
UNIVERSITY OF NORTH TEXAS
HEALTH SCIENCE CENTER AT FORT WORTH
1970

ANALYSIS OF LOW COPY NUMBER DNA USING PROFILER PLUS AT INCREASED AMPLIFICATION CYCLES AND MODIFICATIONS IN SAMPLE INJECTION PARAMETERS

Jody Lynn Hynds, B.S.

APPROVED:

Major Professor

Committee Member

Committee Member

Internship Practicum Supervisor

University Member

Chair, Department of Pathology and Anatomy

Dean, Graduate School of Biomedical Sciences

ANALYSIS OF LOW COPY NUMBER DNA USING PROFILER PLUS AT INCREASED AMPLIFICATION CYCLES AND MODIFICATIONS IN SAMPLE INJECTION PARAMETERS

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

Jody Lynn Hynds, B.S.

August 2003

ACKNOWLEDGEMENTS

I would like to thank the following people for their assistance for this Internship Practicum Report:

Rick Staub, Ph.D., Director of Operations, Orchid Cellmark

Judith Floyd, B.S., Forensic Laboratory Manager, Orchid Cellmark

Arthur Eisenberg, Ph.D., Major Professor, UNTHSC

John Planz, Ph.D., Graduate Advisor and Committee Member, UNTHSC

Joseph Warren, Ph.D., Committee Member, UNTHSC

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

LIST OF FIGURES AND TABLES

CHAPTER I

INTRODUCTION

The analysis of short tandem repeat (STR) loci using commercial Polymerase Chain Reaction (PCR) multiplex kits is standard in the forensic community for typical forensic casework samples containing bloodstains, hair roots, sexual assault evidence, and saliva stains. A commonly used amplification kit, AmpFlSTR™ Profiler Plus, has a recommended quantity of template DNA of 1-2ng (1). The amplification products are visualized by capillary electrophroresis. Due to the variability of the sensitivity between genetic analyzers, often less than the optimum amount of input DNA can produce a complete DNA profile. However, at Orchid Cellmark Dallas (OCD), 1.25ng of template DNA added to the amplification reaction.

It is difficult to obtain complete STR profiles from forensic evidence with low quantities of DNA or low copy number samples. Samples with low copy number DNA contain a small amount of DNA and have a limited number of copies of the genome. Previous studies have indicated that 100pg (0.1ng) or less of extracted DNA should be treated as low copy number samples (2). Trace DNA is considered minute amounts of DNA present in a sample. Some examples of evidence that may contain trace amounts of DNA are fingerprints, shoelaces and clothing in contact with skin. Items presumed to be trace evidence do not always contain low copy number DNA, the amount of DNA deposited on an item is dependent on many factors and need to be examined on a case by case basis (3). These samples usually display partial to no DNA profiles (8).

Optimizing the protocols currently in use in forensic laboratories and DNA laboratories for the analysis of low copy number DNA samples could increase the amount of reliable data obtained from limited or compromised evidence collected at crime scenes. The methodologies may only require modifications to the amplification number and injection parameters, eliminating the necessity of purchasing of new expensive laboratory equipment. The implementation of low copy number techniques would be relatively simple and cost effective for the average crime laboratory.

There are many DNA testing techniques that can be utilized for samples with low quantities of DNA. Mitochondrial DNA testing is designed for successful DNA sequencing of hair shafts, degraded and burned samples. Newly developed SNP (single nucleotide polymorphisms) testing is also designed for the analysis of challenging samples. The increased interest in the analysis of low copy number DNA samples using STR testing is necessitated since the national database, CODIS (Combined Data Index System) currently only accepts the DNA profiles analyzed with the 13 core STR loci. CODIS contains DNA profiles of evidence found at crime scenes, convicted offender and missing persons DNA profiles (4). The goal of this project is to develop methodologies to increase the success rate of LCN DNA samples using STR testing.

Increasing the number of cycles during PCR can increase the amount of amplified product; therefore, increasing the relative fluorescence units (RFUs) of the STR alleles detected during capillary electrophoresis (CE). The RFUs for alleles may ve above the required detection threshold for making an allele call. Increasing the number of PCR cycles can also augment many unwanted amplification artifacts resulting from the

stochastic amplification of limited amounts of template DNA. The PCR cycle number chosen for amplifying LCN samples must obtain a balance between an increase in detected STR loci with the unwanted characteristics of PCR stochastic effects (5). In samples exhibiting partial DNA profiles, the larger loci do not amplify efficiently. Increasing the PCR cycle number theoretically should result in more copies of the desired STR product. Typically, when low copy number samples are amplified and analyzed on a genetic analyzer utilizing to the manufacturer's specifications, there is not enough amplified product generated to meet a laboratory established detection threshold. LCN samples amplified at 34 cycles exhibit a variety of stochastic effects. Samples display heterozygous peak height imbalance, high stutter ratio (compared to associated allele peak) and allelic dropout. Thirty-four amplification cycles appears to be the upper boundary for increasing cycle numbers for LCN samples (2). There has been very limited research performed examining the amplification of LCN samples between 28 and 34 cycles. The available data shows approximately a 55% success rate for samples of 25-100pg amplified at 30 cycles, approximately a 70% success rate for samples of 25-100pg amplified at 32 cycles and approximately a 95% success rate for samples of 25-100pg amplified at 34 cycles. The range of 25-100pg is relatively broad and the averages shown may be misleading as to the success of achieving a complete profile at those low quantities. Samples amplified at 34 cycles contained complete profiles at approximately 25-50pg. The ability to obtain a complete DNA profile with less than 25-50pg is improbable (7).

Manipulation of the CE instrument sample injection parameters were used to obtain the best possible results from the DNA amplified from LCN DNA samples. If the LCN DNA sample is amplified according to the kit manufacturer's specifications, increased amount of amplified product can be injected into the CE instrument with the optimized sample injection parameters (6). The latest CE instruments have higher sensitivity than previous electrophoresis technology and this increased sensitivity can be an invaluable asset in analyzing smaller amounts of DNA. Injection times on the ABI Prism 310 were examined at 5, 10, 15, and 20 seconds. It appears as the injection seconds were increased, the peak heights and base widths also increased. It does not appear that the injection voltage was modified in the study (6).

The amount of STR data obtained from forensic samples with low copy number DNA can be improved by increasing the polymerase chain reaction cycle number. Manipulation of the CE sample injection parameters can maximize the detection of amplified product from low copy number samples. Combining both increased PCR cycles and sample injection parameter manipulation may be used to obtain the maximum number of STR loci in a low copy number sample.

Because of the inconsistency of amplifying low copy number samples, it is suggested to replicate the amplification to ensure the correct results. However, the remaining amount of sample for DNA testing may not be sufficient for replicating the test.

Mixtures pose a problem for low copy number samples. A mixture is defined as two or more individuals contributing to a sample (17). Because low copy number

samples occasionally display imbalanced peak height ratios, it may be difficult to discern two or more separate profiles in those cases with limited amounts of mixed DNA. Mixtures are interpreted based on how many alleles are present at each locus and the peak heights of the alleles present in a sample. There are several ways of interpreting the data when two alleles with a substantial peak height imbalance are present at a locus. It could indicate either: one individual, with improper amplification of that locus' two alleles; two individuals, each homozygous for that allele, or two individuals that share one of the alleles present. If there is a tendency toward heterozygote peak height imbalance in low copy number samples, determining whether poor amplification occurred or the presence of another contributor may be difficult to resolve. All of the loci must be analyzed for the presence of a mixture to determine whether the sample is a single source sample.

Contamination can also be a problem associated with processing low copy number samples. Contamination can occur during collection of the evidence at the crime scene or during processing of the evidence in the laboratory. In samples with large quantities of DNA present, small amounts of extraneous DNA may not be detected. Samples with a large amount of DNA from a single source will amplify preferentially over the minute extraneous DNA present. However, when there are minute amounts of DNA present, all DNA is amplified equally, both the extraneous and probative DNA are amplified equally. All individuals involved in the collection and the analysis of evidentiary material must take precautions when processing samples with possible low amounts of DNA. With the increased occurrence of amplification artifacts associated

with increased amplification cycles, extraction reagent blanks and negative controls may appear to contain contamination when, in fact, there is no contamination present. Research into the reproducibility of the artifacts in negative samples may show true contamination or a by-product of increased cycle numbers during amplification.

Samples may contain low copy amounts of DNA as a result of the degradation of the sample. If the larger loci are highly degraded, the process of increasing the cycle number during amplification may not prove successful with these loci. If the DNA is too highly degraded to amplify, it cannot generate amplified product. In those instances, different testing strategies such as mitochondrial DNA and SNP testing may be employed. Caution must be used when consuming evidence for low copy number DNA testing in those cases when the sample appears to be highly degraded.

The experimental design for this study involved the amplification of DNA isolated from buccal swabs using the Profiler Plus multiplex kit at two different DNA input quantities: 0.0156ng (15.6pg) and 0.0312ng (31.2pg). Four separate amplifications of these DNA samples were done at: 28, 30, 32 and 34 cycles. The manufacturer's recommended cycle number for AmpFlSTR™ Profiler Plus is 28 cycles. These samples were analyzed on both the ABI Prism 310 Genetic Analyzer and the ABI Prism 3100 Genetic Analyzer using OCD standard protocols for loading samples. The injection time and voltage were modified for each of the number of PCR cycles. The best combination of cycle number and injection parameters was chosen for the low copy number reproducibility study.

CHAPTER II

MATERIALS AND METHODS

Buccal swabs were collected from twelve individuals and extracted using the Chelex™ extraction method (9). Quantification of the first six samples (001-006) was performed using the Orchid Cellmark's standard protocol for quantitation which is similar to Applied Biosystems Quantiblot™.

Orchid Cellmark protocol for the quantitation of samples (12):

1. A slot blot loading sheet is filled out for each slot blot to be done.
2. Warm Hybridization and Wash Solutions to 50° C prior to starting procedure.
3. Vortex and spin down all standards, calibrators, and samples.
4. Label tubes or a 96-well plate to represent those standards, calibrators, and samples to be quantified. Add 150 μl of spotting solution to each well or tube that will contain either standard/calibrator, blank, or sample.
5. Add 5 μl of each prepared standard and calibrator to the appropriate sample tube or well. Use 5 μl of water or TE for the blank.
    a. The standard used for comparison are the following quantities:
        10ng, 5ng, 2.5ng, 1.25ng, 0.625ng, 0.312ng, and 0.156ng
6. Add 1 μl of each sample to the appropriate sample tube or well.
7. Place a labeled nylon membrane (Amersham Hybond-N+) 11.0 cm x 7.9 cm, with the upper right corner notched, in a container containing 50 ml of Pre-Wetting Solution. Incubate at room temperature for 1 to 30 minutes.
8. Assemble the slot blotter to be used according to the manufacturer's instructions.
9. Apply the membrane to the base of the slot blot apparatus (Bethesda Research Laboratories), notched corner in upper right. Place the top on the membrane and turn on the clamp vacuum followed by the sample vacuum for 10 seconds.

10. Apply the entire sample to the appropriate wells in the slot blot apparatus. When all samples have been added, turn on the sample vacuum very slowly (until air movement is heard). Leave sample vacuum on for 1 minute. If sample volume has not drawn through, gently draw up fluid with a pipetter and re-apply to sample well to dislodge air bubbles. Vacuum again until liquid is drawn through. Avoid touching the pipette tip to the membrane.

11. Turn clamp and sample vacuums off. Remove membrane and place in a plastic box (box should be just slightly larger than the membrane itself-approx. 6 in x 6 in) containing 100 ml of pre-warmed Hybridization Solution. Add 5 ml of 30% hydrogen peroxide. Rotate with moderate agitation in a 50° C water bath for 15 minutes. Pour off the solution.

12. Add 30 ml of pre-warmed (50° C) Hybridization Solution to the tray containing the membrane. Tilt the tray to one side and add 20 μl of D17Z1 Biotinylated Probe (Invitrogen) to the Hybridization Solution. Place the lid on tray. Place tray in a rotating water bath at 50° C for 20 minutes. Pour off the solution.

Note: TURN ON DEVELOPER.

13. Rinse the membrane briefly in enough pre-warmed (50° C) Wash Solution to cover the membrane (approximately 50 ml) by rocking the tray for several seconds. Pour off the solution.

14. Add 30 ml of pre-warmed (50° C) Wash Solution to the tray. For West Femto detection, tilt the tray to one side and add 3 μl of the Enzyme Conjugate:HRP-SA (Horse Radish Peroxidase/Streptavidin) (Pierce Biotechnology) to the Wash Solution. Place the lid on tray. Rotate at 50° C in water bath for 10 minutes. Pour off the solution.

15. Rinse the membrane thoroughly for 1 minute in enough pre-warmed (50° C) Wash Solution to cover the membrane (approximately 50 ml) by rocking the tray by hand or rotating it on an orbital shaker at room temperature. Pour off the Solution. Rinse again for 1 minute. Pour off the Solution.

16. Wash the membrane by adding enough pre-warmed (50° C) Wash Solution to cover the membrane (approximately 50 ml) and gently rotating at room temperature on an orbital shaker for 15 minutes. Pour off the solution.

17. Rinse the membrane briefly in enough Citrate Buffer to cover the membrane (approximately 50 ml) by rocking the tray. Pour off the solution.

BLOT DETECTION

Note: West Femto substrates 1 and 2 must be stored separately (at 2° C to 8° C) and not allowed to cross-contaminate each other.

1. Add 5 ml of West Femto Substrate 1 to one corner of a tray and 5 ml of West Femto Substrate 2 to the opposite corner. Place the membrane in the tray. Shake for 1 minute at room temperature to allow for thorough mixture of the substrates over the membrane. Pour off the solution. Do NOT prepare this mixture more than 5 minutes before use.

   Note: For maximum sensitivity, expose the membrane to X-ray film within 10 minutes of incubation in West Femto substrates.

2. Place membrane DNA-side up on a sheet of pre-cut plastic film and cover with another piece of plastic wrap. Use a paper towel to smooth out any air bubbles.

3. Tape covered membrane into film cassette to prevent slipping.

4. In a darkroom, place a piece of Kodak film in the cassette on top of the plastic covered membrane. Be careful not to move the film once it is in contact with the membrane. It is also important that the film is in tight so that uniform contact between the film and the covered membrane is achieved.

5. Expose the film for 15 seconds at room temperature. Additional exposures may be performed if needed.

INTERPRETATION

1. Estimate the amount of DNA in each sample by comparison of the blot intensity of the casework samples to that of the standards. A proportional decreasing level of intensity should be observed for each standard proceeding from 10ng through the blank.

2. Compare the intensity of the calibrators C1 (3.5 ng) and C2 (0.5 ng) to the dilution standards. Each calibrator intensity should fall between the appropriate DNA standards [i.e., C1 between "B" (5 ng) and "C" (2.5 ng) and C2 between "E" (0.625 ng) and "F" (0.3125 ng)].

3. If an estimation of DNA yield cannot be accomplished due to sample intensity excessively exceeding that of the standards, the sample may be diluted appropriately and the slot blot procedure repeated.

4. The approximate amount of DNA in the samples multiplied by the dilution factor will give the total DNA yield.

The first six samples were diluted with deionized, distilled water to 0.0156ng/μl (15.6pg/μl). To ensure that the proper dilutions were made, the diluted samples were requantitated using 10μl of the 0.0156ng/μl diluted sample. If the diluted sample's signal matched the 0.156ng standard, the sample was diluted utilized in the amplification process.

The six samples were amplified using AmpFlSTR™ Profiler Plus multiplex amplification kit (10). For each sample, the amplification reaction contained:

- 15μl of Master Mix: 10.5μl Reaction Mix

  5.5μl Primers

  0.5μl *Taq*Gold Polymerase
- 1μl of DNA template for samples amplified at 0.0156ng, 9μl of distilled, deionized water
- 2μl of DNA template for samples amplified at 0.0312ng, 8μl of distilled, deionized water
- Negative controls: 10μl of distilled, deionized water and 15μl of Master Mix
- Positive Controls – 9947A: Three different quantities of 9947A amplified: 1ng, 0.0156ng, and 0.0312ng.

The samples were amplified at 28, 30, 32 and 34 cycles. The Profiler Plus amplification program is as follows (10).

Hold	Cycle			Hold	Hold
95°C	94°C	59°C	72°C	60°C	4°C
11 min.	1 min.	1 min.	1 min.	60 min.	∞

AmpFlSTR™ Profiler Plus multiplex amplification kit contains the following STR loci (17):

Locus	Base Pair Range	Locus	Base Pair Range
D3S1358	97-145	D21S11	186-224
vWA	152-212	D18S51	264-344
FGA	196-288	D5S818	134-170
Amelogenin	103-109	D13S317	193-237
D8S1179	123-171	D7S820	253-293

The six samples amplified at 28 cycles, 0.0156ng and 0.0312ng, positive and negative controls were loaded on the ABI Prism® 310 Genetic Analyzer (Applied Biosystems, Foster City, CA) using the standard operating procedures at Orchid Cellmark Dallas (11):

- 1.5μl of amplified product
- 25μl of Formamide/Rox 500 (Applied Biosystems)
- 5 second injection/15 kilovolts (kV) injection voltage

Formamide is added to amplified product before loading on a genetic analyzer to prevent the single stranded DNA from renaturing. Rox 500 is added to each sample as an internal lane standard. Rox is a red colored fluor that contains fragments of DNA at specific base pair sizes. It migrates simultaneously with the sample during electrophoresis.

The amplification and analysis of the low copy number samples amplified and loaded were done at the standard operating protocols in order to establish a baseline from which experimentation with cycle number and injection parameters could be compared.

The six samples each at 0.0156ng and 0.0312ng, amplified at 28 cycles and the positive and negative controls were loaded on the ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) using the standard operating procedures at Orchid Cellmark Dallas (13):

- 1.0μl of amplified product
- 9μl of Formamide/Rox 500 (Applied Biosystems)
- 5 second injection/ 15 kV injection voltage

The six samples each at 0.0156ng and 0.0312ng, amplified at 28 cycles, and the positive and negative controls were loaded on the 310 Genetic Analyzer using the same loading setup procedures as the previous 310 Genetic Analyzer run. The injection time and injection voltages were modified in the following manner:

- 5 second injection; 15 kV injection, 10 kV injection, 5 kV injection
- 10 second injection; 15 kV injection, 10 kV injection, 5 kV injection
- 15 second injection; 15 kV injection, 10 kV injection, 5 kV injection
- 20 second injection; 15 kV injection, 10 kV injection, 5 kV injection

For each injection time, the injection voltage was tested at 15kV, 10kV, and 5kV.

Data was analyzed using GeneScan 2.1 and Genotyper 2.5 analyzing software (14, 15). The data was compared from this experiment to the samples amplified at 28 cycles, and injected using the standard OCD protocol (16). This was to determine if there was an improvement of the DNA profiles of the samples based solely on the changing of the injection parameters.

The samples amplified at 30, 32, and 34 cycles were loaded on the 310 Genetic Analyzer with the following standard operating procedure:

- 1.5μl of amplified product
- 25μl of Formamide/Rox 500 (Applied Biosystems)
- 5 second injection
- 15 kilovolts (kV) injection voltage

Two samples (001 and 005) were chosen from the six samples to run on the 310 Genetic Analyzer, for the increased amplification cycles and injection parameters experiment. Sample 001 was chosen because it produced the best results from the previous run based on the number of loci detected in the sample. Sample 005 was chosen because it produced the worst results from the previous run. These two samples, 001 and 005 at 0.0156ng and 0.0312ng were amplified at 30, 32, and 34 cycles, and the positive and negative controls were loaded on the 310 Genetic Analyzer, using the same loading preparation procedures as the previous 310 Genetic Analyzer run. The injection time and injection voltages were manipulated in the following manner:

- 5 second injection; 15 kV injection, 10 kV injection

- 10 second injection; 15 kV injection, 10 kV injection
- 15 second injection; 15 kV injection, 10 kV injection

The two samples amplified at 30, 32, and 34 cycles, 0.0156ng and 0.0312ng, positive and negative controls were loaded on the 3100 Genetic Analyzer using the standard operating procedures at Orchid Cellmark Dallas:

- 1.0μl of amplified product
- 9μl of Formamide/Rox 500 (Applied Biosystems)
- 5 second injection/ 15 kV injection voltage

All of the previous experiments were run both on the 310 and 3100 Genetic Analyzers in duplicate for more accurate results.

The data from these experiments were reviewed in order to determine which cycle number and injection parameters gave not only the most complete profile, but also the best quality data. A reproducibility experiment based on one cycle number and one set of injection parameters was then designed.

Six different known buccal swab extracts were quantitated for dilution. The six samples were diluted six times (total 36 dilution tubes) to 0.0156ng/μl. These tubes were requantitated by adding 10μl to the quantitation procedure as done previously. The signal on a quantiblot from all 36 dilutions was computed to verify they matched the 0.156ng standard. All 36 tubes were amplified at 0.0156ng and 0.0312ng as described previously using 32 amplification cycles. The samples (36 samples amplified at 0.0156ng and 36 samples amplified at 0.0312ng) were loaded on the 310 Genetic Analyzer with a 15 second injection time and a 10 kV injection voltage.

Sample Handling Procedures

Contamination precautions were taken during the processing of low copy number samples. A clean lab coat was worn at all times when working with the LCN samples. Gloves were always worn and when a new pair of gloves was taken out of the box, 10 percent bleach was applied to clean the gloves. All of the laboratory areas used in processing the samples were cleaned with 10 percent bleach. Aerosol pipette tips were also used to prevent any sample from contaminating the end of the pipette or any end of the pipette contaminating the sample. Deionized distilled water used for dilutions and amplifications was dedicated to low copy number samples and not used for any other laboratory practices.

One sample tube was opened at a time during the quantitation, dilution, and amplification steps to prevent cross contamination of samples. The Profiler Plus kit used in the low copy number amplifications was a dedicated kit used only for these LCN experiments.

Evaporation of liquid in the tubes containing the dilted DNA samples and amplified product trays was a concern during the course of the research. Accurate quantitation and dilution of the samples was imperative to the success of the experiments performed. When the DNA extracts were diluted to 0.0156ng/μl, they were quantitated immediately. Shortly following the verification of the correct concentration, amplification of the samples was performed. When additional amplifications were needed, new dilutions were made and quantitated to make certain the proper

concentration was amplified. The amplified products in 96-well trays were sealed in plastic bags and frozen in the post amplification laboratory and thawed for loading. It was necessary to prevent evaporation of amplified sample, since the same amplification product was loaded several times over a two month period.

Heterozygote peak height ratios were calculated by taking the RFU peak height given by Genotyper software (15) for the two alleles present in a locus, and dividing the allele with the smaller peak height by the allele with the larger peak height. This calculation was performed for all loci with heterozygous alleles present.

CHAPTER III

RESULTS

Low copy number DNA data was analyzed from the different experiments consisting of variations in both the amplification cycle number and capillary injection parameters.

1. **Amplification: 28 cycles**

   Load: 310 Genetic Analyzer **5 second injection, 15kV injection voltage**

The buccal swabs (001-006) amplified at the Profiler Plus recommended 28 cycles, and injected at the standard operating procedures of OCD of 5 second injections and injection voltage of 15 kilovolts (kV), demonstrated the appearance of low copy number DNA samples. The minimum threshold at which all samples were analyzed was 100 RFUs. This corresponded to the validated minimum threshold of 100 RFUs used to analyze STRs with the OCD standard operating procedures (16). The samples 001-006 amplified at 0.0156ng exhibited partial profiles. Only the alleles in Amelogenin and D5S818 loci had alleles had RFU values above the minimum threshold. Other alleles in the profile were visible, but they were below the threshold of detection. The samples 001-006 amplified at 0.0312ng also exhibited partial profiles with only the loci D3S1358, Amelogenin and D5S818 above the minimum threshold. The rest of the profile was visible, but the alleles were below the threshold of detection (Table 5, 6; Figures 3 and 7).

2. **Amplification: 28 cycles**

Load: 3100 Genetic Analyzer 5 second injection, 15kV injection voltage

Samples 001-006 amplified at 28 cycles were run on the 3100 ABI Prism Genetic Analyzer with the same injection parameters as the previous 310 Genetic Analyzer run – 5 second injections, 15kV injection voltage. The samples amplified at 0.0156ng and 0.0312ng exhibited additional complete profiles as compared with the number of complete profiles obtained from the samples analyzed on the 310 Genetic Analyzer. The 3100 Genetic Analyzer appeared to be a more sensitive instrument than the 310 Genetic Analyzer. Samples amplified at 0.0156ng had numerous loci in which one of the two heterozygous alleles was below threshold. Samples 003, 004, 005, and 006 amplified at 0.0312ng displayed complete profiles. The baseline of the 3100 Genetic Analyzer data was not as clean as the baseline from the data obtained on the 310 Genetic Analyzer. Partial profiles were obtained from the positive control 9947A amplified at 0.0156ng and 0.0312ng.

3. **Amplification: 28 cycles**

Load: 310 Genetic Analyzer - Injection seconds/Injection voltage experiment

Sample 001 amplified at 0.0156ng and 0.0312ng using 28 cycles was loaded on the 310 Genetic Analyzer; the injection time and voltage was changed from 5 seconds and 15kV to the following different injection times and voltages:

- 5 second injection; 15kV injection, 10kV injection, 5kV injection
- 10 second injection; 15kV injection, 10kV injection, 5kV injection
- 15 second injection; 15kV injection, 10kV injection, 5kV injection

- 20 second injection; 15kV injection, 10kV injection, 5kV injection

Increasing the injection time caused an increase in the peak heights of the alleles, resulting in more alleles above the 100 RFU minimum threshold. The increased injection time also displayed wider peak widths at the larger loci when injected at the recommended 15kV voltage.

A decrease in the injection voltage from 15kV to 10kV inconjuction with increased injection time sharpened the appearance of the alleles (Figures 1a and 1b). The alleles present in the larger loci did not have increased peak heights. However, sharpening of the peaks improved the ability to accurately interpret the alleles. Samples injected at 20 seconds at both 15kV and 10kV show wide peaks. For example, one rounded peak was called by the analyzing software as two peaks; the true allele and an off ladder allele, one base pair away from the true allele. The resolution of two alleles, two base pairs apart was often ambiguous (Figures 2a and 2b). An injection voltage of 5kV was too low to attract enough DNA into the capillary for the 310 Genetic Analyzer. Increased injection time was required at the lower injection voltage to obtain any results. However, the data obtained from the lowered voltage was of poor quality.

The 20 second injection and 5kV injection voltage did not provide useful results, therefore, these injection parameters were excluded from the subsequent experiments.

4. Amplification: 30, 32 and 34 cycles

Load: 310 Genetic Analyzer 5 second injection, 15kV injection voltage

Samples 001-006 were amplified at 30, 32, and 34 amplification cycles and analyzed on the 310 Genetic Analyzer using a 5 second injection time and 15kV injection voltage. The samples amplified at 0.0156ng and 0.0312ng and 30 cycles resulted in additional alleles above the minimum threshold as compared to the samples amplified at 28 cycles. However, the sample profiles were still incomplete. At both quantities, the larger loci, for example, FGA, D18S51 and D7S820 were below the 100 RFU threshold. Heterozygous peak height imbalance was not a critical issue at 30 cycles and the baseline was clean (Figures 4 and 8; Table 1).

The samples (0.0156ng and 0.0312ng) amplified at 32 cycles have more complete profiles than the samples amplified at 30 cycles. The loci that have alleles below threshold were D18S51 and D7S820. This was seen at both DNA concentrations. The heterozygous loci of D18S51 and D7S820 appear homozygous in some samples. Heterozygous peak height imbalance was a concern in some loci, but there was no detected pattern of specific loci having heterozygote peak height imbalance. The baseline of the samples was clean and level (Figures 5 and 9; Table 2).

The samples amplified at 0.0156ng and 0.0312ng using 34 cycles exhibited unwanted amplification stochastic effects. The samples at both quantities contain minus A, and stutter. The heterozygous peak height imbalance was augmented at 34 cycles. Preferential amplification was seen in some of the samples amplified at 0.0156ng and all of the samples amplified at 0.0312ng. Preferential amplification occurs when there are

more copies made of the smaller loci as compared to the copies made of the larger loci. Off scale data, out of the detectible range of the CCD camera, were seen at the smaller loci (11). This can occur when too much DNA template is added to the amplification reaction and the larger loci do not amplify at all. When this occurs, the smaller loci, for example, D3S1358, vWA, and Amelogenin, will have amplification artifacts primarily associated with too much template DNA. These artifacts are minus A, stutter and pull up. Minus A is usually one base pair less than the true allele. A stutter peak is typically seen four base pairs less than the true allele and usually is a small percentage (less than 18 percent) of the true allele's peak height. Pull up occurs when an allele of a certain fluorescent dye is above its optimal fluorescent units, and its spectral dye wavelength overlaps with the spectral range of other fluorescent dyes present in the amplified sample. A smaller peak at the exact location of the true allele can be identified in many of the dyes present in the sample. These artifacts were identified in samples amplified at 34 cycles (Figures 6 and 10; Table 3). The cause of stochastic effects in the low copy number DNA samples does not result from too much DNA template added to the amplification reaction, but rather from the number of cycles used to amplify the samples.

5. **Amplification: 30, 32, and 34 cycles**

   Load: 3100 Genetic Analyzer 5 second injection, 15kV injection voltage

Samples 001-006 amplified at two quantities using 30, 32, and 34 cycles were loaded on the 3100 Genetic Analyzer using the standard operating procedures at OCD of 5 second injection and a 15kV injection voltage.

The samples amplified at 0.0156ng using 30 cycles had one complete profile. The baseline of the 3100 Genetic Analyzer data looked flat and clean and many samples with heterozygous loci had alleles below threshold. Samples amplified at 0.0312ng had complete profiles and clean baseline.

The samples amplified at 0.0156ng and 0.0312ng using 32 cycles show complete profiles. Minor heterozygote peak height imbalance occurred in some loci, as was found in the 310 Genetic Analyzer data. The baseline of samples amplified at 0.0156ng was flat and clean. Elevated baseline, including pull up, was a minor concern in samples amplified at 0.0312ng.

The samples amplified at 0.0156ng and 0.0312ng using 34 cycles exhibit complete profiles, however, poor baseline and pull up were a major problem in these samples. Samples amplified at 0.0312ng have the same stochastic effects seen in samples amplified with too much template DNA, showing a high instance of minus A, pull up and stutter.

6. Amplification: 30, 32, and 34 cycles

Load: 310 Genetic Analyzer - Injection seconds/Injection voltage experiment

Two samples were chosen from six samples analyzed on the 310 Genetic Analyzer amplified at 30, 32, and 34 cycles and injected at varying time and voltage. The sample that appeared to have the best results (001) and the sample that appeared to have the poorest results (005) were chosen for the injection parameter experiment.

Sample 001 and 005 at 0.0156ng and 0.0312ng from each cycle number was loaded on the 310 Genetic Analyzer with the following injection parameters:

- 5 second injection; 15kV injection
- 10 second injection; 15kV injection
- 15 second injection; 15kV injection, 10kV injection

The injection parameters that exhibited the best results as compared to the samples amplified at 28 cycles was at a 15 second injection and a 10kV injection voltage. Therefore, only the 15 second injection was paired the 10kV injection parameter because of the number of samples processed and time constraints.

Sample 005 produced poor results with the modification of the injection parameters. Sample 005 at 0.0156ng did not produce a complete profile at any cycle number. There were major heterozygous peak height imbalances at many loci and the baseline data was uneven. Sample 005 at 0.0312ng was only able to achieve a complete profile at 32 cycles with the injection parameters set at 10 seconds and 15kV, and 15 seconds and 10kV. The same amplification product was loaded in both this experiment and the previous 310 Genetic Analyzer experiment; therefore, it appears less template DNA was amplified in sample 005 as compared to samples 002-004 and 006.

Sample 001 amplified at 0.0156ng using 30 cycles produced partial profiles at all injection parameters except a 10 second injection and 15kV. With increased injection time, the alleles at the larger loci exhibited wider peaks as with the samples analyzed at 28 cycles. All samples including those that gave a complete profile at a 10 second injection and a 15kV injection voltage had heterozygote peak height imbalance.

However, all injections of sample 001 amplified at 0.0312ng exhibited partial DNA profiles. Locus D18S51 was below the minimum threshold in all injections.

Sample 001 amplified at 0.0156ng and 0.0312ng using 32 cycles had complete profiles at the following injection parameters:

- 10 second injection; 15kV injection voltage
- 15 second injection; 15kV injection voltage
- 15 second injection; 10kV injection voltage

Data obtained from the samples giving a complete profile at the 15 seconds and 10 kV injection parameter displayed quality results. At the other experimental injection parameters, the alleles in the larger loci had "plateau" shaped peaks, slightly above the minimum allelic threshold. At the 15 second injection and 10kV injection voltage parameter, the alleles located at larger loci were more distinguishable and the resolution between two alleles, two base pairs apart was clear (Figures 11a and 11b).

Sample 001 amplified at 0.0156ng using 34 cycles had complete profiles at all the parameters tested, but the quality of the data was poor. Preferential amplification was apparent in samples loaded at 10 and 15 second injections and 15kV injection voltage. Heterozygous peak height imbalance was observed in this sample. Complete profiles were obtained from sample 001 amplified at 0.0312ng at all injection parameters tested except the 5 second injection, 15kV injection voltage. However, the complete profiles obtained from the sample amplified at 0.0312ng did not produce quality data. The data displayed minus A, stutter, ambiguous shaped peaks, and pull up. One injection exhibited inaccurate electrophoretic migration of an allele at the locus D18S51.

7. **General observations of the samples amplified at 30, 32, and 34 cycles and analyzed at various injection parameters are:**

- Samples amplified at 0.0156ng display greater heterozygote peak height imbalance than samples amplified at 0.0312ng.
- Samples amplified at 30 cycles exhibited the best balance of heterozygote alleles.
- Samples amplified at 34 cycles exhibited the most unwanted amplification stochastic effects at both 0.0156ng and 0.0312ng.
- Samples amplified at 32 cycles displayed virtually no unwanted amplification stochastic effects.
- Analyzing all cycle numbers, at both quantities, and run in duplicate on the 310 Genetic Analyzer, the 15 second injection and 15kV injection voltage exhibited baseline artifacts not related to the alleles present at locus D8S1179. The baseline artifacts were not present when the sample was injected at 15 seconds and a 10kV injection voltage.
- Increasing the injection seconds and changing the voltage to 10kV does not considerably affect the heterozygote peak height ratio. For example, sample 001 amplified using 32 cycles with 0.0312ng DNA at locus D3S1358 had a heterozygous peak height ratio of 0.467 at the 5 second injection/15kV injection voltage. At a 10 second injection/15kV injection voltage, the heterozygous peak height ratio was 0.461; at 15 second injection/15kV injection voltage, the

heterozygous peak height ratio was 0.480; at 15 second injection/10kV injection voltage, the heterozygous peak height ratio was 0.482.

- Heterozygote peak height ratios did not greatly differ between the 310 Genetic Analyzer and the 3100 Genetic Analyzer.

After analyzing the data obtained in the low copy number DNA experiments performed, a single amplification cycle and injection parameter was chosen for a reproducibility experiment. The number of cycles that gave a balance between the most complete profile with minimal stochastic effects was 32 cycles. The injection parameter best suited for low copy number DNA samples amplified at a higher cycle number was a 15 second injection and a 10kV injection voltage. The 15 second injection increases the amount of amplified product entering the capillary of the Genetic Analyzer, while the 10kV injection voltage controls the width of the peak.

8. Reproducibility: 32 cycles - 15 second injection, 10kV injection voltage

Known extracts 007-012 was chosen for the reproducibility experiment. The six samples were diluted six different times to 0.0156ng and amplified at 0.0156ng and 0.0312ng. The purpose of examining six samples, diluted the same way six times, amplified and injected using the chosen parameters was to investigate in what way sample processing affect the reproducibility of a DNA profile in low copy number DNA samples.

The variability of the data obtained from the results of the reproducibility experiment demonstrated the unpredictability of amplifying low quantities of DNA.

Table 1, labeled *32 cycles 15second injection/10kV injection voltage,* display the alleles present and the RFU values of those alleles. Table 2, labeled *32 cycles 15 second injection/10kV injection voltage – ratios,* exhibit the heterozygote peak height ratios of the alleles present in the reproducibility study. These tables demonstrate the inconsistency of the results from low copy number samples.

The main problem with the samples is inconsistent amplification of heterozygous alleles (Figures 12-17; Tables 4, 7-12). In addition, sample 011 exhibits a possible "drop in" of an allele (Figure 18). Amplifying low copy number samples can amplify an allele not present in the true DNA profile. The following table shows the quantity of the samples amplified, and the number of complete profiles obtained from the analyzed data. Each sample was amplified six times at the same quantity of DNA. Some samples do not have data for all six profiles, for example, under the "Complete Profile" column; results are displayed as "0 out of 5". This indicates one sample analyzed displayed capillary failure and results were reported for only five profiles. Sample 012 exhibited poor results. In the majority of the repetitive amplifications of sample 012, the analysis software identified an "off ladder allele" at the vWA locus.

Sample	Complete Profiles
007-0.0156ng	0 out of 6
008-0.0156ng	1 out of 5
009-0.0156ng	0 out of 5
010-0.0156ng	1 out of 5
011-0.0156ng	3 out of 6
012-0.0156ng	0 out of 5

Sample	Complete Profiles
007-0.0312ng	5 out of 6
008-0.0312ng	4 out of 6
009-0.0312ng	4 out of 6
010-0.0312ng	6 out of 6
011-0.0312ng	6 out of 6
012-0.0312ng	1 out of 5

Samples 007-012, amplified at 0.0156ng displayed complete profiles in 15.6% of the amplifications. Samples 007-012, amplified at 0.0312ng displayed complete profiles in 68.6% of the amplifications.

9. Negative Controls

The reagent blank extracted along with samples 001-012 showed no detectable amplified product at 28 cycles on either the 310 and 3100 Genetic Analyzers. The same aliquot of deionized, distilled water was used for all low copy number amplifications. Two negative controls were amplified with samples 001-006 at 28 cycles; both negative controls exhibited no amplified product on the 310 and the 3100 Genetic Analyzers. Two negative controls were amplified with samples 001-006 at each increased cycle number.

At 30 cycles, one negative control showed no detectable amplified product, but the other negative control contained amplified product at the Amelogenin and D3S1358 loci. This was replicated in the 3100 Genetic Analyzer.

At 32 cycles, one negative control was free of amplified product and the other negative control contained an X allele at the Amelogenin locus. The X allele in later runs was not consistently present. The 3100 Genetic Analyzer often shows spikes at the Amelogenin locus, therefore, the presence of an X allele at that locus cannot be considered as a true contaminate. These spikes do not appear as true alleles; spikes have no peak width and usually are not reproducible.

The negative controls amplified at 34 cycles showed one with no detectable amplification products and the other negative control was clean and one appeared to have a possible allele and a spike. These results were not reproducible on the 3100 Genetic Analyzer or additional 310 Genetic Analyzer runs.

The negative controls amplified with the reproducibility experiment were both free of amplified product.

CHAPTER IV

DISCUSSION

Known buccal swabs were diluted to 0.0156ng/μl (15.6pg/μl) and 0.0312ng/μl (31.2pg/μl) and minimal to no results were expected from samples amplified at AmpFlSTR™ Profiler Plus recommendation of 28 cycles. These two quantites were chosen for low copy number experimentation because the two lowest detection standards on the quantitation procedure are 0.156ng and 0.312ng. By adding 10μl of diluted DNA extract to the quantitation procedure, the signal should equal the two lowest standards. Accurate DNA quantitation was imperative to the success of this project.

After varying the amplification cycle number and injection parameters on the 310 Genetic Analyzer, it was determined that the best number of cycles for the amplification of low copy number DNA samples was 32 cycles. The injection parameters chosen for analysis on the 310 Genetic Analyzer was a 15 second injection and a 10kV injection voltage. These parameters produced the most consistent results out of all the conditions tested. Samples diluted to 0.0156ng did not produce consistent results most likely due to the low quantity of DNA present. Complete profiles were obtained from 0.0312ng of DNA, but heterozygote peak height imbalance was an intermittent concern. LCN samples with 0.0312ng of DNA amplified at 32 cycles can produce reliable DNA results.

Low copy number DNA samples did, in fact, show that amplified product was generated at 28 cycles at both quantities of DNA. However, the results were below the threshold established at OCD (16). The same samples amplified at 0.0312ng loaded on

the 3100 Genetic Analyzer produced complete profiles at 28 cycles. Analysis of samples amplified at cycles 30, 32, and 34 on the 3100 Genetic Analyzer demonstrated similar results to those obtained from the 310 Genetic Analyzer data. These results consisted of: higher RFUs with additional alleles detected above the minimum threshold of 100 RFUs. There are a few possible reasons for the inconsistent results between the 310 and the 3100 Genetic Analyzer. First, the sensitivity is very different from instrument to instrument. The sensitivity of the 3100 Genetic Analyzer in use at Orchid Cellmark Dallas is much greater than the 310 Genetic Analyzer. The preparation of amplified sample for loading on both instruments is very different. Because the 310 Genetic Analyzer has one capillary, the time required for the analysis of a sample is approximately 30 minutes. Additional amounts of formamide are added to the amplified product to keep the DNA strands denatured. For a 310 Genetic Analyzer run, 24μl of formamide is added to a sample compared to the 9μl of formamide added to a sample for a run on the 3100 Genetic Analyzer. A sample loaded on the 310 Genetic Analyzer may be more dilute than the same sample loaded on the 3100 Genetic Analyzer.

One concern was the evaporation of amplified product. As the time between amplification and analysis is increased, evaporation of amplified product occurs, leaving the sample more concentrated than when it was initially amplified. Precautions were taken to prevent the evaporation of amplification product as described in Materials and Methods.

Heterozygote peak height imbalance is an adverse result of the amplification of small quantities of DNA. Therefore, increasing the number of amplification cycles in

low copy number samples can magnify heterozygote peak height imbalance. Heterozygote peak height imbalance was the greatest concern with the data obtained from the low copy number research. If heterozygote peak height imbalance is present in a sample, caution must be taken when deducing a DNA profile. The presence of a mixed profile must be checked by examining each locus for the presence of more than two alleles. Also, calculate the heterozygote peak height ratio for all loci. A minimum ratio will have to be established specifically for low copy number DNA. If a locus does not meet the minimum ratio established, it should be called inconclusive. The whole profile does not be discarded; those loci meeting the established criteria can be reported. If the sample does not produce conclusive data, do not attempt to over interpret the data. The report must accurately convey those results.

Based on the reproducibility study, it is recommended to run duplicate amplifications of a LCN sample, if the amount of extracted DNA permits. Amplification of samples 007-012 was duplicated six times, and the profiles obtained were not consistent. Allelic drop out of heterozygous alleles was a concern, especially in the locus D18S51. If a sample is amplified in duplicate and one sample displays heterozygote peak height imbalance in a locus, but the additional amplification shows proper ratio between heterozygote alleles in that same locus, those alleles can be reported as the true DNA typing.

Drop in of alleles not present in the true profile was not observed often in the samples tested, but it did possibly occur in the reproducibility study. Analysts should examine the data with possible drop in of alleles with caution. Examining the other loci

within a DNA profile can help determine whether the profile is a mixed sample, possible contamination or allele drop in. Duplicate reactions would also be important when possible drop in of alleles is suspected in a sample.

More than one negative control should be amplified with LCN DNA samples amplified at 32 cycles. As was seen with the results of the increased cycle number experiments, when two negative controls were amplified, one was clean while the other had possible alleles present. Sometimes the alleles present in the negative controls could not be reproduced. Dedicated deionized, distilled water supply should always be employed for LCN DNA sample amplification.

Often when samples do not exhibit any signal on the quantitation procedure, which does not always indicate low copy number samples. The quantitation procedure utilized can only quantitate samples down to 0.156ng/μl (156pg/μl). Samples with no signal on the Quantiblot can still produce a complete profile when amplified at the recommended 28 cycles. Those samples suspected to have low amounts of DNA; for instance, telogen hair roots, stamp/envelope extractions, ancient or aged samples, one-fifth to one-tenth of the sample extract should be quantitated. The amount added to the Quantiblot is dependent on the amount of extract the sample contains.

Future considerations for low copy number research:

1. Experiment with reducing the amount of ROX and formamide added to the sample on the 310 Genetic Analyzer. The ROX may be competing with the LCN DNA for injection into the capillary. Less ROX may make the DNA more prominent in the data. With less formamide, the LCN DNA may be more

concentrated in the load tray and produce more complete LCN results. Reducing the ROX on the 3100 Genetic Analyzer may also be a consideration.

2. Since the 3100 Genetic Analyzer is a more sensitive machine, diluting low copy number samples after amplification at 32 cycles, before loading onto the 3100 Genetic Analyzer would make the baseline cleaner and possibly a better appearing profile. Experimenting with how much to dilute the amplification product could be a beneficial consideration.
3. The next step in LCN research is to perform the parameters chosen in this research on actual non-probative forensic casework, increase the variety of types of samples (hair shafts, DNase treated degraded samples, etc).
4. Validation of lowering the minimum RFU threshold for LCN samples should be a future LCN research consideration.
5. A mixture study with known samples and different ratios of mixed DNA would determine at what point two individuals cannot be separated in low copy number samples.

CHAPTER V

FIGURES AND TABLES

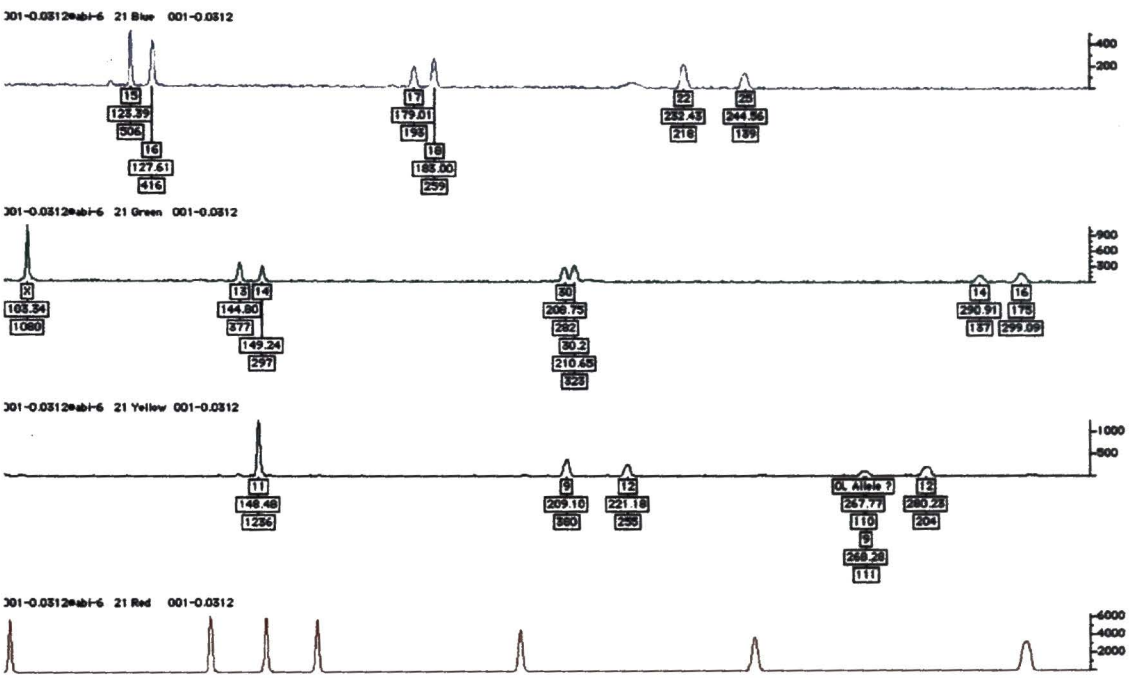

Figure 1a: Sample 001 was amplified at 28 cycles and with 0.0312ng of input DNA. The sample was injected on the 310 Genetic Analyzer using 15sec/15kV.

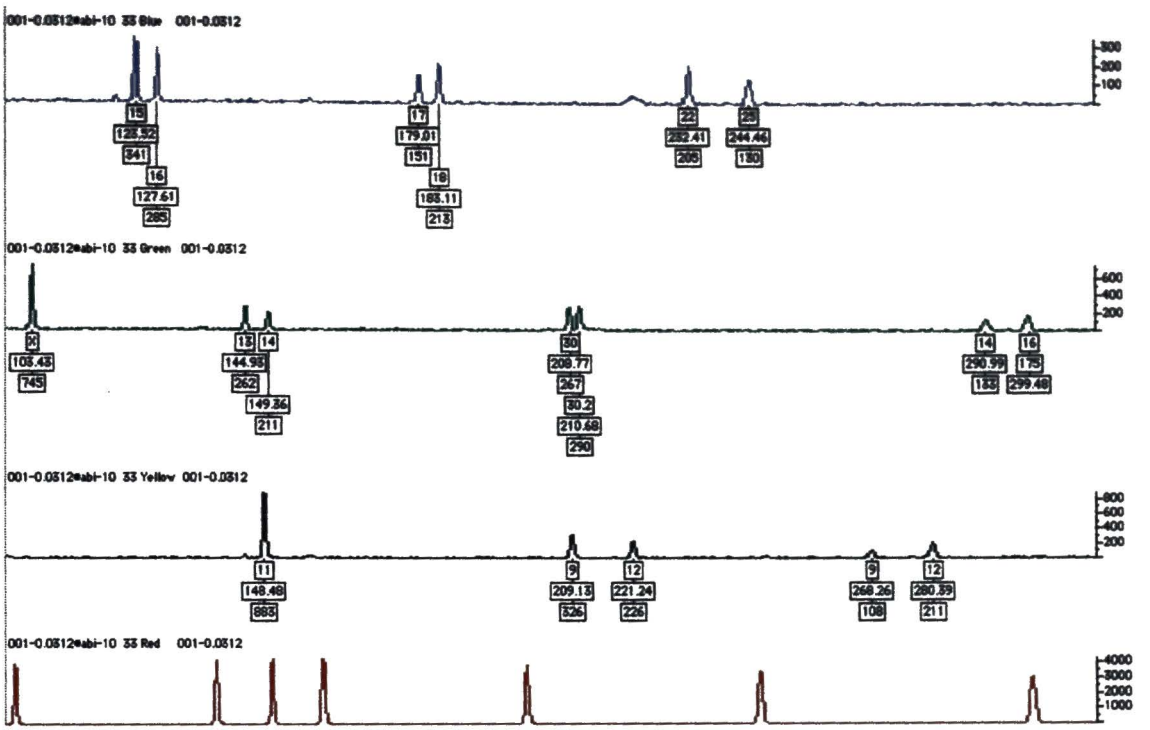

Figure 1b: Sample 001 was amplified at 28 cycles and with 0.0312ng of input DNA. The sample was injected on the 310 Genetic Analyzer using 15sec/10kV.

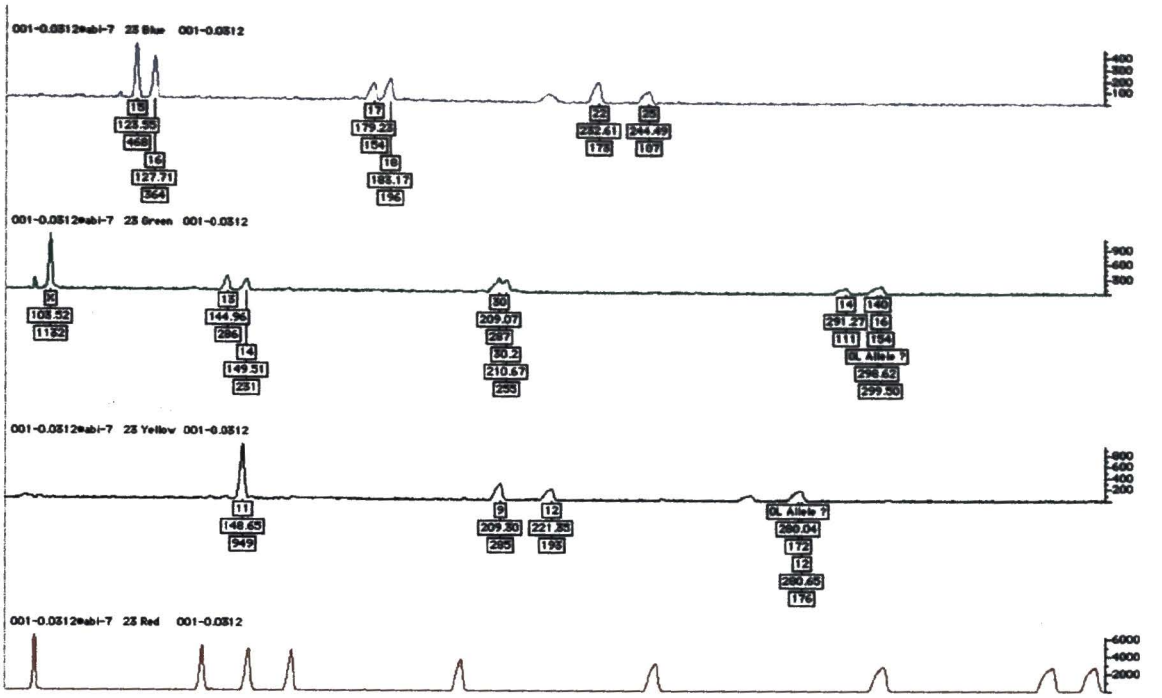

Figure 2a: Sample 001 was amplified at 28 cycles and with 0.0312ng of input DNA. The sample was injected on the 310 Genetic Analyzer using 20sec/15kV.

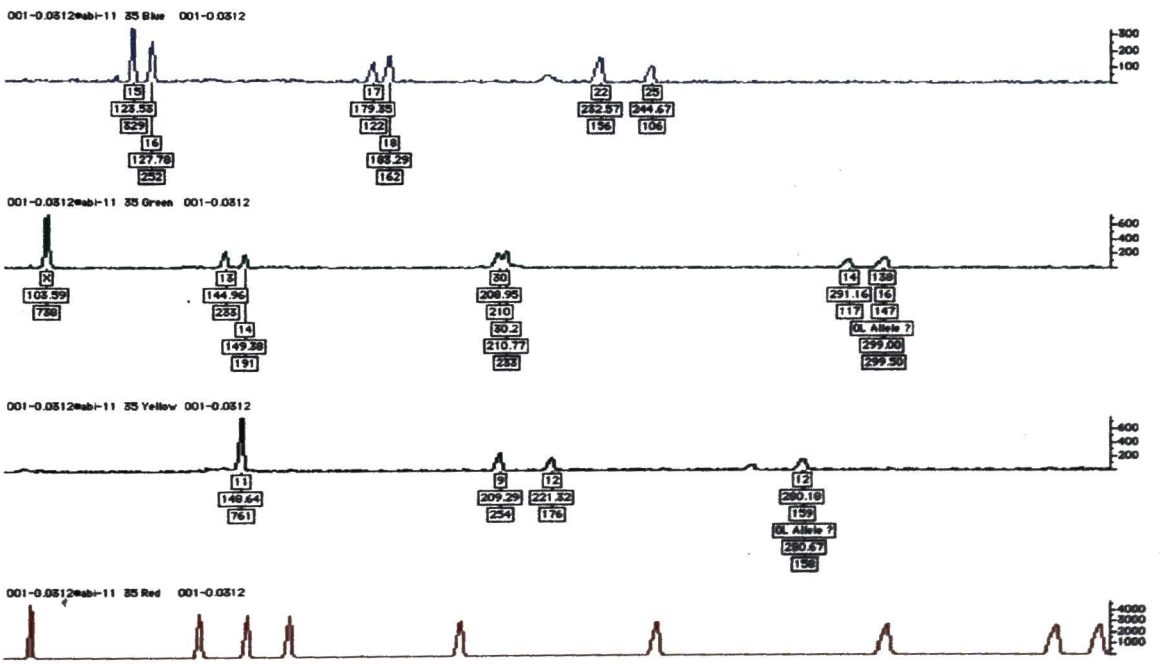

Figure 2b: Sample 001 was amplified at 28 cycles and with 0.0312ng of input DNA. The sample was injected on the 310 Genetic Analyzer using 20sec/10kV.

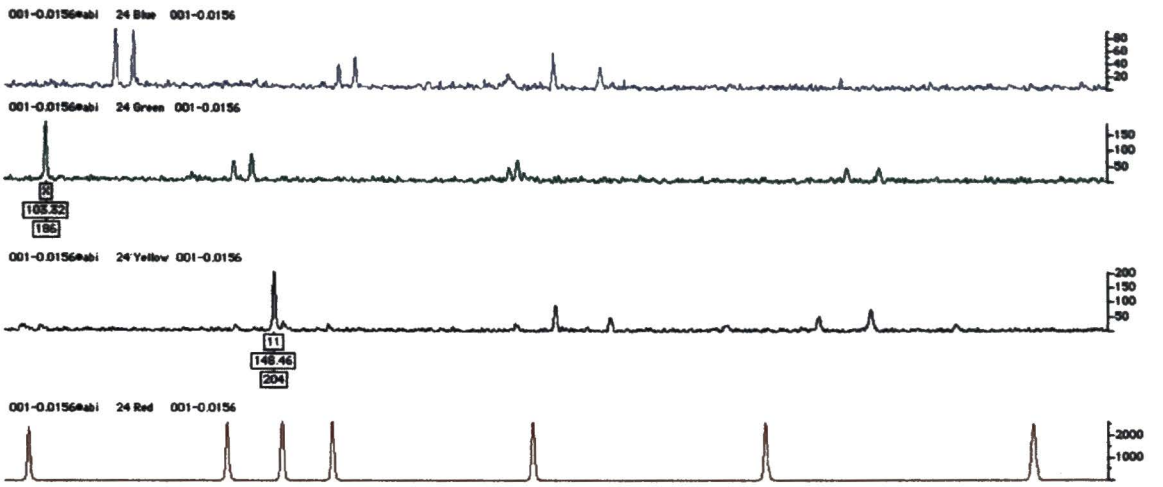

Figure 3: Sample 001 was amplified at 28 cycles and with 0.0156ng of input DNA.

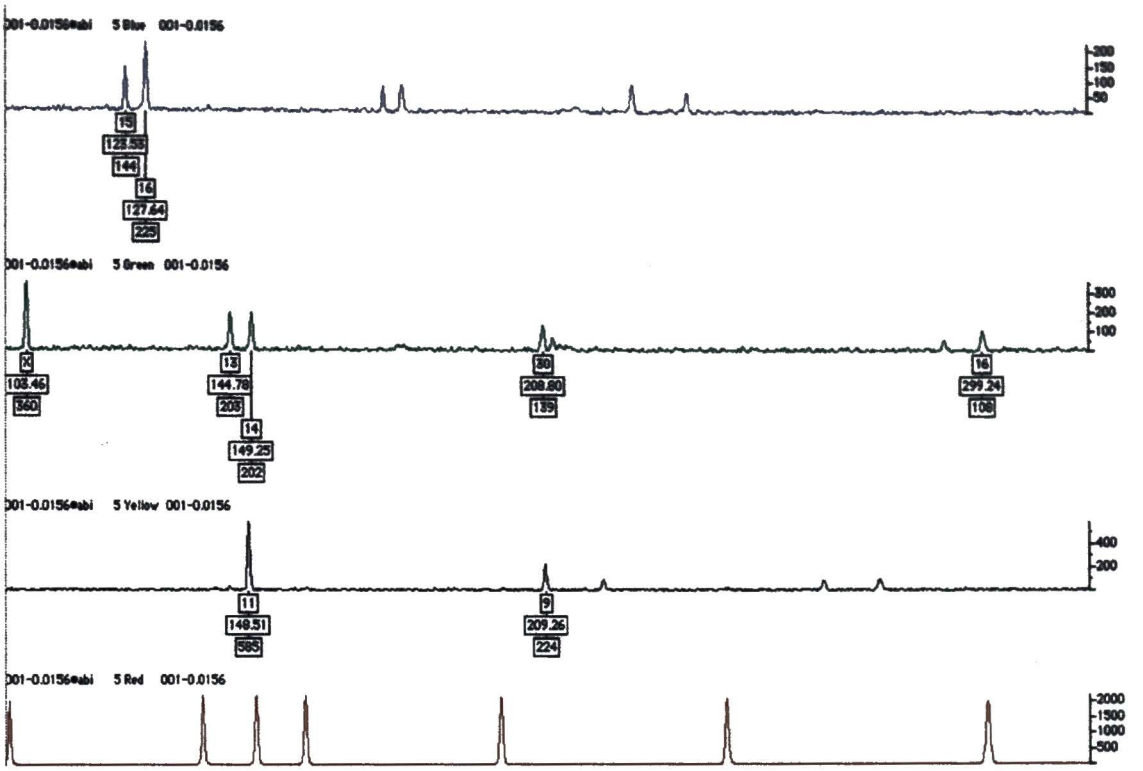

Figure 4: Sample 001 was amplified at 30 cycles and with 0.0156ng of input DNA.

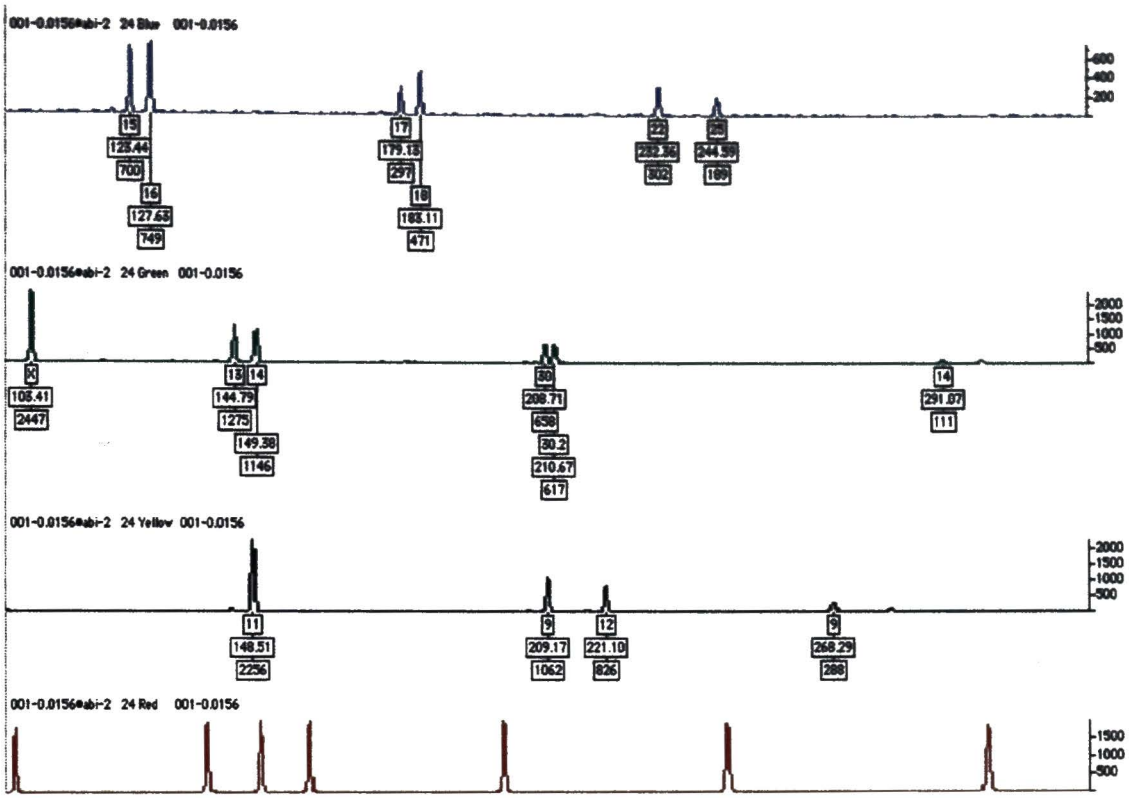

Figure 5: Sample 001 was amplified at 32 cycles and with 0.0156ng of input DNA.

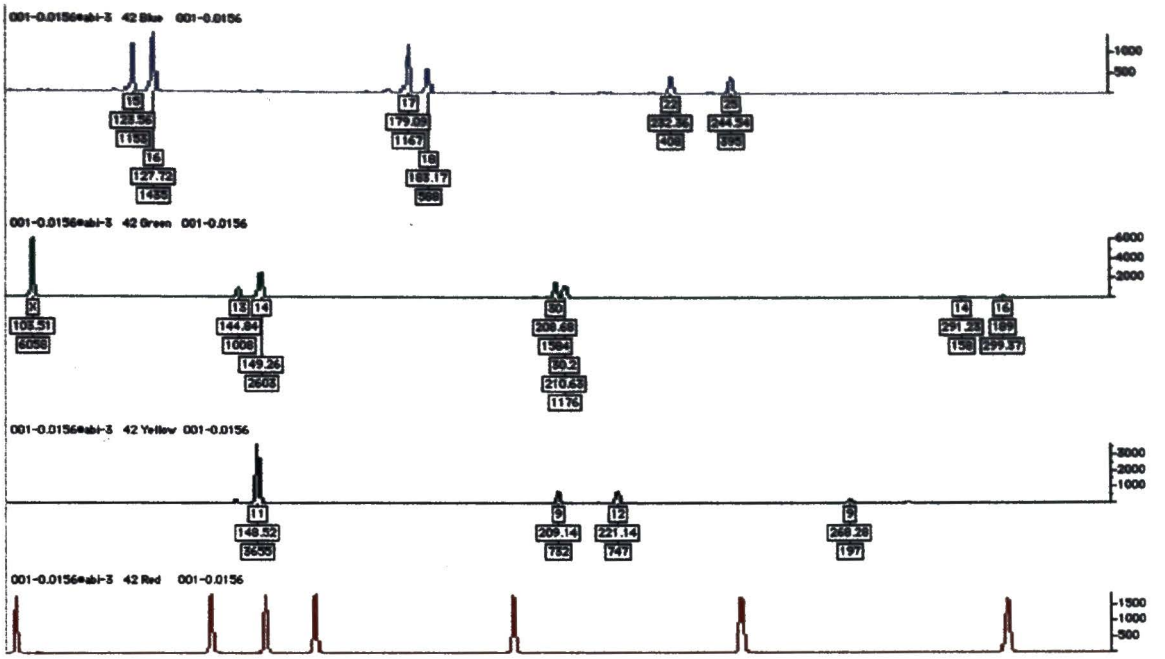

Figure 6: Sample 001 was amplified at 34 cycles and with 0.0156ng of input DNA.

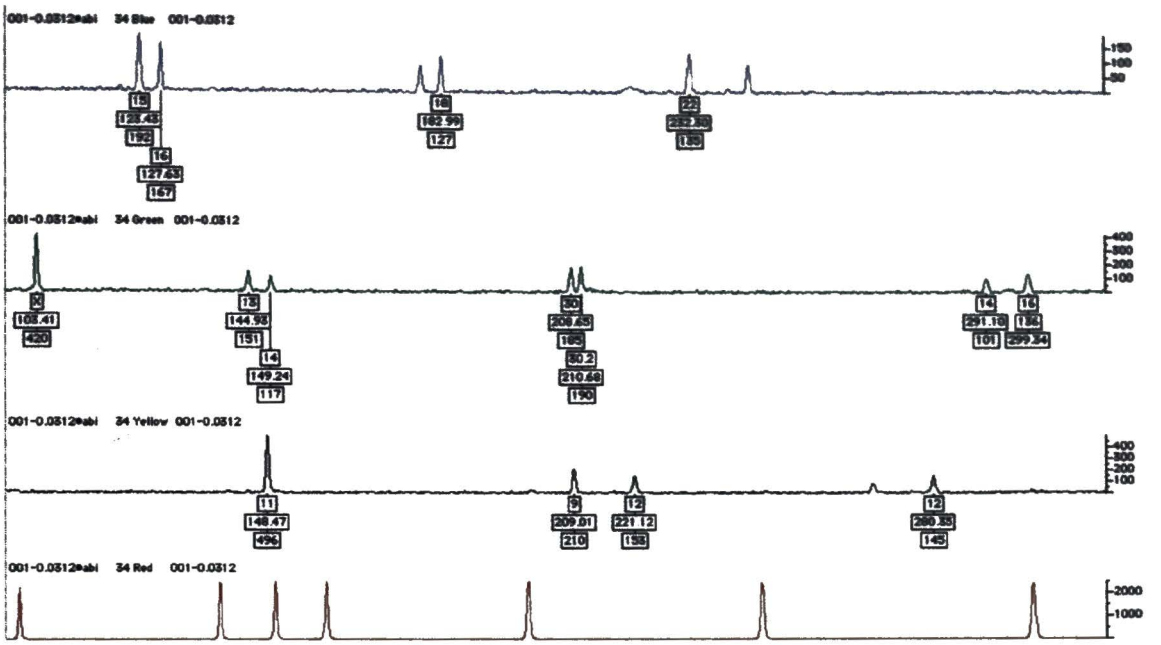

Figure 7: Sample 001 was amplified at 28 cycles and with 0.0312ng of input DNA.

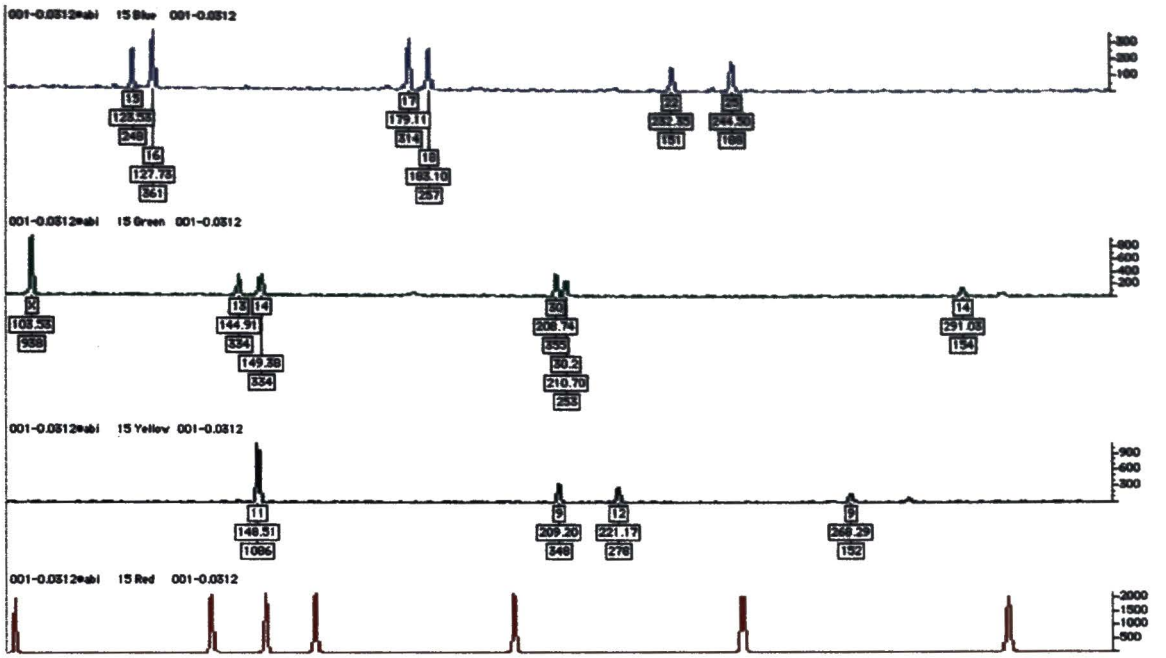

Figure 8: Sample 001 was amplified at 30 cycles and with 0.0312ng of input DNA.

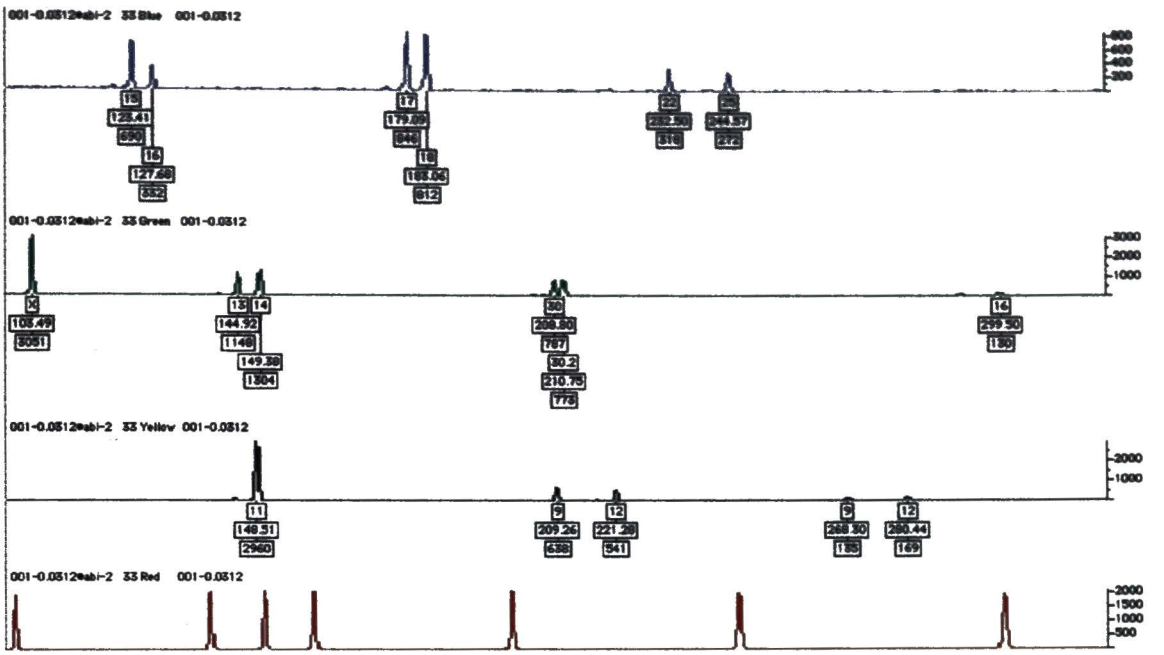

Figure 9: Sample 001 was amplified at 32 cycles and with 0.0312ng of input DNA.

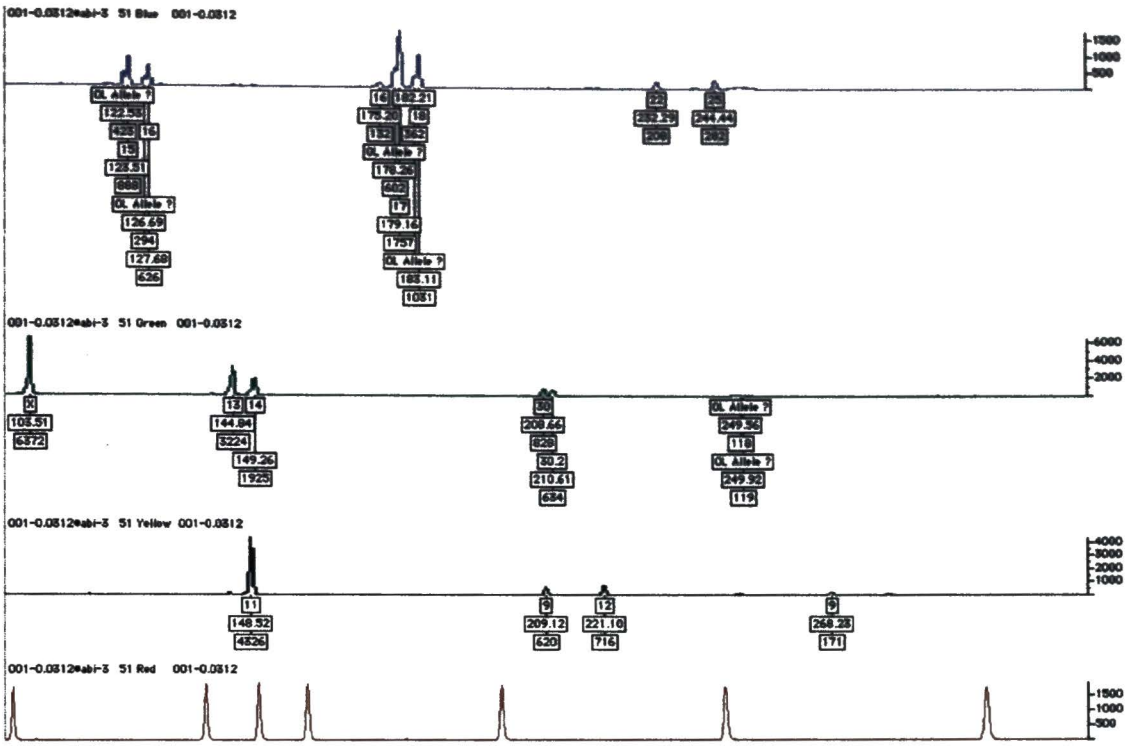

Figure 10: Sample 001 was amplified at 34 cycles and with 0.0312ng of input DNA.

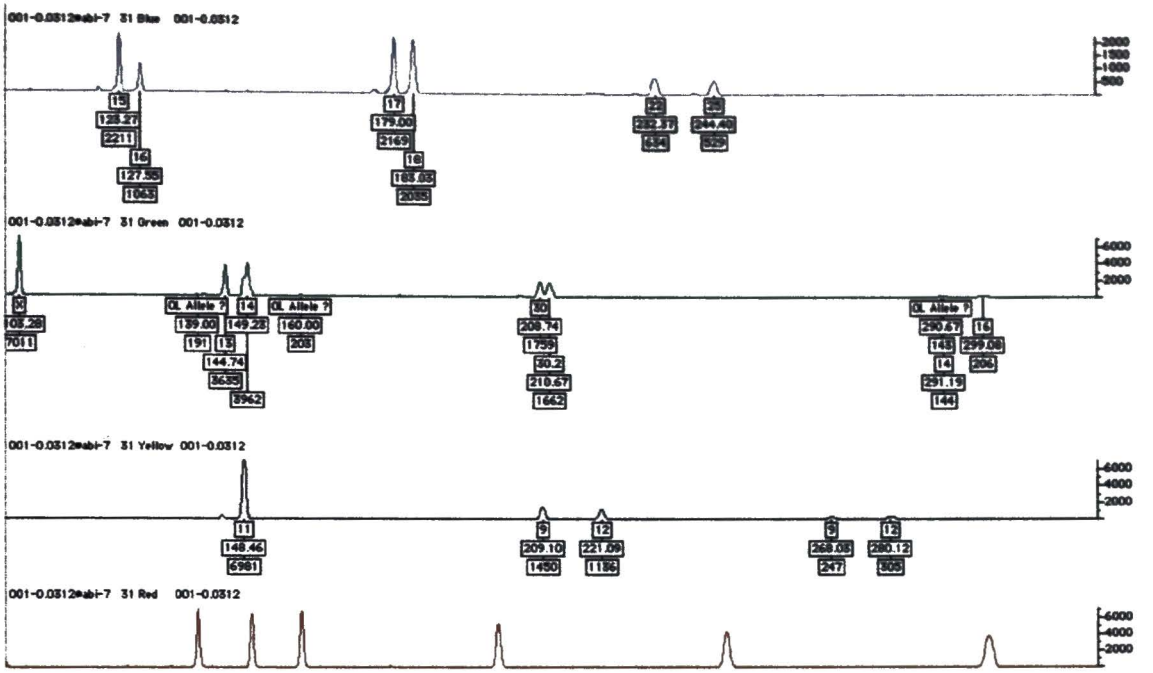

Figure 11a: Sample 001 was amplified at 32 cycles with 0.0312ng of input DNA. It was then injected for 15sec/15kV

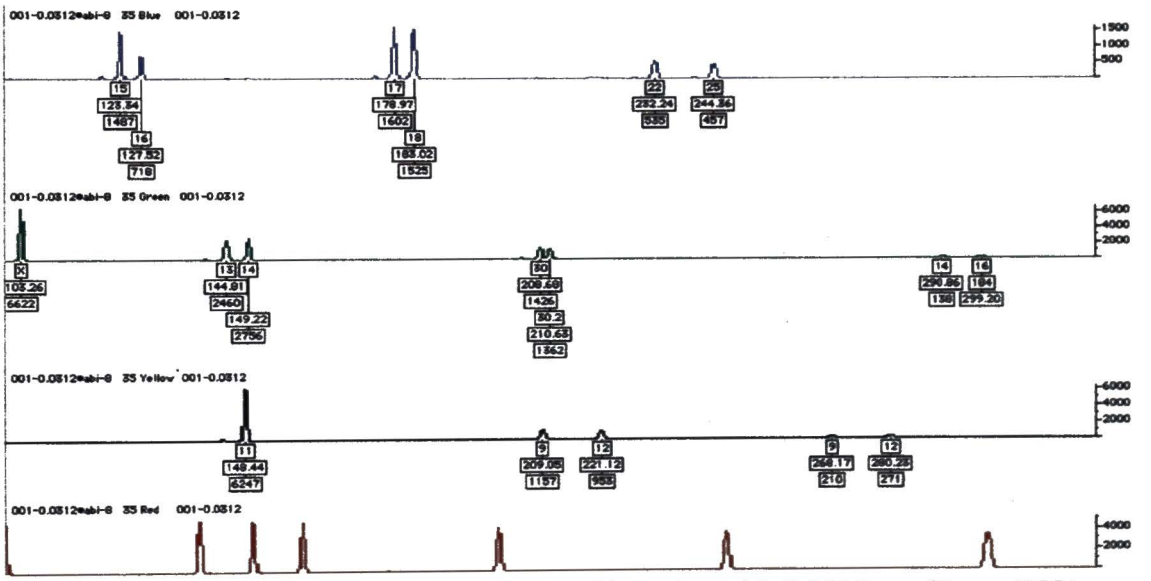

Figure 11b: Sample 001 was amplified at 32 cycles with 0.0312ng of input DNA. It was then injected for 15sec/10kV

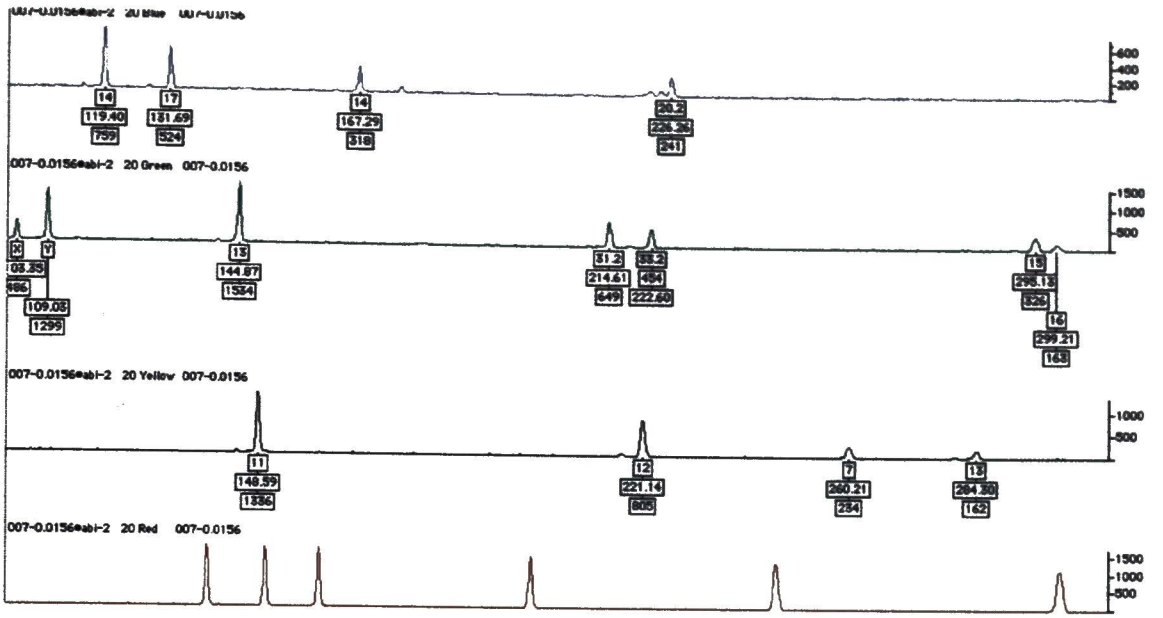

Figure 12: Reproducibility Study
Sample 007 was amplified at 32 Cycles using 0.0156ng of input DNA
Figures 12-14 are examples of sample 007 amplified at 32 cycles using 0.0156ng of input DNA in three separate reactions

(Notice the heterozygote peak height imbalances in figures 12-17)

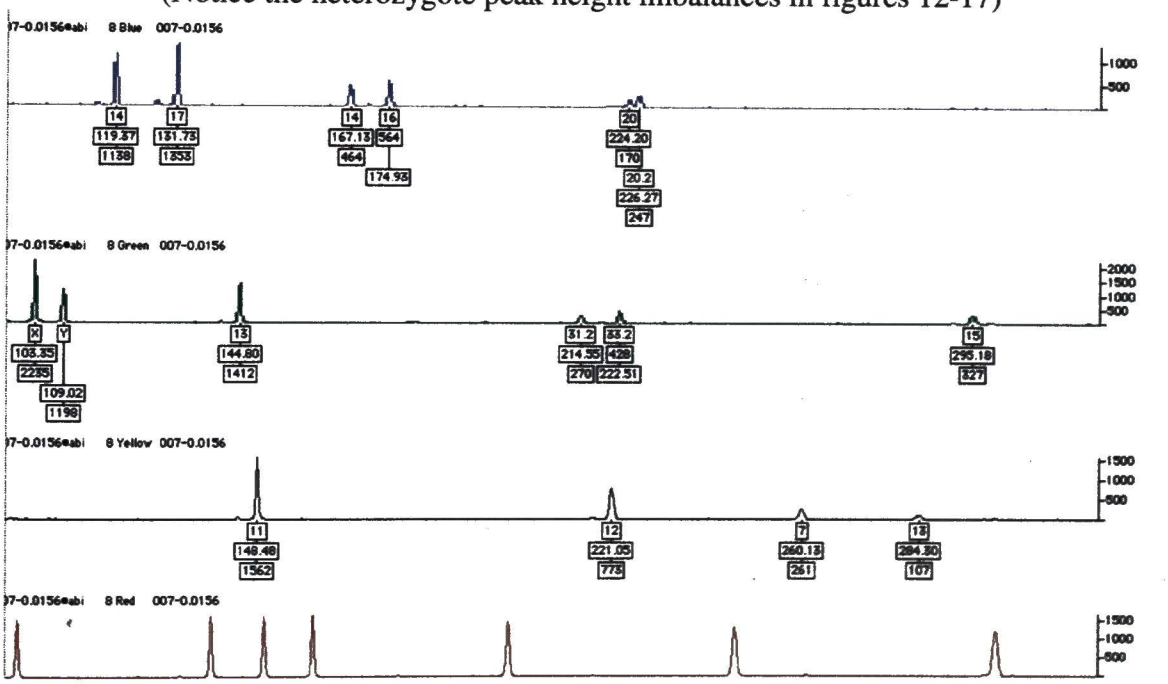

Figure 13: Reproducibility Study
Sample 007 was amplified at 32 Cycles using 0.0156ng of input DNA

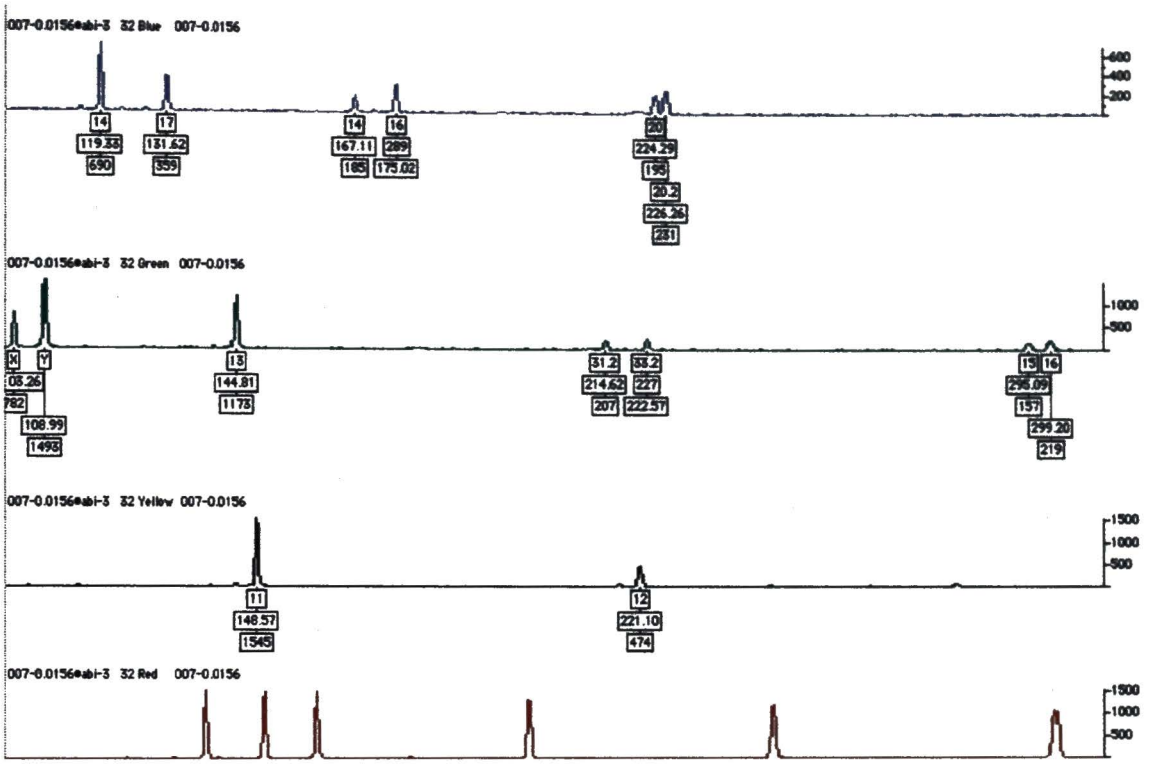

Figure 14: Reproducibility Study
Sample 007 was amplified at 32 Cycles using 0.0156ng of input DNA

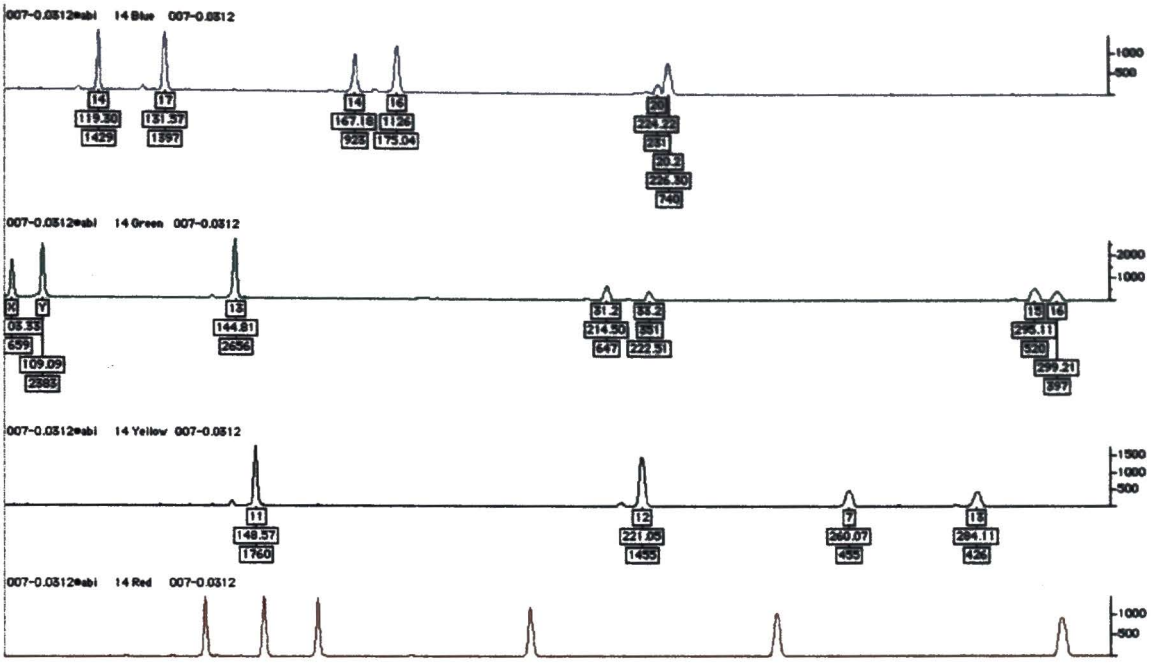

Figure 15: Reproducibility Study
Sample 007 was amplified at 32 Cycles using 0.0312ng of input DNA.
Figures 15-17 are examples of sample 007 amplified at 32 cycles using 0.0312ng of input DNA in three separate reactions

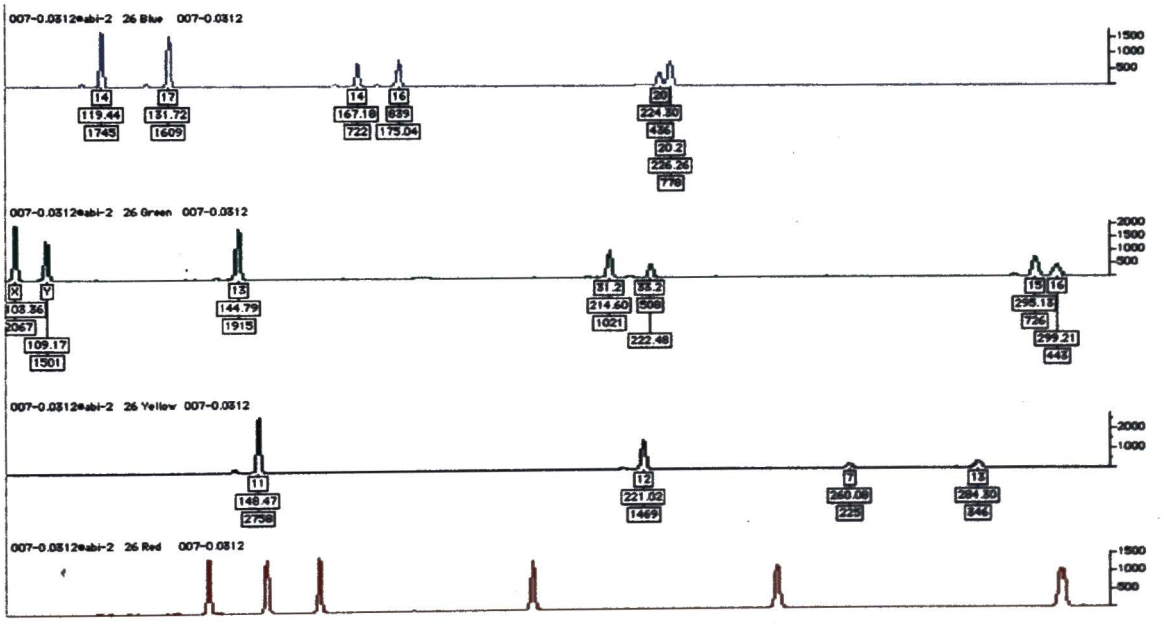

Figure 16: Reproducibility Study
Sample 007 was amplified at 32 Cycles using 0.0312ng of input DNA.

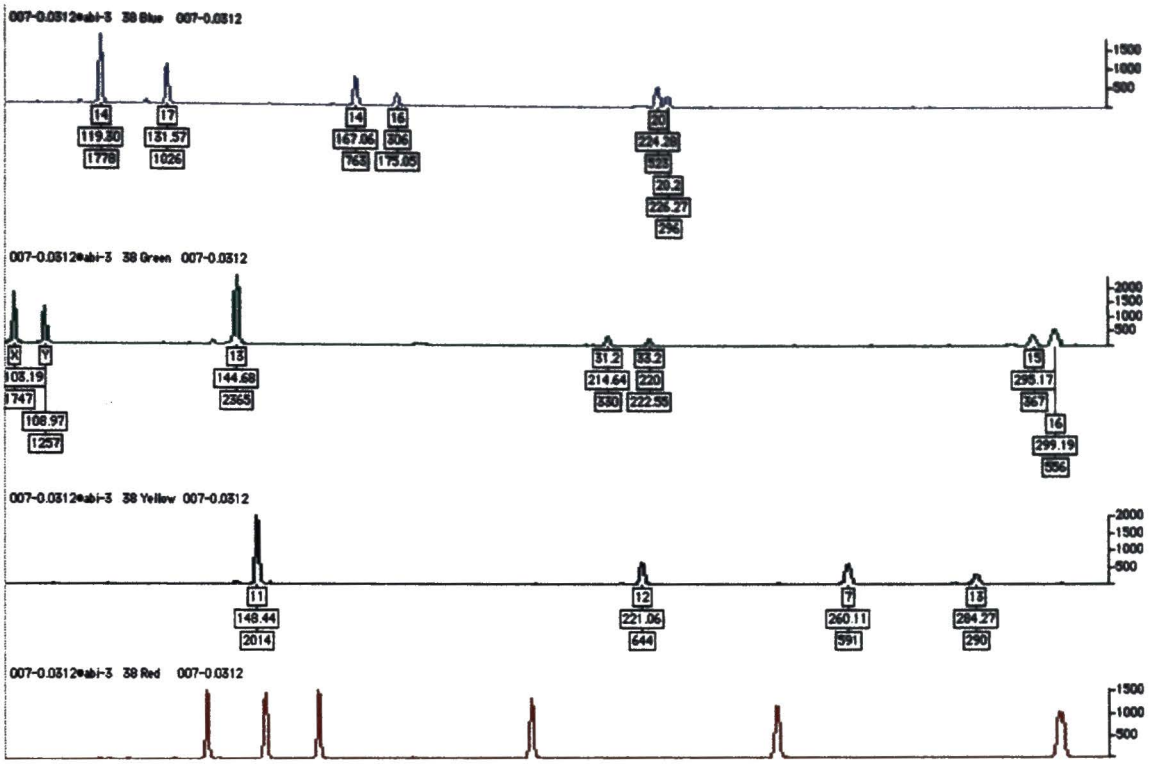

Figure 17: Reproducibility Study
Sample 007 was amplified at 32 Cycles using 0.0312ng of input DNA.

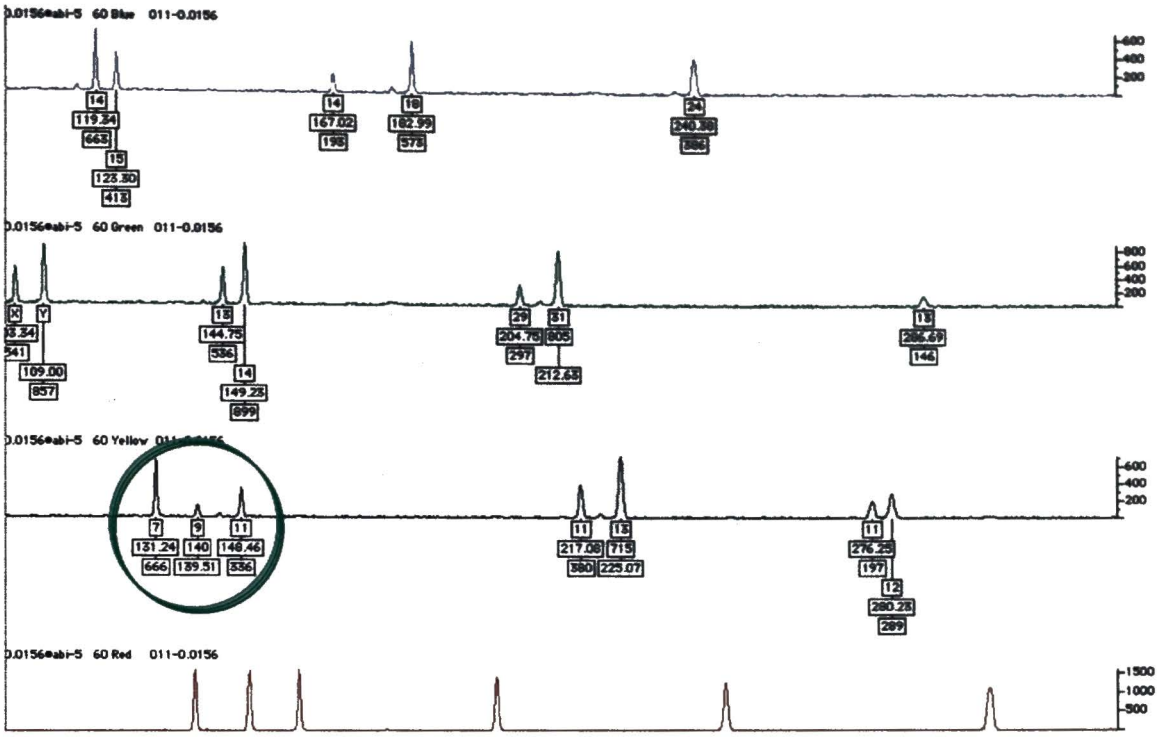

Figure 18: Reproducibility Experiment: Sample 011 was amplified at 32 cycles with 0.0156ng of input DNA. This sample shows possible drop in at locus D5S818. The correct alleles at this locus is 7 and 11.

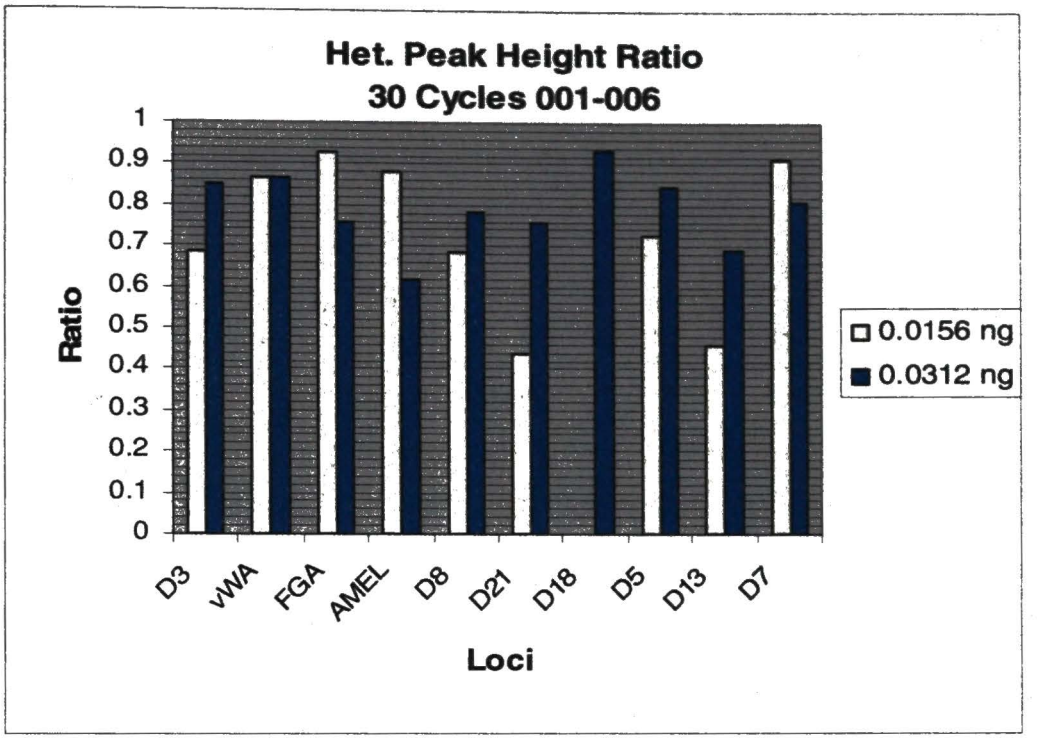

Table 1: Average Heterozygote Peak Height Ratio
Profiler Plus Loci and its corresponding heterozygote peak height ratios for samples 001-006 amplified at 30 cycles

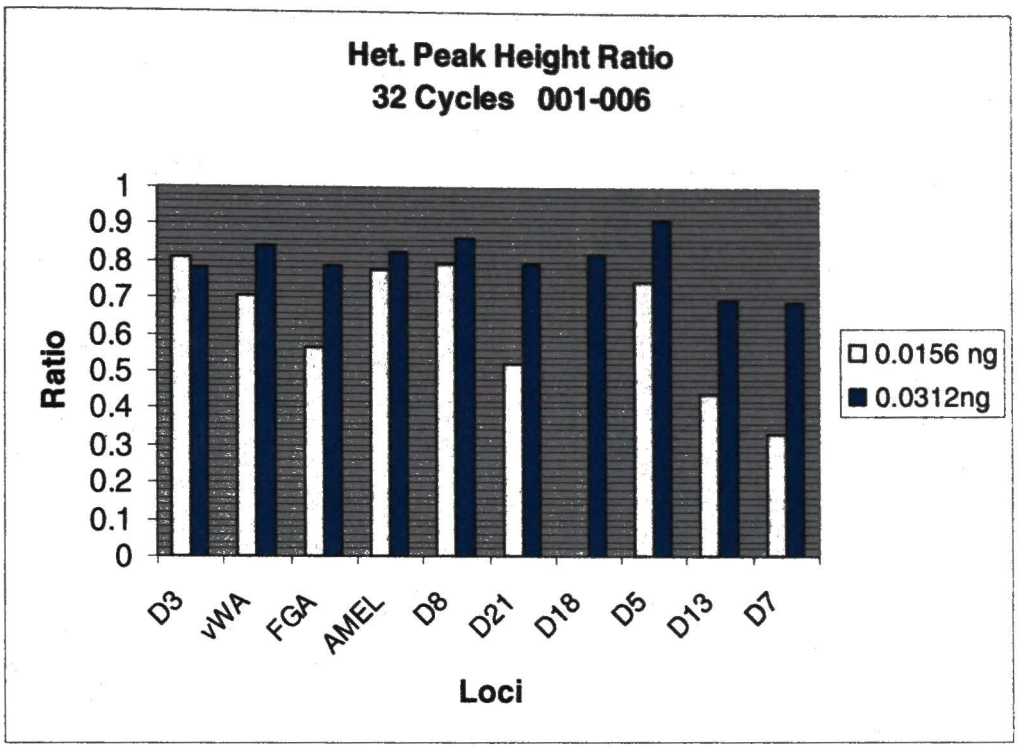

Table 2: Average Heterozygote Peak Height Ratio
Profiler Plus Loci and its corresponding heterozygote peak height ratios
for samples 001-006 amplified at 32 cycles

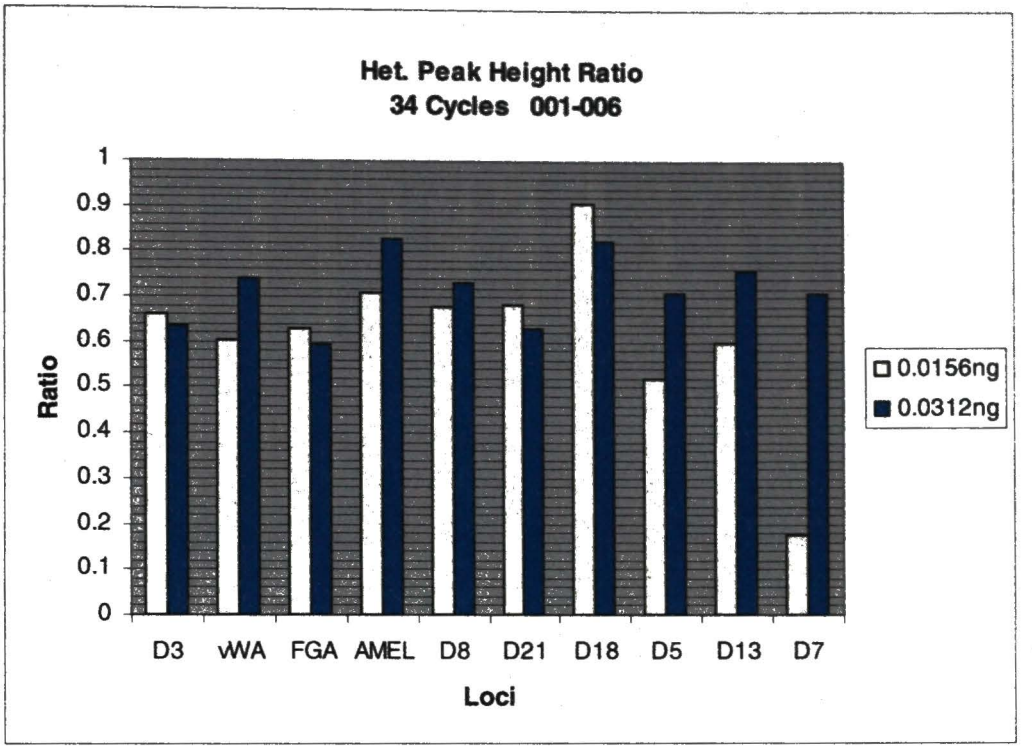

Table 3: Average Heterozygote Peak Height Ratio
Profiler Plus Loci and its corresponding heterozygote peak height ratios
for samples 001-006 amplified at 34 cycles

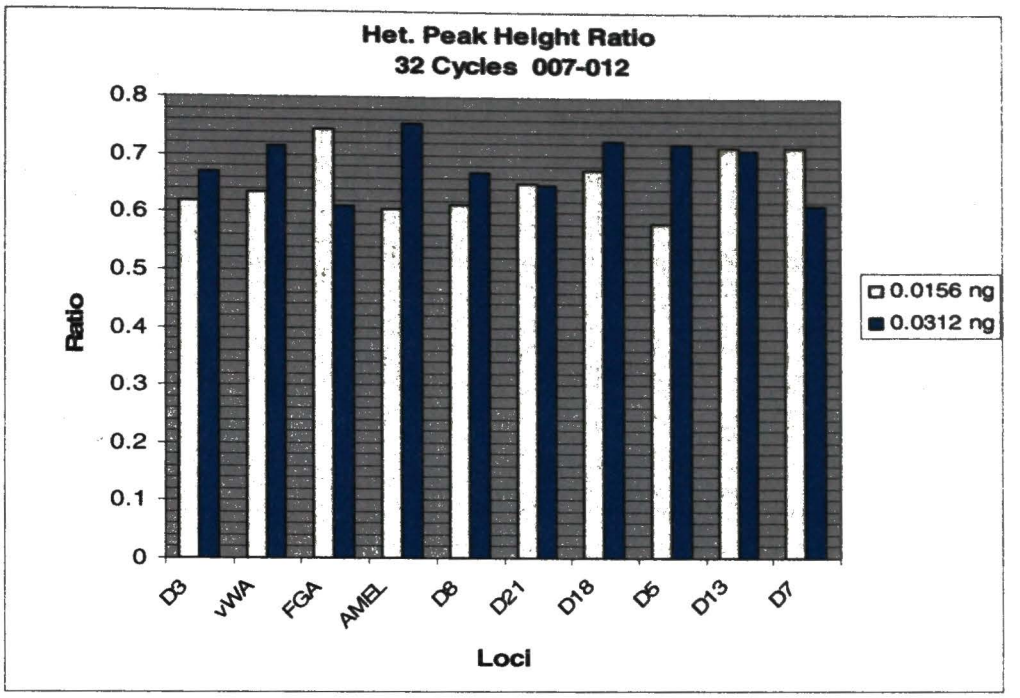

Table 4: Reproducibility Study
Average Heterozygote Peak Height Ratio
Profiler Plus Loci and its corresponding heterozygote peak height ratios
for samples 007-012 amplified at 32 cycles

TABLE 5

RUN 1 310 — 28 cycles

	D3	vWA	FGA	AMEL	D8	D21	D18	D5	D13	D7
Allele(s)	**14/15**	**17/18**	**23/24**	**X**	**13**	**30**	**15/19**	**11**	**11**	**10/11**
RFUs	1799/1749	1307/1270	1121/1105	4417	3786	2872	1635/1378	4787	3589	1410/1298
Allele(s)	*			*			*	**11**		
RFUs								137		
Allele(s)	*	*	*	**X**	*	*	*	**11**	*	*
RFUs				186				204		
Allele(s)	*	*	*	*	*	*		**9***	*	*
RFUs								174		
Allele(s)	*	*	*	*	*	*	*	**13**		
RFUs								101	*	**
Allele(s)	*	*	*	**X**	*	*	*	**12***	*	*
RFUs				138				115		
Allele(s)	*	*	*	**X**	*	*	*	**11**	**8/11**	*
RFUs				109				106	107/100	
Allele(s)	**17***	*	*	**X**	*	**30/31.2**	*	**11/13**	**14***	*
RFUs	108			264		117/123		229/147	133	
CLEAN										
CLEAN										

*: possible alleles below threshold

Cell blank: nothing seen in system

Loaded: 1.5µl of amplified product, 24µl Formamide, 1µl of ROX500 ILS

5 second injection time/15kV injection voltage

TABLE 6

RUN 1 310 28 cycles

	D3	vWA	FGA	AMEL	D8	D21	D18	D5	D13	D7
Allele(s)	**14/15**	**17/18**	**23/24**	**X**	**13**	**30**	**15/19**	**11**	**11**	**10/11**
RFUs	1745/1713	1235/1302	1228/1042	3734	3266	2818	1555/1369	4005	3685	1340/1184
Allele(s)	*	*	*	**X**		**30**	*	**11**	**11**	*
RFUs				141		138		189	119	
Allele(s)	**15/16**	**18***	**22***	**X**	**13/14**	**30/30.2**	**14/16**	**11**	**9/12**	**12***
RFUs	192/167	127	135	420	151/117	185/190	101/136	496	210/153	145
Allele(s)	*	*	*	**XY**	*	*	**12***	**9/12**	**8***	**9***
RFUs				145/136			102	228/171	114	101
Allele(s)	**15/18**	**15***	*	**XY**	**10***	**30/31.2**	**11***	**12/13**	**10/12**	**9***
RFUs	223/136	136		223/205	139	229/143	166	271/241	210/145	145
Allele(s)	**15/16**	**18***	**22***	**X**	**14***	**30**	**18**	**11/12**	**11***	**10***
RFUs	129/226	104	155	295	117	253	152	193/241	175	101
Allele(s)	**15/16**	*	**18***	**X**	**10***	**29***	*	**11**	**8/11**	*
RFUs	168/139		119	422	146	181		369	154/158	
Allele(s)	**16/17**	**16***	**21/22**	**X**	**10/12**	**30/31.2**	**14***	**11/13**	**8/14**	**10/11**
RFUs	216/189	116	169/138	564	194/168	180/136	117	353/349	145/215	153/114
CLEAN										
CLEAN										

*: possible alleles below threshold

Cell blank: nothing seen in system

Loaded: 1.5µl of amplified product, 24µl Formamide, 1µl of ROX500 ILS

5 second injection time/15kV injection voltage

TABLE 7

RUN 1
310

0.0156ng — 32 CYCLES 15 SEC INJECTION 10kV INJECTION

Sample		D3	vWA	FGA	AMEL	D8	D21	D18	D5	D13	D7
007-0.0156	Allele(s):	**14/17**	**14/16**	**20/20.2**	**XY**	**13**	**31.2/33.2**	**15**	**11**	**12**	**7/13**
	RFUs:	1138/1353	464/564	170/247	2235/1198	1412	270/428	327	1562	773	261/107
007-0.0156	Allele(s):	**14/17**	**14***	**20.2**	**XY**	**13**	**31.2/33.2**	**15/16**	**11**	**12**	**7/13**
	RFUs:	759/524	318	241	486/1299	1534	649/454	326/163	1336	805	234/162
007-0.0156	Allele(s):	**14/17**	**14/16**	**20/20.2**	**XY**	**13**	**31.2/33.2**	**15/16**	**11**	**12**	*
	RFUs:	690/359	185/289	195/231	782/1493	1173	207/227	157/219	1545	474	
007-0.0156	Allele(s):	**14/16/17**	**14/16**	**20/20.2**	**XY**	**13**	**31.2/33.2**	**16***	**11**	**12**	**7***
	RFUs:	1137/109/563	210/273	451/170	865/844	1767	416/319	247	1201	1198	315
007-0.0156	Allele(s):	**14/17**	**14/16**	**20/20.2**	**XY**	**13**	**31.2/33.2**	**16***	**11**	**12**	**7/13**
	RFUs:	813/973	867/607	531/139	1028/1353	1382	462/921	207	1538	549	239/344
007-0.0156	Allele(s):	**14/17**	**14/16**	**20/20.2**	**XY**	**13**	**31.2/33.2**	**15/16**	**11**	**12**	**7**
	RFUs:	1477/849	541/354	431/366	1044/1916	2388	332/485	395/671	654	1511	313
008-0.0156	Allele(s):	**BAD**	**INJECTION**								
	RFUs:										
008-0.0156	Allele(s):	**14/16**	**16/17**	**20/21**	**X**	**10/13**	**29/31.2**	**12/17**	**12/13**	**11**	**9***
	RFUs:	665/442	684/564	155/283	1762	496/293	457/357	184/193	787/750	947	172
008-0.0156	Allele(s):	**14/16**	**16/17**	**20/21**	**X**	**10/13**	**29/31.2**	**12***	**12/13**	**11**	**9/11**
	RFUs:	1236/711	194/354	163/267	2186	771/464	312/323	353	807/785	1437	209/209
008-0.0156	Allele(s):	**14/16**	**16/17**	**20/21**	**X**	**10/13**	**29/31.2**	**12/17**	**12/13**	**11**	**9/11**
	RFUs:	598/479	588/300	469/384	1493	180/378	395/221	424/150	569/852	829	335/242
008-0.0156	Allele(s):	**14/16**	**16/17**	**20/21**	**X**	**13***	**29/31.2**	**12/17**	**12/13**	**11**	**9/11**
	RFUs:	541/307	268/248	318/293	1398	305	447/386	182/161	612/263	507	246/179
008-0.0156	Allele(s):	**14/16**	**16/17**	**20/21**	**X**	**10/13**	**29/31.2**	**17***	**12/13**	**11**	**9**
	RFUs:	491/277	377/264	150/151	1383	244/564	522/414	227	777/330	487	241

Loaded: 1.5µl of amplified product, 24µl Formamide, 1µl of ROX500

Stutter

B: baseline peaks

TABLE 8

RUN 1
310

0.0156ng — 32 CYCLES 15 SEC INJECTION 10kV INJECTION

Sample		D3	vWA	FGA	AMEL	D8	D21	D18	D5	D13	D7
009-0.0156	Allele(s):	**BAD**	**INJECTION**								
	RFUs:										
009-0.0156	Allele(s):	**15/17**	**17/18**	**22/25**	**X**	**13**	**30/31.2**	**18***	**10/12**	**9/12**	**9***
	RFUs:	213/543	206/267	168/246	763	444	460/297	130	638/256	807/149	169
009-0.0156	Allele(s):	**15/17**	**17/18**	**25***	**X**	**13**	**31.2**	**14/18**	**10/12**	**9/12**	**8/9**
	RFUs:	431/193	241/214	499	842	277	456	406/252	657/1421	299/539	242/209
009-0.0156	Allele(s):	**15/17**	**17/18**	**25***	**X**	**13**	**30/31.2**	**18**	**10/12**	**9***	**8/9**
	RFUs:	160/440	297/473	370	587	775	375/804	139	138/822	272	263/177
009-0.0156	Allele(s):	**15***	**17/18**	**22***	**X**	**13**	**30/31.2**	**14***	**10/12**	**9/12**	**8/9**
	RFUs:	143	189/257	101	531	614	352/198	118	418/517	189/270	169/228
009-0.0156	Allele(s):	**15/17**	**17/18***	**22/25**	**X**	**13**	**31.2***	**14/18**	**10/12**	**9/12**	**8***
	RFUs:	244/233	165/127	223/236	1036	1135	641	384/115	956/146	364/325	226
010-0.0156	Allele(s):	**BAD**	**INJECTION**								
	RFUs:										
010-0.0156	Allele(s):	**15**	**15/17**	**24***	**XY**	**12/15**	**28/30**	**12***	**11/12**	**12/13**	**10/12**
	RFUs:	1132	277/336	221	1395/1018	411/245	667/369	353	1063/395	497/598	154/277
010-0.0156	Allele(s):	**15**	**15/17**	**19/24**	**XY**	**12/15**	**28/30**	**12/13**	**11/12**	**12/13**	**10/12**
	RFUs:	1307	268/130	402/434	854/314	533/725	441/241	370/268	1441/969	268/262	287/490
010-0.0156	Allele(s):	**15**	**15/17**	**19***	**XY**	**12/15**	**28/30**	**12/13**	**11/12**	**12/13**	**10***
	RFUs:	551	397/251	161	486/923	756/474	233/104	192/528	1166/576	405/196	138
010-0.0156	Allele(s):	**15**	**15/17**	**19/24**	**XY**	**12/15**	**28/30**	**12/13**	**11/12**	**12***	**10**
	RFUs:	1272	562/367	190/194	782/888	220/403	626/447	156/214	620/644	503	200
010-0.0156	Allele(s):	**15**	**15***	**19/24**	**XY**	**12/15**	**30***	**	**11/12**	**12/13**	**10/12**
	RFUs:	1493	531	251/204	909/1251	182/543	467		469/704	251/282	151/153

Loaded: 1.5µl of amplified product, 24µl Formamide, 1µl of ROX500

*: alleles below threshold **ambigious peak (not clearly defined peak)

TABLE 9

RUN 1

0.0156ng 310 32 CYCLES 15 SEC INJECTION 10kV INJECTION

Sample		D3	vWA	FGA	AMEL	D8	D21	D18	D5	D13	D7
011-0.0156	Allele(s):	**14/15**	**14/18**	**24**	**XY**	**13/14**	**29/31**	**13/14**	**7/11**	**11/13**	**12**
	RFUs:	1153/773	556/233	501	707/388	441/1035	738/492	193/350	1776/662	405/500	624
011-0.0156	Allele(s):	**14/15**	**14/18**	**24**	**XY**	**13/14**	**29/31**	**13/14**	**7/11**	**11/13**	**11/12**
	RFUs:	917/721	664/309	724	432/1478	405/231	335/388	330/338	632/844	415/470	274/404
011-0.0156	Allele(s):	**14/15**	**14/18**	**24**	**XY**	**13/14**	**29/31**	**13/14**	**7/11**	**11/13**	**11**
	RFUs:	518/1300	257/206	674	330/581	582/526	576/493	459/398	618/931	273/226	218
011-0.0156	Allele(s):	**14/15**	**14/18**	**24**	**XY**	**13/14**	**29/31**	**13/14**	**7/11**	**11/13**	**11***
	RFUs:	1117/827	885/162	352	597/797	310/433	424/173	441/374	1219/716	680/424	291
011-0.0156	Allele(s):	**14/15**	**14/18**	**24**	**XY**	**13/14**	**29/31**	**13**	**7/9/11**	**11/13**	**11/12**
	RFUs:	663/413	193/573	386	541/857	536/899	297/805	146	666/140/336	380/715	197/289
011-0.0156	Allele(s):	**14/15**	**14/18**	**24**	**XY**	**13/14**	**29/31**	**13/14**	**7/11**	**11/13**	**11/12**
	RFUs:	379/915	103/220	552	432/822	345/701	241/336	697/568	489/139	402/347	277/378
012-0.0156	Allele(s):	**16**	**OLA/17**	**27***	**X**	**13**	**29/34.2**	**13/14**	**12/13**	**11/12**	*
	RFUs:	1463	103/494	149	3916	683	444/221	100/177	817/480	210/160	
012-0.0156	Allele(s):	**16**	**14/17**	*	**X**	**13/14**	**29/34.2**		**12/13**	**11**	*
	RFUs:	1254	304/397		2479	561/387	505/313		1089/470	147	
012-0.0156	Allele(s):	**BAD**	**INJECTION**								
	RFUs:										
012-0.0156	Allele(s):	**16**	**OLA/17**	**22***	**X**	**13/14**	**29***	**13***	**12/13**	**11/12**	**9**
	RFUs:	1319	258/219	152	1986	340/170	910	164	413/661	246/196	247
012-0.0156	Allele(s):	**16**	**14/17**	**22/27**	**X**	**13/14**	**29/34.2**	**13/14**	**12/13**	**11**	*
	RFUs:	1035	333/262	115/137	2693	494/509	456/448	166/124	429/329	451	
012-0.0156	Allele(s):	**16**	**17***	**22/27**	**X**	**13/14**	**29/34.2**	**13/14**	**12/13**	**11/12**	**8***
	RFUs:	973	209	440/531	2940	572/506	940/203	179/251	1302/1197	236/429	240

Loaded: 1.5µl of amplified product, 24µl Formamide, 1µl of ROX500 DROP IN

TABLE 10

RUN 1

0.0312ng 310 32 CYCLES 15 SEC INJECTION 10kV INJECTION

Sample		D3	vWA	FGA	AMEL	D8	D21	D18	D5	D13	D7
007-0.0312	Allele(s):	**14/17**	**14/16**	**20/20.2**	**XY**	**13**	**31.2/33.2**	**15/16**	**11**	**12**	**7/13**
	RFUs	1429/1397	923/1126	231/740	1659/2383	2656	647/351	520/397	1760	1455	455/426
007-0.0312	Allele(s):	**14/17**	**14/16**	**20/20.2**	**XY**	**13**	**31.2/33.2**	**15/16**	**11**	**12**	**7/13**
	RFUs	1745/1609	722/829	436/778	2067/1501	1915	1021/508	726/443	2758	1469	225/346
007-0.0312	Allele(s):	**14/17**	**14/16**	**20/20.2**	**XY**	**13**	**31.2/33.2**	**15/16**	**11**	**12**	**7/13**
	RFUs	1778/1026	783/306	523/296	1747/1259	2365	330/220	367/556	2014	644	591/290
007-0.0312	Allele(s):	**14/17**	**14/16**	**20/20.2**	**XY**	**13**	**31.2/33.2**	**15/16**	**11**	**12**	**7/13**
	RFUs	771/572	768/1018	615/250	1118/1734	2282	612/359	227/268	2883	754	276/423
007-0.0312	Allele(s):	**14/17**	**14/16**	**20/20.2**	**XY**	**13**	**31.2/33.2**	**15/16**	**11**	**12**	**13**
	RFUs	1649/1659	730/778	297/336	1643/1355	2654	1174/780	261/512	3132	1048	260
007-0.0312	Allele(s):	**14/17**	**14/16**	**20/20.2**	**XY**	**13**	**31.2/33.2**	**15/16**	**11**	**12**	**7/13**
	RFUs	1293/1967	632/589	306/380	1252/1402	1855	366/502	331/550	2042	865	326/189
008-0.0312	Allele(s):	**14/16**	**16/17**	**20/21**	**X**	**10/13**	**29/31.2**	**17***	**12/13**	**11**	**9/11**
	RFUs	1147/1417	705/749	420/791	2474	1157/756	725/862	463	1158/1392	2524	580/546
008-0.0312	Allele(s):	**14/16**	**16/17**	**20/21**	**X**	**10/13**	**29/31.2**	**12/17**	**12/13**	**11**	**9***
	RFUs	1247/844	716/546	545/260	2902	851/981	712/675	378/261	1615/679	907	342
008-0.0312	Allele(s):	**14/16**	**16/17**	**20/21**	**X**	**10/13**	**29/31.2**	**12/17**	**12/13**	**11**	**9/11**
	RFUs	1543/1384	729/1003	721/409	2084	759/1362	1257/310	449/607	1658/1387	1584	253/412
008-0.0312	Allele(s):	**14/16**	**16/17**	**20/21**	**X**	**10/13**	**29/31.2**	**12/17**	**12/13**	**11**	**9/11**
	RFUs	1526/197	629/532	246/553	2570	1099/917	760/664	417/473	1432/428	737	285/258
008-0.0312	Allele(s):	**14/16**	**16/17**	**20/21**	**X**	**10/13**	**29/31.2**	**12/17**	**12/13**	**11**	**9/11**
	RFUs	668/977	744/541	372/268	2059	428/863	456/157	322/459	961/981	1274	361/226
008-0.0312	Allele(s):	**14/16**	**16/17**	**20/21**	**X**	**10/13**	**29/31.2**	**12/17**	**12/13**	**11**	**9/11**
	RFUs	598/924	450/247	239/501	1878	1204/423	645/101	257/442	484/882	466	239/121

Loaded: 1.5µl of amplified product, 24µl Formamide, 1µl of ROX500

*: alleles below threshold **ambigious peak (not clearly defined peak)

TABLE 11

RUN 1

0.0312ng 310 32 CYCLES 15 SEC INJECTION 10kV INJECTION

Sample		D3	vWA	FGA	AMEL	D8	D21	D18	D5	D13	D7
009-0.0312	Allele(s):	**15/17**	**17/18**	**22/25**	**X**	**13**	**30/31.2**	**14/18**	**10/12**	**9/12**	**8/9**
	RFUs	1041/724	765/534	756/580	2209	974	779/726	397/404	990/1243	732/196	315/865
009-0.0312	Allele(s):	**15/17**	**17/18**	**22/25**	**X**	**13**	**30/31.2**	**14/18**	**10/12**	**9/12**	**8/9**
	RFUs	739/1094	614/519	272/175	925	1503	399/548	333/396	1619/901	907/1039	271/444
009-0.0312	Allele(s):	**15/17**	**17/18**	**22/25**	**X**	**13**	**30/31.2**	**14/18**	**12**	**9/12**	**8/9**
	RFUs	905/213	555/420	614/136	1690	1532	689/441	613/445	831	443/1136	320/524
009-0.0312	Allele(s):	**15/17**	**17/18**	**22/25**	**X**	**13**	**30/31.2**	**14/18**	**10/12**	**9/12**	**8/9**
	RFUs	1158/739	504/218	575/331	1566	1350	349/763	503/273	1036/747	611/447	572/408
009-0.0312	Allele(s):	**15/17**	**17/18**	**22/25**	**X**	**13**	**30/31.2**	**14/18**	**10/12**	**9/12**	**8***
	RFUs	476/848	491/780	285/309	2429	1451	968/365	676/401	1283/600	523/823	320
009-0.0312	Allele(s):	**15/17**	**17/18**	**22/25**	**X**	**13**	**30/31.2**	**14/18**	**10/12**	**9/12**	**8/9**
	RFUs	737/936	304/680	547/402	1732	855	166/403	206/179	1170/2299	914/533	577/432
010-0.0312	Allele(s):	**15**	**15/17**	**19/24**	**XY**	**12/15**	**28/30**	**12/13**	**11/12**	**12/13**	**10/12**
	RFUs	1883	1054/463	726/526	1486/1508	1199/238	914/713	879/534	1725/2021	622/676	535/319
010-0.0312	Allele(s):	**15**	**15/17**	**19/24**	**XY**	**12/15**	**28/30**	**12/13**	**11/12**	**12/13**	**10/12**
	RFUs	2322	222/455	361/392	1271/556	769/1022	926/651	449/445	1227/999	621/719	132/404
010-0.0312	Allele(s):	**15**	**15/17**	**19/24**	**XY**	**12/15**	**28/30**	**12/13**	**11/12**	**12/13**	**10/12**
	RFUs	1619	712/867	531/397	1061/980	968/448	582/708	264/505	1211/1327	686/653	291/200
010-0.0312	Allele(s):	**15**	**15/17**	**19/24**	**XY**	**12/15**	**28/30**	**12/13**	**11/12**	**12/13**	**10/12**
	RFUs	1821	740/798	697/574	1312/964	581/652	667/722	631/146	457/719	888/520	592/198
010-0.0312	Allele(s):	**15**	**15/17**	**19/24**	**XY**	**12/15**	**28/30**	**12/13**	**11/12**	**12/13**	**10/12**
	RFUs	2297	589/394	475/199	855/1156	679/663	758/733	323/298	679/908	767/341	664/317
010-0.0312	Allele(s):	**15/16**	**15/17**	**19/24**	**XY**	**12/15**	**28/30**	**12/13**	**11/12**	**12/13**	**10/12**
	RFUs	2287/138	982/506	947/358	1331/1488	917/996	890/890	252/299	1175/1120	579/738	367/548

Loaded: 1.5µl of amplified product, 24µl Formamide, 1µl of ROX500 **Stutter present**

*: alleles below threshold **ambigious peak (not clearly defined peak)

TABLE 12

RUN 1
310 32 CYCLES 15 SEC INJECTION 10kV INJECTION

0.0312ng		D3	vWA	FGA	AMEL	D8	D21	D18	D5	D13	D7
011-0.0312	Allele(s):	**14/15**	**14/18**	**24**	**XY**	**13/14**	**29/31**	**13/14**	**7/11**	**11/13**	**11/12**
	RFUs	960/1808	686/456	1282	788/901	474/1736	1073/1290	397/456	1344/974	616/668	301/536
011-0.0312	Allele(s):	**14/15**	**14/18**	**24**	**XY**	**13/14**	**29/31**	**13/14**	**7/11**	**11/13**	**11/12**
	RFUs	1601/1330	914/451	1005	1980/1530	903/469	928/572	511/565	1605/1334	798/1006	561/539
011-0.0312	Allele(s):	**14/15**	**14/18**	**24**	**XY**	**13/14**	**29/31**	**13/14**	**7/11**	**11/13**	**11/12**
	RFUs	1750/735	667/580	1354	1051/1378	794/762	452/358	707/571	1144/1174	935/937	496/188
011-0.0312	Allele(s):	**14/15**	**14/18**	**24**	**XY**	**13/14**	**29/31**	**13/14**	**7/11**	**11/13**	**11/12**
	RFUs	1102/1091	576/620	848	890/1160	892/689	1052/282	694/428	1949/1076	692/646	123/473
011-0.0312	Allele(s):	**14/15**	**14/18**	**24**	**XY**	**13/14**	**29/31**	**13/14**	**7/11**	**11/13**	**11/12**
	RFUs	644/1360	784/1104	1088	1909/850	1193/1277	1155/998	521/452	1677/1135	764/936	607/653
011-0.0312	Allele(s):	**14/15**	**14/18**	**24**	**XY**	**13/14**	**29/31**	**13/14**	**7/11**	**11/13**	**11/12**
	RFUs	1452/733	693/455	1380	1373/1016	267/1148	237/1464	460/655	1330/1614	616/1353	352/152
012-0.0312	Allele(s):	**16**	**14/17**	**22/27**	**X**	**13/14**	**29/34.2**	**13/14**	**12/13**	**11/12**	**9**
	RFUs	2191	447/598	240/100	4452	508/731	659/436	173/150	1758/1086	404/309	317
012-0.0312	Allele(s):	**16**	**OLA/17**	**22/27**	**X**	**13/14**	**29/33.2 /34.2**	**13/14**	**12/13**	**11***	**8***
	RFUs	3269	414/314	366/171	4833	841/1013	1458/111/567	130/210	1094/1241	203	308
012-0.0312	Allele(s):	**16**	**OLA/17**	**22/27**	**X**	**13/14**	**29/34.2**	**13/14**	**12/13**	**11/12**	**9***
	RFUs	2419	676/529	351/285	5507	719/705	852/714	352/268	1227/1154	382/240	213
012-0.0312	Allele(s):	**16**	**14/17**	**22/27**	**X**	**13/14**	**29/34.2**	**13***	**12/13**	**11/12**	**9***
	RFUs	2489	566/642	428/244	4071	490/729	981/584	224	1211/1351	270/404	106
012-0.0312	Allele(s):	**16**	**14/17**	**22/27**	**X**	**13/14**	**29/34.2**	**13/14**	**12/13**	**11/12**	**8/9**
	RFUs	2601	615/373	218/177	3161	425/707	816/691	206/144	601/876	374/231	278/149
012-0.0312	Allele(s):	**Capillary**	**Failure**								
	RFUs										

Loaded: 1.5µl of amplified product, 24µl Formamide, 1µl of ROX500 Stutter

NC Blank PC-0.5ng Preferential Amplification, partial profiles

REFERENCES

1. FrankWE, Llewellyn BE, Fish PA, Riech AK, Marcacci TL, Gandor DW, Parker D, Carter RR, Thibault SM. "Validation of the AmpF*l*STR™ Profiler Plus PCR Amplifcation Kit for Use in Forensic Casework." *J Forensic Sci* 2001;46(3):642-646.

2. Whitaker JP, Cotton EA, Gill P. "A comparison of the characteristics of profiles produced with the AMPF*l*STR® SGM Plus™ multiplex system for both standard and low copy number (LCN) STR DNA analysis." *Forensic Sci. Int.* 123(2001):215-223.

3. Wickenheiser RA. "Trace DNA: A Review, Discussion of Theory, and Application of the Transfer of Trace Quantities of DNA Through Skin Contact." *J Forensic Sci* 2002;47(3):442-450.

4. U.S. Department of Justice. "NIJ Special Report: Using DNA to Solve Cold Cases." July 2002.

5. Gill P. "Application of Low Copy Number DNA Profiling." *Croat Med J* 2001;42:229-232.

6. Moretti TR, Baumstark AL, Defenbaugh DA, Keys KM, Brown AL, Budowle B. "Validation of STR Typing by Capillary Electrophoresis." *J Forensic Sci* 2001;46(3):661-676.

7. Gill P, Whitaker J, Flaxman C, Brown N, Buckleton J. "An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA." *Forensic Sci. Int.* 112(200)17-40.

8. AAFS 55th Annual Meeting. "Low Copy Number DNA Analysis: A Communuty Forum." Workshop #17.

9. Walsh PS, Metzger DA, Higuchi R. "Chelex 100 as a Medium for Simple Extraction of DNA for PCR-Based Typing from Forensic Material." *Biotechniques* 1(1991)91-98.

10. PE Applied Biosystems. "AmpF*l*STR® Profiler Plus™ PCR Amplification Kit User's Manual." 1998.

11. PE Applied Biosystems. "ABI PRISM™ 310 Genetic Analyzer User's Manual." 1998.

12. Perkin-Elmer Applied Bioststems Quantiblot™ Human DNA Quantitaion Kit User's Manual, 1996.

13. PE Applied Biosystems. "ABI PRISM™ 3100 Genetic Analyzer User's Manual." 2001.

14. GeneScan 2.1 (computer program) Macintosh version, 1996.

15. Genotyper (computer program) Macintosh version, 1998.

16. Orchid Cellmark Dallas Standard Operating Procedures (SOP) Guidelines.

17. Butler JM. "Forensic DNA Typing." 2001(103-106, 274-283).