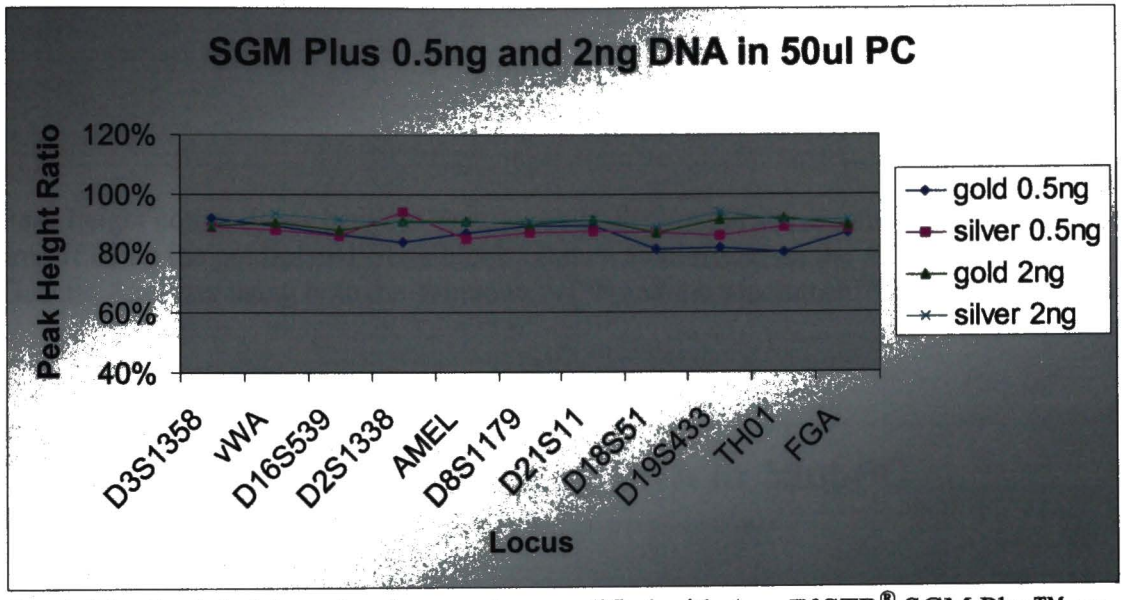
Graph 18 - Peak height ratios for samples amplified with AmpFℓSTR® Profiler Plus *ID*™ on both silver and gold-plated silver sample blocks.



Graph 19 - Peak height ratios for samples amplified with AmpFℓSTR® COfiler™ on both silver and gold-plated silver sample blocks.



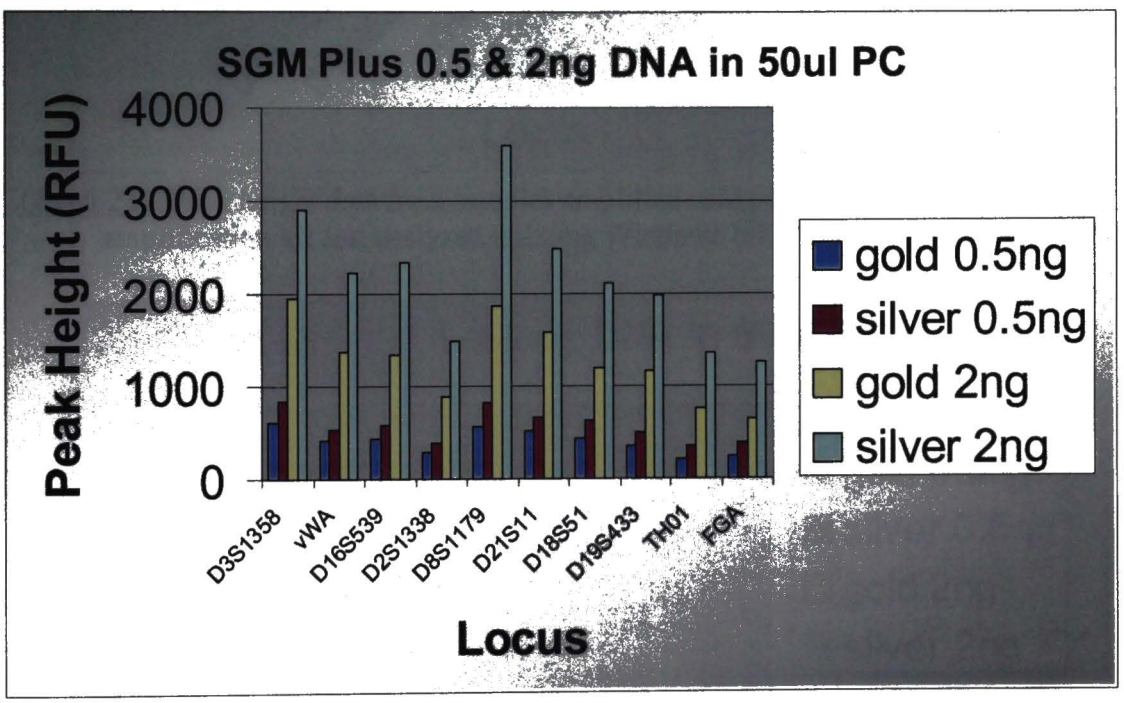
Graph 20- Peak height ratios for samples amplified with AmpFℓSTR® COfiler™ on both silver and gold-plated silver sample blocks.



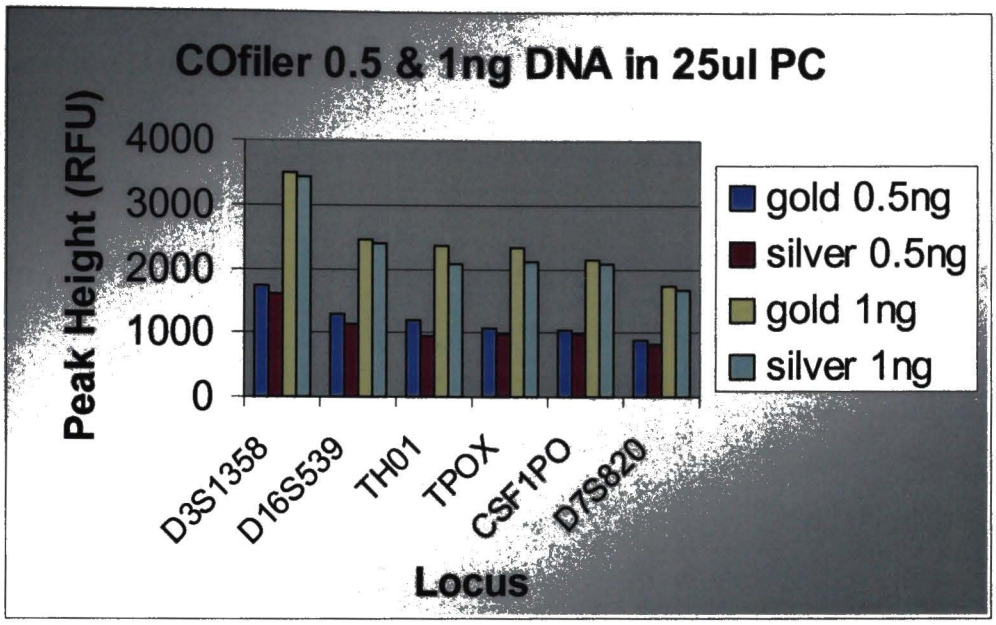
Graph 21 – Peak height ratios for samples amplified with AmpFℓSTR® SGM Plus™ on both silver and gold-plated silver sample blocks.

APPENDIX B

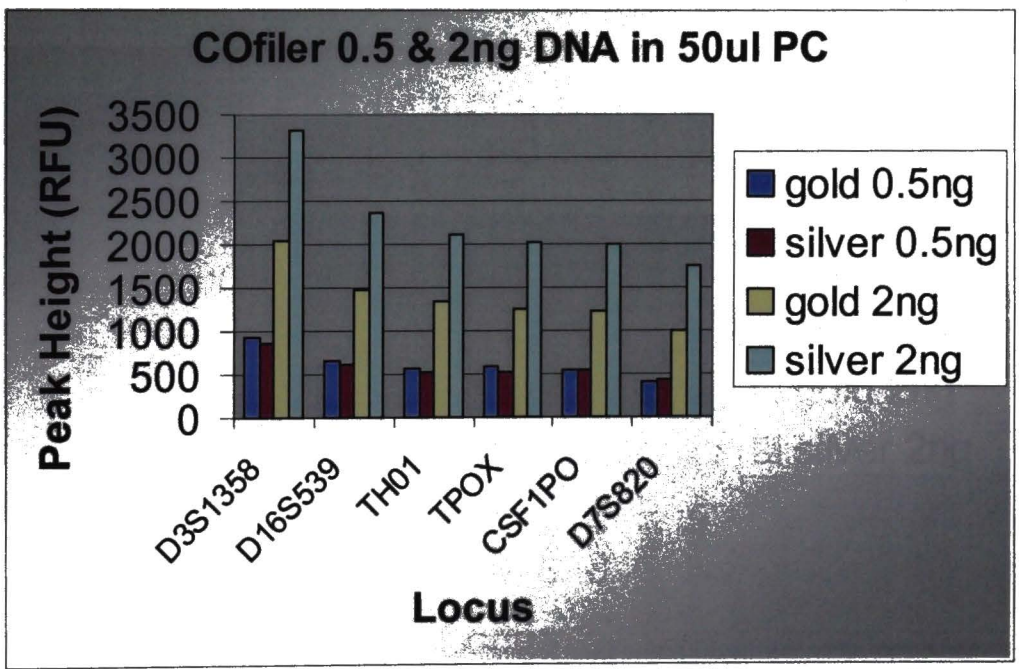
Peak height comparisons between samples amplified on the silver sample block and those amplified on the gold-plated silver block. Data was analyzed on the ABI PRISM™ 310 Genetic Analyzer using both the Windows NT™ and the Macintosh™ operating systems.



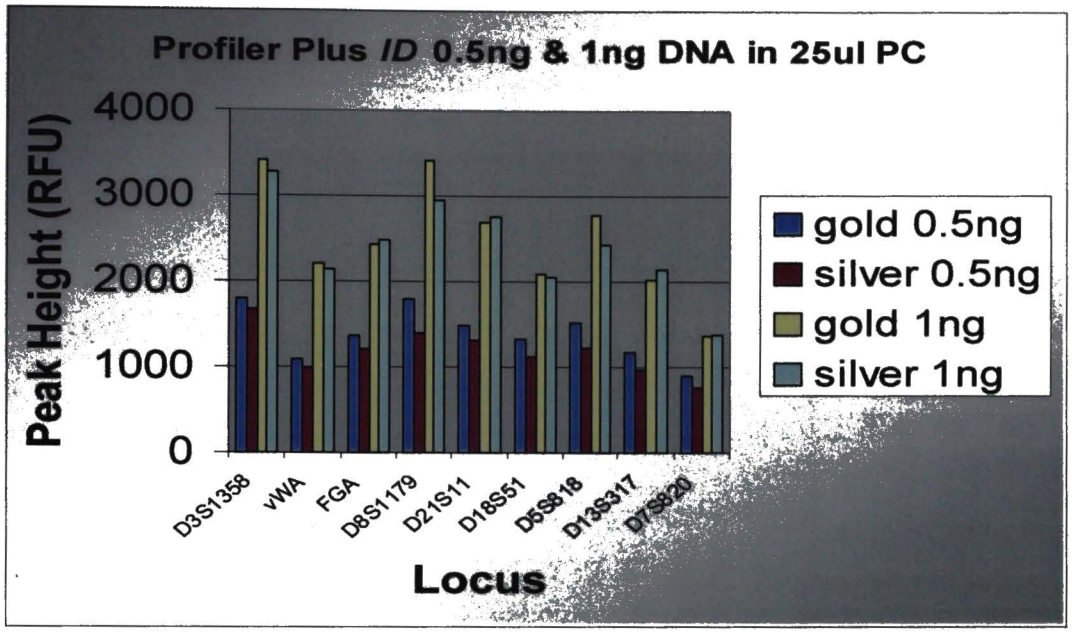
Graph 22 – Peak height data from samples amplified using the AmpFℓSTR® SGM Plus™ amplification kit and analyzed utilizing Windows NT™ operating system.



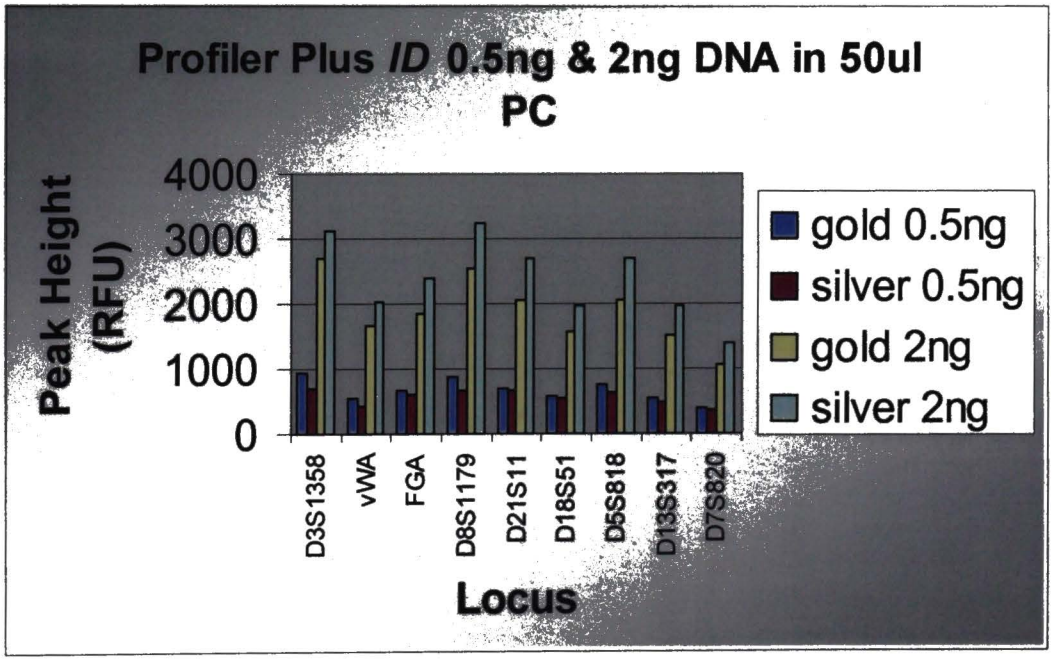
Graph 23 – Peak height data from samples amplified using the AmpFℓSTR® COfiler™ amplification kit and analyzed utilizing Windows NT™ operating system.



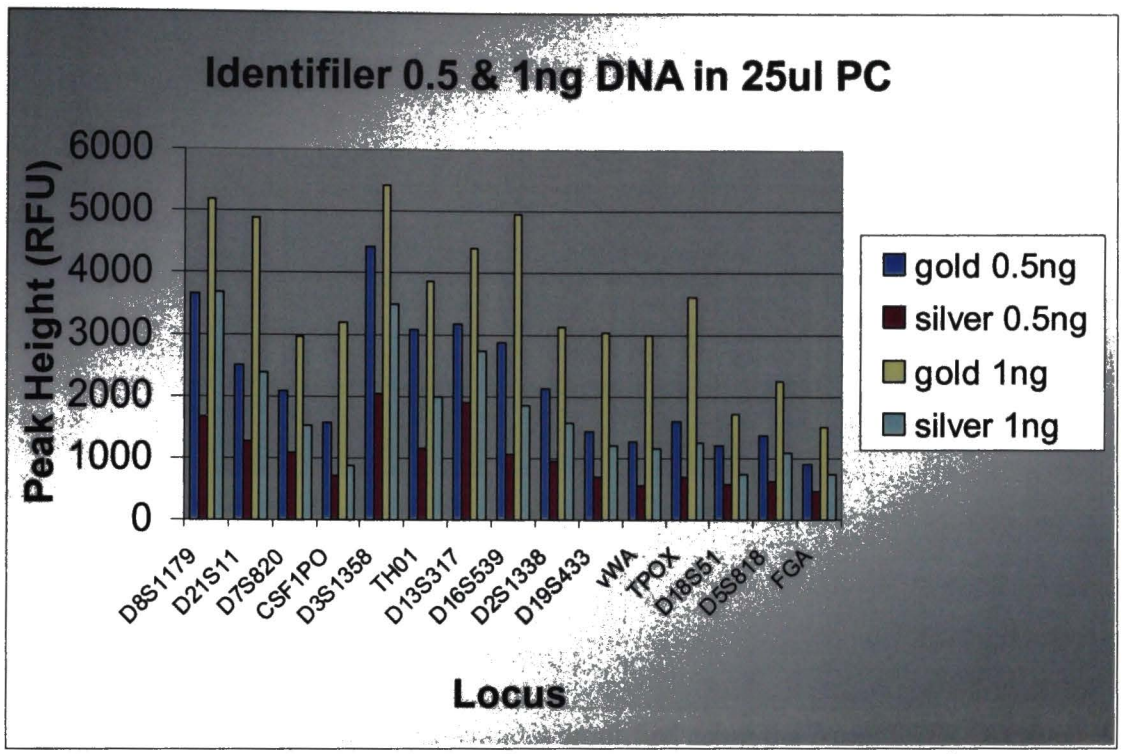
Graph 24 – Peak height data from samples amplified using the AmpFℓSTR® COfiler™ amplification kit and analyzed utilizing Windows NT™ operating system.



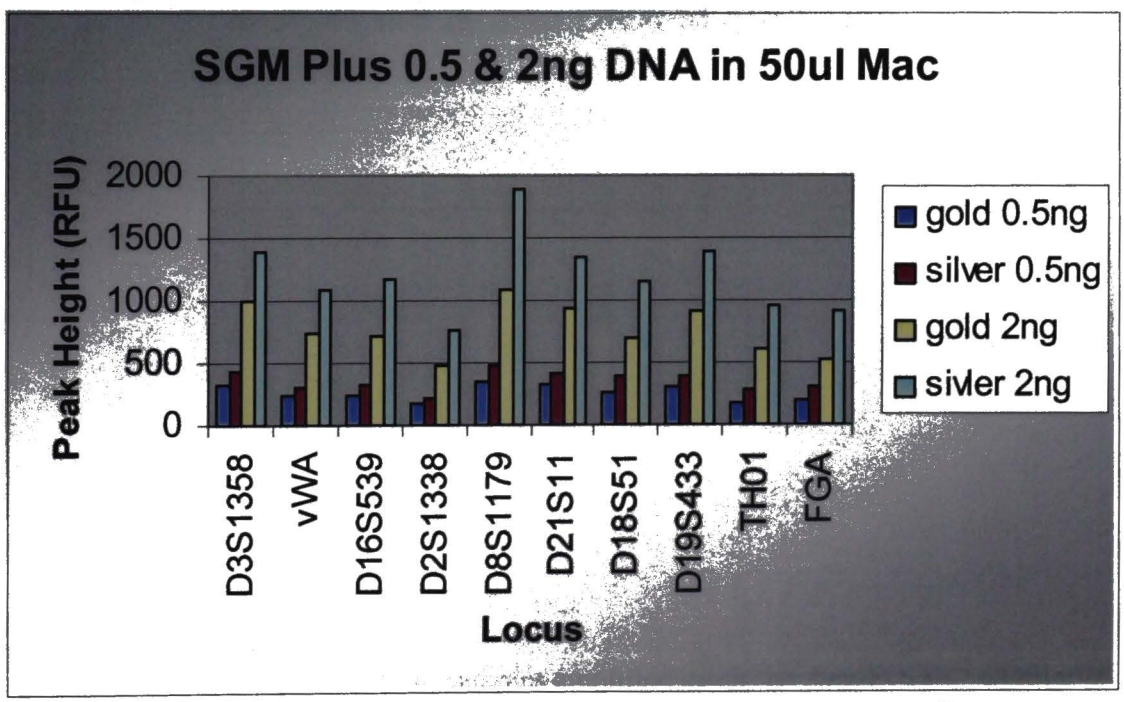
Graph 25 – Peak height data from samples amplified using the AmpFℓSTR® Profiler Plus *ID*™ amplification kit and analyzed utilizing Windows NT™ operating system.



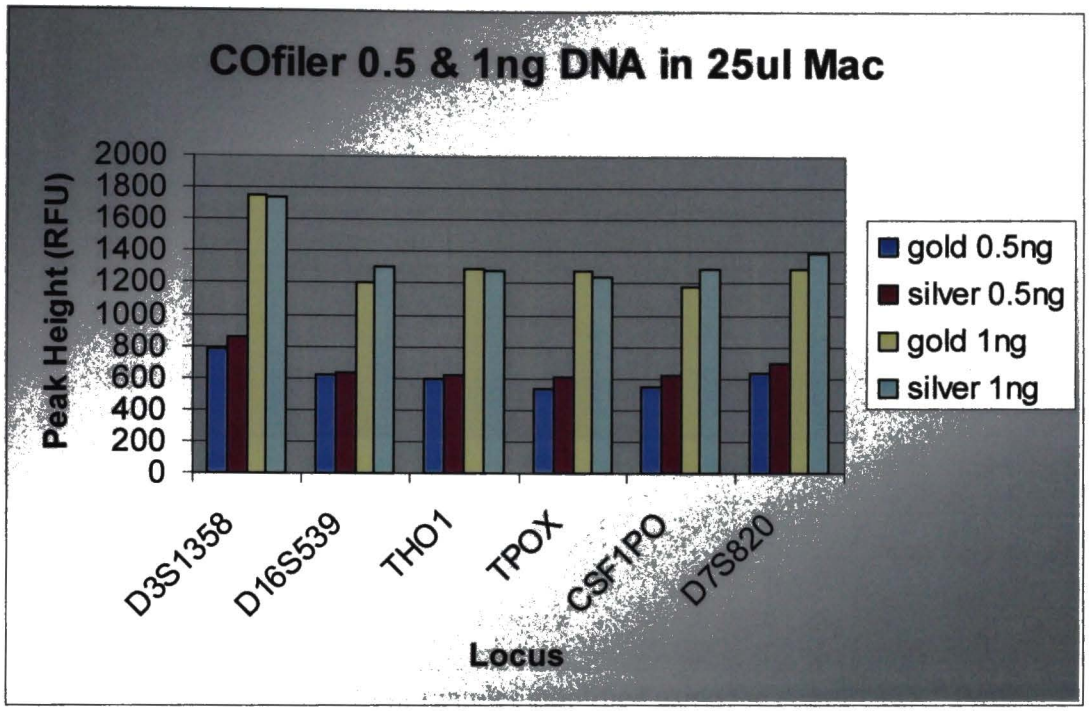
Graph 26 – Peak height data from samples amplified using the AmpFℓSTR® Profiler Plus *ID*™ amplification kit and analyzed utilizing Windows NT™ operating system.



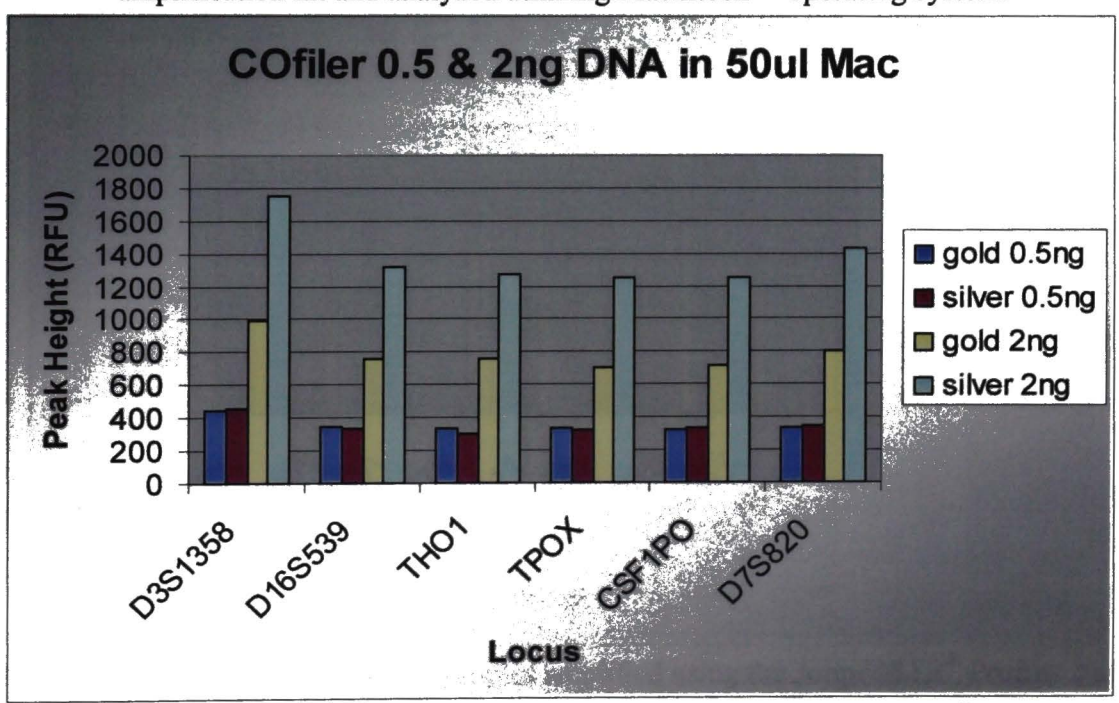
Graph 27 – Peak height data from samples amplified using the AmpFℓSTR® Identifiler™ amplification kit and analyzed utilizing Windows NT™ operating system.



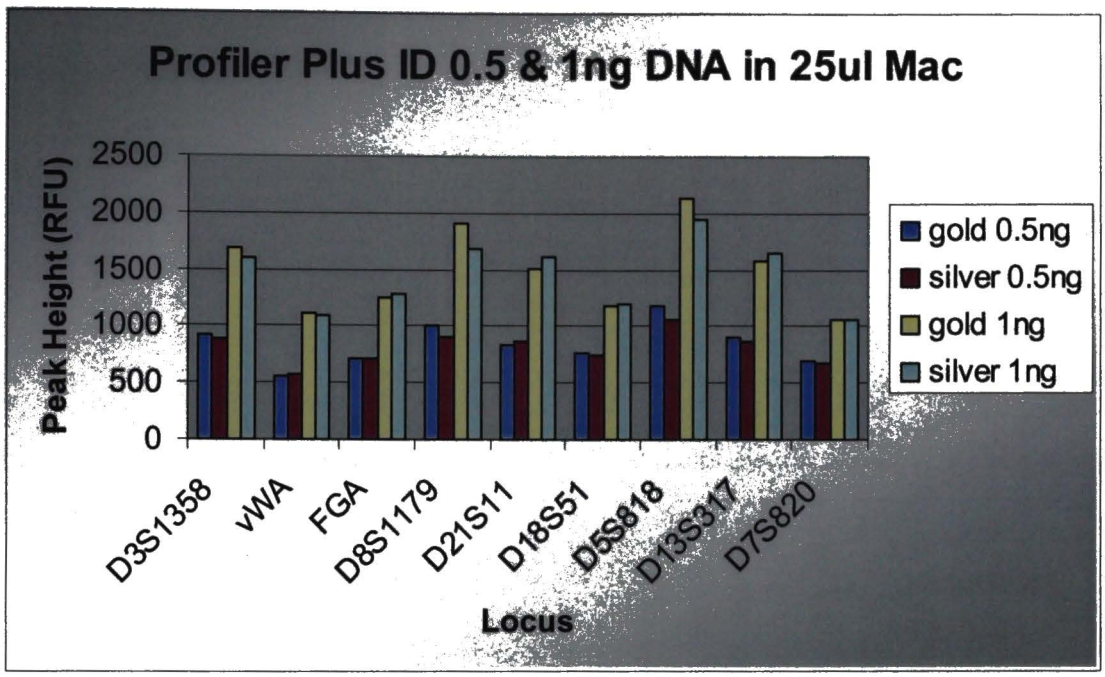
Graph 28 – Peak height data from samples amplified using the AmpFℓSTR® SGM Plus™ amplification kit and analyzed utilizing Macintosh™ operating system.



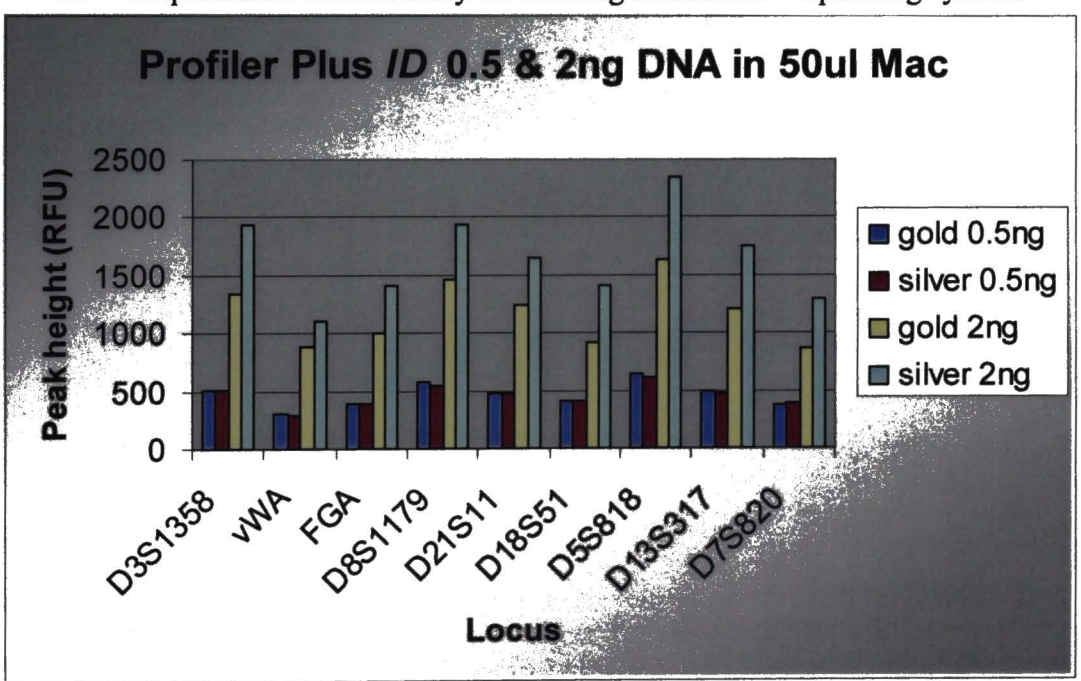
Graph 29 – Peak height data from samples amplified using the AmpFℓSTR® COfiler™ amplification kit and analyzed utilizing Macintosh™ operating system.



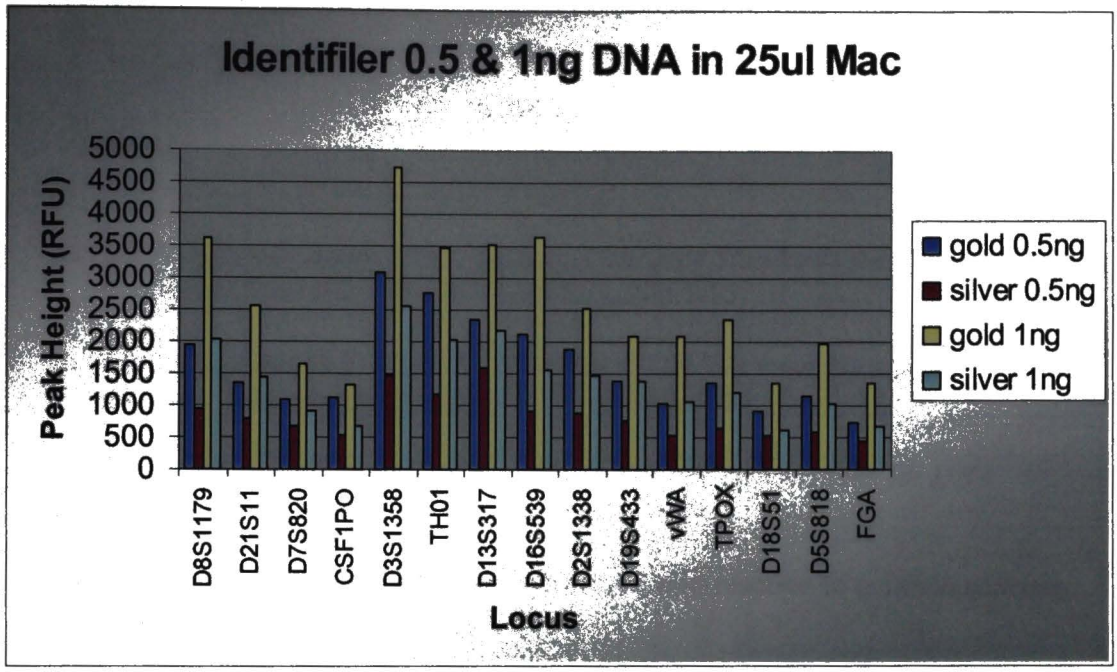
Graph 30 – Peak height data from samples amplified using the AmpFℓSTR® COfiler™ amplification kit and analyzed utilizing Macintosh™ operating system.



Graph 31 – Peak height data from samples amplified using the AmpFℓSTR® Profiler Plus *ID*™ amplification kit and analyzed utilizing Macintosh™ operating system.



Graph 32 – Peak height data from samples amplified using the AmpFℓSTR® Profiler Plus *ID*™ amplification kit and analyzed utilizing Macintosh™ operating system.



Graph 33 – Peak height data from samples amplified using the AmpFℓSTR® Identifiler™ amplification kit and analyzed utilizing Macintosh™ operating system.

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