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The goal was to evaluate the performance of a novel miniSTR multiplex system for the analysis of degraded and low quantity DNA samples. Three studies were designed to evaluate this new miniSTR kit: 1. a concordance study to insure that the profiles generated are identical to those with currently used STR kits; 2. a dilution study to identify the sensitivity limits of the multiplex system, and 3. the ability to generate profiles from DNA isolated from skeletal remains which had previously given incomplete profiles using conventional STR kits. The results indicate that the Applied Biosystems new miniSTR multiplex system will provide a valuable tool for forensic scientists to obtain genetic data from challenging casework samples.

EVALUATION OF A NOVEL MULTIPLEX MINISTR

SYSTEM FOR ANALYSIS OF DEGRADED AND LOW COPY

DNA SAMPLES

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EVALUATION OF A NOVEL MULTIPLEX MINISTR SYSTEM FOR ANALYSIS OF DEGRADED AND LOW COPY DNA SAMPLES

INTERNSHIP PRACTICUM REPORT

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LIST OF COMMON ABBREVIATIONS USED

ABI – Applied Biosystems, Inc.

CODIS – Combined DNA Indexing System

DNA – Deoxyribonucleic Acid

dNTPs – Deoxyribonucleotide Tri-Phosphates

LCN – Low Copy Number

mtDNA – Mitochondrial Deoxyribonucleic Acid

PCIA – Phenol Chloroform Isoamyl Alcohol

PCR – Polymerase Chain Reaction

RFUs – Reflective Fluorescence Units

STR – Short Tandem Repeat

SWGDAM – Scientific Working Group on DNA Analysis Methods

UNTHSC – University of North Texas Health Science Center

I. SUMMARY

The goal of this project was to evaluate the performance of a novel miniSTR multiplex system. Data generated by conducting a concordance study, a dilution/sensitivity study, and a degraded/low copy number sample study was used for this evaluation. The concordance study involved the comparison of reference sample DNA profiles generated by the miniSTR system with DNA profiles generated by commercially available STR multiplex kits currently used for forensic DNA analysis. The dilution study tested the ability of the miniSTR system to generate DNA profiles at low quantities of template DNA and determine the lower and upper DNA quantity limitations of the system. Analysis of DNA obtained from skeletal remains tested the ability of the miniSTR system to generate a DNA profile from bone samples which had previously given incomplete DNA profiles using standard STR typing kits.

II. PROBLEM/HYPOTHESIS

DNA extracted from environmentally challenged forensic evidentiary samples, especially those involving unidentified skeletal remains, is often limited in quantity and highly degraded. Amplification of these DNA samples with currently available STR kits often results in partial DNA profiles, with limited statistical value, or worse no genetic data.

A new STR multiplex system has been developed by Applied Biosystems containing PCR primers that have been moved closer to the variable repeat target region of eight STR loci. The scope of this project was to determine if: 1) the miniSTR multiplex kit will produce concordant profiles with currently available multiplex kits; 2) the kit will display a greater sensitivity to low amounts of input DNA; and 3) the new kit will demonstrate an increased ability to amplify DNA recovered from skeletal remains as compared to those generated using standard STR kits.

III. SIGNIFICANCE

In forensic casework, investigators are often faced with the challenge of obtaining genetic information from biological samples that are less than ideal. Throughout the United States there may be 40,000 or more skeletal remains stored by medical examiners, coroners and law enforcement agencies that cannot be identified by conventional means (informal survey conducted by U.S. Department of Justice). Especially challenging are unidentified human remains cases where the only source of DNA must be obtained from bone or highly degraded tissue. Standard STR typing methods currently in practice are not optimal for use on these types of samples, and often produce incomplete genetic profiles or profiles that are difficult to interpret. The PCR primers used in making the standard STR typing kits are not optimally designed to work with degraded DNA or low quantities of DNA. There is a need for an STR multiplex system that is both sensitive and robust so that useful genetic information can be obtained from highly degraded and low copy DNA samples. The miniSTR multiplex system that was evaluated in this project includes primers which were redesigned to amplify a subset of the CODIS STR loci. The amplified products generated by these primers are shorter in length, which could increase the likelihood of successfully obtaining DNA profiles from degraded and low copy number samples. If shown to reliably and accurately perform, this miniSTR system could become a valuable tool for wide use in forensic casework.

IV. BACKGROUND

Over the past two decades, DNA typing has become the most prominent tool used for human identification in the field of forensic science. Like fingerprints, DNA profiles are unique to each individual on earth. With the exception of identical twins, no two individuals share the exact same genetic makeup. Forensic scientists have taken advantage of this feature of human genetics in order to provide the legal system with powerful evidence associating an individual with a crime. A set of thirteen core short tandem repeat (STR) loci have been established as the standard markers for the field. DNA databases containing profiles from convicted criminals, crime scene evidence, and missing persons reference samples are used by law enforcement agencies around the country to find links between individuals and crimes that might otherwise go unsolved.

Advances in technology have allowed genetic information to be used in a myriad of ways. In the field of forensic science, genetic profiles are most often used to identify suspects and victims associated with particular crime scenes. DNA profiles can be used in a number of civil legal applications, including paternity testing for child support issues. Parentage and kinship analysis can also be used in cases of missing persons or unidentified human remains by comparing reference samples from closely related individuals. Genealogies have been developed using genetic information in order to trace the lineage of individuals back to certain ancestors, as well as assisting researchers studying human migration patterns. Archeologists also rely on DNA collected from

ancient remains to provide clues about the history and background of the human remains they excavate. In the medical field, genetics have seemingly unlimited applications including disease studies, cancer research, and drug testing.

In the forensic science field, technology allows scientists to obtain DNA profiles from an ever-growing number of biological sources. Methods that previously required a substantial amount of blood for successful analysis have given way to techniques that produce genetic profiles from some very unlikely materials. Skeletal remains such as bone fragments, teeth, and degraded tissue are now routinely utilized for DNA analysis. Hair analysis that previously required the root or skin tag to be present can now be performed on shed hair shafts. Saliva deposited on licked stamps and envelopes as well as in bite marks on victims is a good source of DNA. Fingerprints and other contact points found on gun triggers, steering wheels, paper, or just about any surface provide not only a unique fingerprint, but may yield enough biological material to develop a DNA profile from as well. Even highly degraded or damaged samples from cold cases or bodies recovered from harsh environments can now be successfully typed. In some cases, only a few sloughed cells from body surfaces, such as epithelial, dandruff, and fecal matter, may be sufficient to produce a full or partial DNA profile (1, 2, 6, 9, 10, 13, 14).

Commercially available STR multiplex kits will produce a complete genetic profile of the 13 Core CODIS loci with a minimum input of approximately 250 pg of DNA. Many of the biological sources previously mentioned may actually yield less than 100 pg of DNA, and these samples are termed low copy number (LCN) samples. LCN

samples may be the only source of biological material available at a crime scene, so the ability to successfully amplify them may be crucial to a forensic investigation. In order to increase the ability of the forensic DNA analyst to develop useful genetic profiles from these LCN samples, alternative or modified systems to conventional STR typing have been developed.

The STR multiplex kits used in forensic DNA typing use primer pairs that produce amplicons of approximately 100 to 450 base pairs in length. In samples that have been compromised or environmentally challenged, the nuclear DNA material is often damaged or degraded DNA, resulting in fragments of 200 base pairs or less, making amplification of the larger loci improbable at best. The amplification pattern observed in these samples is reminiscent of a "decay curve" where the height of the alleles in the STR profile is inversely proportionate to the amplicon's length. As a result, the loci with larger amplicons will likely fall below the detection threshold, thus generating a partial STR profile (6, 7).

Standard STR analysis typically works most efficiently when PCR is performed under the conditions specified by the kit manufacturer. Currently available multiplex STR kits have a lower limit of sensitivity at about 250 pg of input/template DNA using a range of 28-30 PCR cycles. These systems have been optimized with these limits in order to obtain the most reliable profile possible while avoiding potential amplification artifacts. Using these amplification parameters, a forensic analyst will have the greatest opportunity to associate the profiles obtained from evidentiary samples with known individuals (9). As discussed above, however, low copy number or trace samples yield

only a minute amount of template DNA and therefore tend to fail to produce complete or even partial profiles when standard STR analysis is performed. The sensitivity of these methods can be increased simply by increasing the number of amplification cycles during the PCR reaction (2, 8, 9, 11). Nested PCR may also be utilized to increase sensitivity (11, 16). Using two primer sets and two separate PCR reactions, this method amplifies the STR and adjacent flanking regions in the first round of PCR, and then in the second round, the product of the first round is used as a template to amplify a smaller final product. Nested PCR theoretically has the ability to amplify the DNA from a single cell while reducing the occurrence of non-specific products and stochastic effects; however, it also requires transferring the PCR product into a separate tube which increases the risk of sporadic contamination (11).

A number of investigators have reported success in developing STR profiles from a wide range of samples containing low amounts of DNA using increased PCR cycle numbers. Gill (9) reported on a number of such findings including: epithelial cells deposited by a strangler using 30-31 cycles (Wiegand and Kleiber); fingerprints on tool grips using 28-40 cycles (Van Hoofstat et al.); a 70-year old bone from the Romanov family using 38-43 cycles (Gill et al.); and bones thousands of years old using 50 cycles (Burger et al.) and 60 cycles (Schmerer et al). McNevin et al. (16) conducted a study on obtaining STR profiles from telogen phase hair shafts using both increased PCR cycles (34-36 cycles) and the nested PCR method (2 x 28 cycles) and found that, in combination with their optimized extraction procedure, usable profiles of similar quality were obtained from both PCR methods. The authors recommended the single 34-36 cycle procedure

since it is the simpler of the two to perform. McNevin et al. also report on the findings of Brandstatter and Parson that better quality profiles were obtained from a single PCR with increased cycle number (35 cycles) when compared to two rounds of nested PCR.

While raising the number of PCR cycles can increase the success of amplifying LCN samples (Gill et al. (11) report the ability to obtain full profiles down to 25-50pg, or four to ten cells), these methods unfortunately are not the ultimate solution to the problems associated with LCN DNA analysis. As the number of PCR cycles is increased, so follows the incidence of negative stochastic effects as well as the likelihood of amplifying biological sources resulting from contamination and/or adventitious transfer. Allele dropout can occur when one allele at a heterozygous locus is not detected. As a result, the locus erroneously appears to be homozygous. This is known to occur approximately 10% of the time when dealing with LCN conditions. Generally, the lower molecular weight allele will preferentially drop out (19). Increasing amplification cycles can often result in the recovery of these alleles in the profiles of these samples. However, increasing PCR cycles may only result in preferential amplification of one heterozygous allele and actually increase the occurrence of allele dropout.

Slippage by the DNA polymerase (*Taq*, etc.) during amplification results in the production of amplicons that are four base pairs smaller that the true allele in tetrameric STR alleles. These are known as stutter peaks and are a common occurrence in DNA profiles even under standard STR amplification conditions. Stutter peaks are detected in approximately 30% of PCR amplifications. Stutter peaks are typically observed to have RFU values <15% of the height of the true allele. Stutter peak heights vary depending on

the particular STR locus. The occurrence and range of stutter peaks are used by laboratories to develop interpretations guidelines so their presence is not falsely associated with true alleles in a genetic profile. When dealing with LCN samples, however, there is a reduced amount of template DNA material being amplified, and the proportion of stutter peaks to true alleles can become increased. It is not uncommon to see stutter peaks that are 20-40% the height of the true allele peaks (20). As a result, these stutter peaks exceed the threshold allowed by interpretation guidelines and will likely be called by genotyping software as additional alleles in the DNA profile. The presence of these stutter peaks in LCN samples makes the interpretation of the genetic profiles extremely difficult for the analyst.

Additional false alleles may also be seen as a result of sporadic contamination. These instances of allele drop-in occur when sources other than the stain or sample being tested are amplified and these false alleles are seen along with the profile of the true source. The sensitivity levels of standard STR methods often prevents minor DNA contaminants from being detected since the high amount of template DNA from the target source is preferentially amplified. Increasing the number of PCR cycles will enhance the ability to detect trace DNA from sources other then those associated with the victim or perpetrator of the crime. Detection of DNA profiles from individuals not truly associated with a case will result in a mixture profile, which in conjunction with other PCR artifacts, makes the interpretation of the DNA results exceedingly difficult. Negative controls are routinely used with standard STR methods to ensure that the process is free of any detectable levels of contamination in the reagents or materials being

used. When utilizing increased PCR cycles, casework samples may display minor contaminants that may or may not be observed in the negative controls. These low level contaminants could originate from the evidentiary sample; however, they could also be tube-specific and result from DNA associated with minute dust particles or plastic-ware (11).

The interpretation of LCN samples can be aided by imposing what is known as the duplication rule (5, 9, 10, 11, 20). This guideline stipulates that an allele can only be scored and reported if it is seen in at least two replicate amplifications. Butler (5) reports that the probability of seeing a false allele twice in repeated samples is less than one percent. Gill et al. (11) tested this method and reported that only four out of 1,225 pair-wise comparisons showed the same false allele, which equates to about 0.3%. While the duplication rule can greatly aid an analyst in weeding out false alleles, it is also not the ultimate answer to LCN analysis since it cannot account for drop out of true alleles, or the fact that there may not always be enough source material available to run multiple amplifications.

The introduction of mitochondrial DNA sequencing has provided one alternative method of developing genetic profiles from samples containing very small amounts of DNA. As described by Budowle et al. (4), mitochondria are cellular organelles that contain their own extrachromosomal genome that is separate and distinct from the nuclear genome. Mitochondrial DNA, or mtDNA, are circular, double-stranded, histone-free molecules that comprise a small set of genetic codes for about 13 polypeptides that make up the proteins involved in the oxidative phosphorylation processes of the cell, as

well as several RNA molecules. A single somatic cell may contain up to 1,000 mitochondria, each of which possesses between two and ten copies of mtDNA. The number of copies of mtDNA is significantly higher when compared to the amount of nuclear DNA found in each cell. Therefore in LCN samples, STR typing may fail to provide any significant genetic profiles, while mtDNA analysis will typically provide useful genetic information (3, 4).

Except when a mutation occurs, the mtDNA sequences of all maternally related individuals will be identical. The availability of reference samples from maternal relatives can be used to confirm or refute the identity of unknown samples. This is especially helpful when in missing persons cases, unidentified skeletal remains, or mass disaster remains. The stable inheritance of mtDNA is generally consistent over multiple generations; therefore reference samples can be obtained from maternal relatives multiple generations apart from the source being tested. The most useful reference samples for STR analysis are obtained from close relatives (mother, father, or siblings). The ability to use more distant relatives for mtDNA comparisons can be a significant advantage. The lack of mtDNA repair mechanisms combined with the low fidelity of mtDNA polymerase, results in the mitochondrial genome possessing a higher mutation rate than the nuclear genome. It has been shown that some mtDNA regions have up to a 17 times higher mutation rate as compared with nuclear DNA. This higher mutation rate results in hypervariable regions which have become prime targets for human identity testing. The majority of mtDNA analysis is performed on two specific segments of the control region known as hypervariable regions I and II, or HVI and HVII. The small size of these

regions, along with the observation of an average of eight nucleotide differences between Caucasians and 15 differences between African-Americans, makes them ideal for PCR-based typing for forensic applications (3, 4).

Although mtDNA is maternally inherited and therefore only one sequence should be detected per person, multiple variations within an individual have been observed. This feature is referred to as heteroplasmy and is most often detected as two different nucleotides at a single base position. Multiple heteroplasmic sites have been observed within a single individual, however this has been shown to occur at a much lower frequency. According to Bar et al. (3), there are three basic ways that heteroplasmy is exhibited:

1. An individual may display more than one mtDNA type in a single tissue.
2. An individual may show one mtDNA type in one tissue sample and another type in a different tissue sample.
3. An individual may exhibit one mtDNA type in one tissue type and a different mtDNA type in another tissue type.

In forensic mtDNA analysis, comparison is made between mtDNA types of known exemplars and evidence samples and evaluated based on established interpretation guidelines. A failure to exclude a sample from originating from a known source occurs when the mtDNA sequences for HVI and HVII are the same. As a result of heteroplasmy, individuals are usually not excluded on the basis of a single nucleotide difference.

Mitochondrial DNA typing is used in the forensic arena primarily to assist with LCN applications on items of evidence such as bone, teeth, and degraded stains, as well as hair shafts (12). When combined with other genetic data, such as partial STR profiles, mtDNA analysis can provide additional statistical strength to aid in any conclusions drawn about the source of an evidentiary sample. In the case of bone or hair, mtDNA may be the only genetic data attainable.

In order to reach a sufficient level of certainty about a relationship between an evidentiary item or stain and a particular person, some estimation of the rarity of a given mtDNA profile is needed. As reported by Budowle et al. (4), the current method consists of simply counting how many times a particular mtDNA sequence is observed in a population database. Unfortunately, mtDNA profiles cannot be compared to the well established databases for STRs and separate databases for mtDNA have had to be developed. There are multiple databases consisting of sequence data for over 1,000 individuals compiled for multiple population groups. The largest mtDNA database contains the HVI and HVII sequences for over 14,000 individuals. The FBI has added these mtDNA databases to the CODIS system, which has proved useful in missing persons cases (5). Because of the lack of recombination in mtDNA, more individuals are required to be typed for these databases, as compared with the STR markers, in order to establish an acceptable level of relevance and ensure a complete representation of the degree of variation among the population (4). The development of full HVI and HVII sequences will require a significantly larger investment of effort, time, and money as compared to STR typing. The majority of labs participating in CODIS are only capable of

STR analysis. To date, there are only a very small number of forensic labs performing mtDNA analysis. Therefore, the availability of mtDNA testing limits its utility in forensic casework.

Other factors have prevented the routine utilization of mtDNA analysis in forensic casework. Since mtDNA is maternally inherited, the profiles from maternally related individuals cannot be differentiated from each other, and therefore none can be eliminated as a source of the evidentiary sample. In addition, mtDNA profiles are incompatible with the CODIS STR databases and cannot be searched against the CODIS Offender or Forensic databases in hopes of developing a suspect. Since all of the bases within the mtDNA genome are linked, the data generated from sequence analysis must be treated as a single locus. As a result, mtDNA profiles lack the discriminatory power as compared with independently inherited multiple STR profiles (6, 18). As with increased PCR cycle numbers for STR analysis, mtDNA typing has a number of limitations preventing it from becoming the primary choice for the analysis of LCN samples.

Another approach that has been utilized to increase the success of obtaining genetic profiles from samples containing degraded or limited quantities of template DNA is through the redesign of the amplification primers. The primers have been relocated so that they are closer to the particular genetic marker in order to produce a shorter amplified product. McNevin et al. (15) report on research that supports the utility of such redesigned primers. Mitochondrial DNA studies conducted by Alonso et al. and Allen et al. involved shorter amplicons that were repeatedly able to produce full profiles when longer amplicons for the same mtDNA regions were not. This is consistent with the

finding of Paabo et al. which showed that the majority of DNA detectible by ethidium bromide staining after gel electrophoresis was present in degraded fragments of 50-200bp each. Vigilant demonstrated that fragments less than 300bp could be successfully amplified about 80% of the time, compared to 60% for 400bp fragments and 15% for 500bp fragments (15). The redesign of the primers utilized for the amplification of the CODIS STR loci may be an important tool for LCN analysis in the near future. By moving the flanking region of each STR primer as close as possible to the target repeat region, shorter amplicons can be produced. Redesigned primers that are able generate PCR products from highly degraded DNA samples were first utilized successfully more than ten years ago during the identification of the victims of the Branch Davidian fire in Waco, TX. Investigators were able to co-amplify four STR loci from severely degraded DNA samples taken from human remains that had been exposed to extreme thermal, physical, and chemical insult (19). One major advantage of these smaller STR amplicons, also called miniSTRs, is that the primers can be redesigned for the 13 core CODIS loci, therefore the miniSTR profiles generated are compatible with the CODIS databases. Butler et al. (6) produced new primer pairs for nearly all of the commercially available STR loci, including the 13 core CODIS loci. Studies were conducted in which the redesigned primers were combined into various sets. These sets generally contained primer sets for three loci (one per fluorescent dye color) and were termed "miniplexes." The study evaluated the performance of each of the redesigned primers and the most effective combinations of primers in the miniplexes. The concordance of the between the new miniSTRs and the conventional STR multiplexes were evaluated. In another study,

the same authors looked at how the shorter amplicons would affect the relationship of the DNA template concentration with the signal strength and peak heights of the genetic profiles (7).

In designing the new primers, Butler et al. (6, 7) had attempted to generate amplicons between 80 and 100 base pairs which would require the primers to be located as close as possible to the STR repeat region. This was not possible for all loci due to the presence of polymorphic nucleotides, partial repeats, mononucleotide repeat stretches, or insertions/deletions in the flanking regions of the STRs that might reduce the stability of the primer annealing. The danger with having an insertion or deletion within the primer binding region of an STR flanking region is that full amplification may still occur, but different alleles may be called from those observed when the same sample is amplified with standard STR primers. The authors also discovered that the 3` end of a primer can be positioned up to two full repeats into the STR repeat region and still produce successful amplifications. The authors report full concordance of the results of the miniSTRs with the commercial STR multiplex kits, as well as increased amplification products from degraded DNA samples. The miniSTRs also displayed an ability to amplify template DNA quantities as low as 31 pg for two different miniplexes, with peak heights well above the detection threshold. A larger miniplex consisting of six primer pairs showed a sensitivity of 250pg before allele dropout occurred. Peak height ratios greater than 60% were observed for DNA concentrations as low as 125 pg for the smaller miniplexes and as low as 250 pg for the larger miniplex. Overall, the miniSTR primer sets designed by Butler et al. were shown to be a reliable alternative for the amplification

of low copy number samples and for those samples that previously produced allele dropout of the larger loci.

It has been shown that for some forensic applications that the 13 Core CODIS STR loci may be insufficient to achieve a statistically significant profile for the identification of an unknown sample. In cases involving unidentified human remains or mass disaster victims, there may not be a direct reference sample or even a family member available to provide a genetic sample for comparison. By using miniSTR markers or SNP markers in addition to the core STR markers, investigators can not only increase the likelihood of successfully amplifying a sample, but can also provide additional statistical data to support the association of the profiles. Additional markers can also provide higher levels of exclusionary power that are sometimes needed in both routine and complex paternity cases. In addition to developing redesigned miniSTR primers for the CODIS loci, Coble et al. (8) also developed miniSTR primers for non-CODIS loci for use in analysis of degraded DNA samples. A sensitivity study showed the miniplexes to be consistently reliable down to input DNA amounts of 100pg. The miniplexes also produced alleles at least some of the time at 20pg. By increasing the number of PCR cycles and concentration of AmpliTaq Gold polymerase, the authors were able to improve the number of observed alleles when adding less than 50pg of DNA. In correlation to standard STR profiling methods, full miniplex profiles were developed for fifteen bone samples in contrast to only one full profile developed by the PowerPlex 16 kit (Promega). The authors stated that the new miniSTR loci will likely become useful in a variety of casework scenarios, possibly providing an alternative to

mtDNA sequencing of degraded DNA samples, as well as becoming useful in analysis of hair shafts. Coble et al. concluded that miniSTR assays offer a new tool for recovering useful genetic information from samples for which standard STR multiplex kits are only able to generate partial profiles.

Unfortunately, commercial miniSTR miniplex kits are not yet available to the forensic community. Individual analysts or agencies who wish to utilize the processes developed for miniSTRs can purchase the individual redesigned primers, but must then develop and optimize their own miniplexes and perform their own developmental validation. Even though miniSTR primers have been developed for the 13 core CODIS loci, only three or four of the primers have typically been combined in a single PCR reaction, so multiple amplifications are required to analyze all the loci. Since miniSTRs have been developed for use primarily with highly degraded or low copy number samples, there may not be a sufficient amount of template DNA material available to perform multiple amplifications. The potential for primer binding mutations will require additional concordance studies to be performed between the miniSTRs and conventional STR methods to determine the frequency of these occurrences. Other problems can also arise when an increased number of PCR cycles are used to enhance the performance of the miniSTR primers leading to a rise in the occurrence of stutter, allele drop-in, and the increased risk of amplifying contaminating sources of DNA (5, 6, 8, 15).

Applied Biosystems, Inc. has developed a new miniSTR multiplex system in which eight STR loci and the Amelogenin marker are amplified concurrently. The first step in Applied Biosystems' development of the miniSTR multiplex system was selection

of the loci that would be included. Applied Biosystems conducted numerous surveys with laboratories that process unidentified human remains and highly compromised casework samples. The loci chosen are included in the U.S. CODIS database system as well as the European database system. The loci chosen produce amplicons greater than 200 base pairs when amplified with the current multiplex systems, and are therefore those most susceptible to drop-out. The loci selected were Amelogenin, D13S317, D7S820, D2S1338, D21S11, D16S539, D18S51, CSF1PO, and FGA. Each of the primer sets, excluding those for Amelogenin, has been redesigned to produce shorter amplicons than those used in the conventional STR multiplex systems. With the exception of the FGA locus, all of the amplicons produced by this system are less than 240 base pairs in length. Applied Biosystems was able to utilize their five-dye chemistry to incorporate all nine of these loci with these redesigned primers. Figure 4-1 shows the prototype allelic ladder that was developed for the for the miniSTR multiplex system. The first sensitivity study performed by Applied Biosystems demonstrated the ability of the miniSTR multiplex system to produce a full genetic profile at DNA quantities as low as 62pg (Figure 4-2).

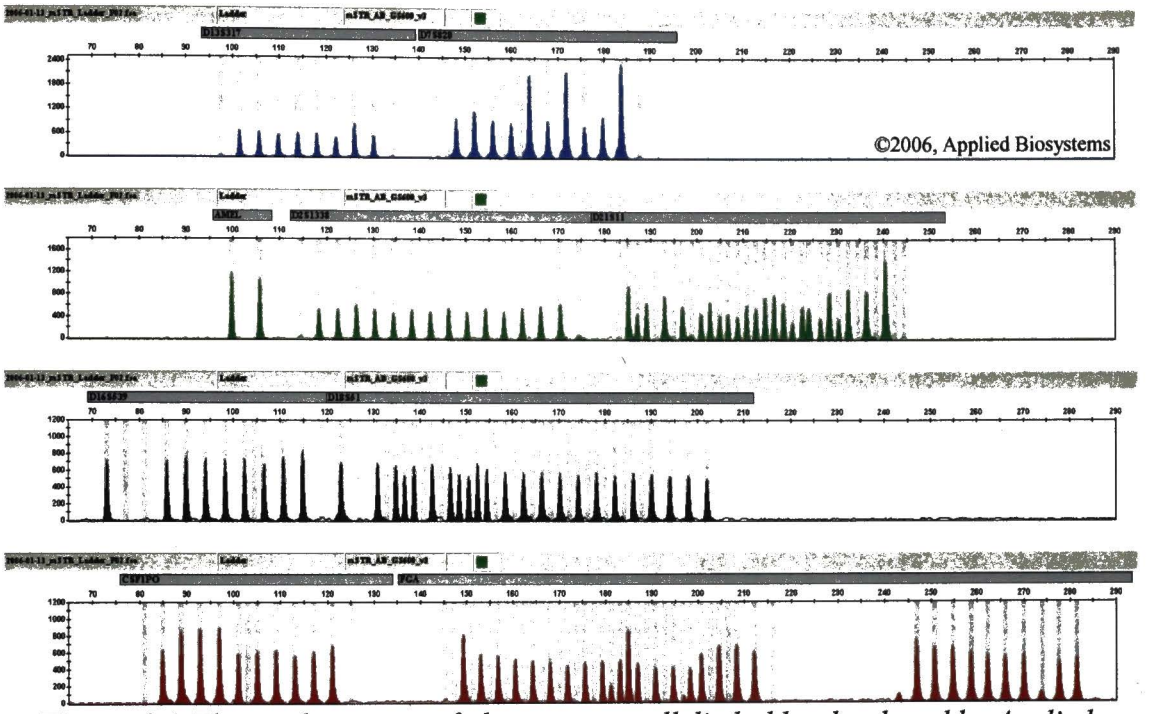

Figure 4-1 *Electropherogram of the prototype allelic ladder developed by Applied Biosystems for the miniSTR multiplex system. Data supplied by Applied Biosystems.*

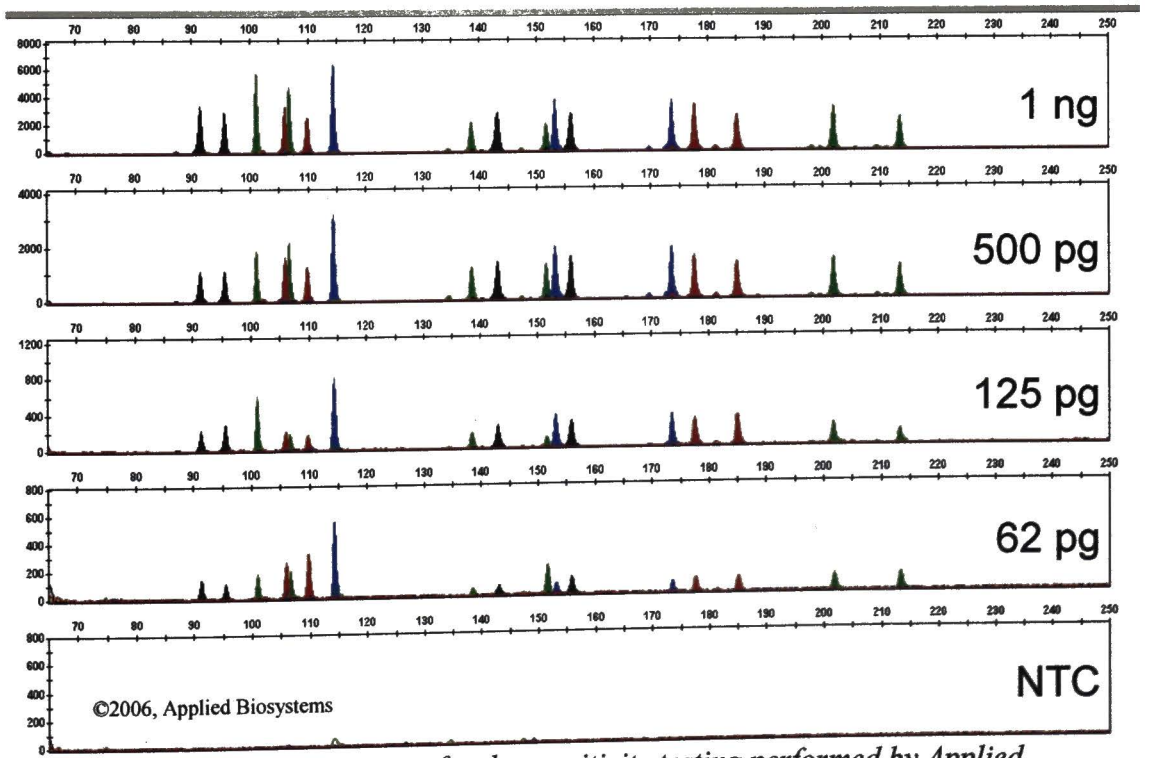

Figure 4-2 *Electropherogram for the sensitivity testing performed by Applied Biosystems for control DNA sample 007 amplified with the miniSTR multiplex system. Data supplied by Applied Biosystems.*

Further testing done by Applied Biosystems demonstrated that amplifications utilizing the new miniSTR primers are far less susceptible to the PCR inhibitors humic acid, commonly associated with buried remains, and hematin, a product of red blood cell breakdown that commonly causes inhibition with DNA isolated from older blood stains (Table 4-1 and 4-2).

Humic Acid	Mini-1	Mini-2	Mini-3	Ident-1	Ident-2	Ident-3
0 ng/ul	17/17	17/17	17/17	17/17	17/17	17/17
10 ng/ul	17/17	17/17	17/17	12/17	11/17	12/17
30 ng/ul	17/17	17/17	17/17	0/17	0/17	0/17
50 ng/ul	17/17	17/17	17/17	0/17	0/17	0/17

Table 4-1 *The results of inhibitor testing performed by Applied Biosystems for DNA samples amplified with the miniSTR multiplex system and the Identifiler multiplex system in the presence of various concentrations of humic acid. Data supplied by Applied Biosystems.*

Hematin	Mini-1	Mini-2	Mini-3	Ident-1	Ident-2	Ident-3
0 mM	17/17	17/17	17/17	17/17	17/17	17/17
20 mM	17/17	17/17	17/17	17/17	17/17	17/17
40 mM	17/17	17/17	17/17	0/17	0/17	0/17
80 mM	0/17	0/17	0/17	0/17	0/17	0/17

Table 4-2 *The results of inhibitor testing performed by Applied Biosystems for DNA samples amplified with the miniSTR multiplex system and the Identifiler multiplex system in the presence of various concentrations of hematin. Data supplied by ABI.*

Applied Biosystems also performed testing on samples artificially degraded by DNAse I treatment, which showed the ability of the miniSTR multiplex system to amplify highly degraded DNA samples much more successfully than the Identifiler system (Table 4-3).

DNAse I	Mini-1	Mini-2	Mini-3	Ident-1	Ident-2	Ident-3
Control	14/14	14/14	14/14	14/14	14/14	14/14
4U DNAse I	14/14	14/14	14/14	5/14	7/14	5/14
5U DNAse I	14/14	14/14	14/14	2/14	3/14	3/14
6U DNAse I	13/14	13/14	13/14	1/14	0/14	2/14

Table 4-3 *The results of degradation testing performed by Applied Biosystems for DNA samples amplified with the miniSTR multiplex system and the Identifiler multiplex system after treatment with various concentrations of DNAse I. Data supplied by Applied Biosystems.*

Additional testing performed by Applied Biosystems demonstrated the ability of the miniSTR multiplex system to amplify DNA isolated from blood samples deposited on denim fabric, which commonly causes inhibition when amplified with conventional multiplex systems.

Applied Biosystems asked the UNTHSC DNA Identity Laboratory to serve as a test site in the development and evaluation of the prototype miniSTR multiplex kit.

V. RESEARCH DESIGN AND METHODOLOGY

A. Studies Conducted

A concordance study was performed to evaluate the ability of the miniSTR system to produce correct DNA profiles for known reference samples, and to evaluate the quality of the profiles produced using the miniSTR system when compared to the DNA profiles produced by conventional multiplex STR typing systems currently used in forensic DNA casework. The types of reference samples used were buccal swabs, blood spots on FTA paper, and buccal smears on FTA paper. Twenty samples for each sample type were taken from casework files and recorded by reference number; the identity of the source of the samples was not known to the investigators. In addition, each of the three sample types was collected from each of the five laboratory investigators who worked on the project. The buccal swab samples were extracted using the PCIA method and then the DNA quantity of each sample was determined using the Quantifiler Human DNA Quantification Kit from Applied Biosystems. The samples were adjusted to 1ng/µl based on the results of the Quantifiler quantification, and 1ul of each sample was used for the amplification process. The FTA blood and FTA buccal samples were amplified directly from a 1.2mm punch taken from each sample card. The buccal swab PCIA extracts adjusted to 1ng/µl, as well as the FTA blood and FTA buccal punches for each of the reference samples were amplified with each of the following STR multiplex systems: AmpFlSTR Profiler Plus, AmpFlSTR COfiler, AmpFlSTR SGM Plus, AmpFlSTR

Identifiler, and the miniSTR multiplex system version 3. Genetic profiles were generated using capillary electrophoresis on the ABI 3130xl Genetic Analyzer instrument. The profiles generated for each sample were compared to ensure that all the typing systems, including the miniSTR multiplex system produced the same genetic profile with concordant results in the areas of: allele calls, peak height, heterozygous peak height balance, stutter peaks, etc.

A dilution/sensitivity study was conducted in order to evaluate the ability of the miniSTR multiplex system to amplify low levels of input template DNA and to determine the lowest threshold DNA quantity for which the miniSTR system can be reliably used. Three dilution sets were prepared using three different DNA samples. Blood samples from individuals XGA and AJE were extracted using the PCIA method, then quantified using the Quantifiler Human DNA Quantification Kit from Applied Biosystems and diluted to a 10ng/µl stock. The commercially available control DNA 9947A was purchased from Promega Corporation in a 10ng/µl quantity. A series of dilutions was prepared for each of the three DNA stocks in the following DNA quantities: 1ng/µl, 0.5ng/µl, 0.2ng/µl, 0.1ng/ul, 0.05ng/ul, 0.025ng/ul, and 0.0125ng/ul. Each of the three dilution series were amplified using the miniSTR multiplex system, first following Applied Biosystems recommended protocol using 30 PCR cycles, then following a modified protocol using 32 PCR cycles. 1ul of each sample was added in the amplification reaction. Genetic profiles were generated using capillary electrophoresis on the ABI 3130xl Genetic Analyzer instrument. The profiles were examined to determine the DNA quantity level at which the samples began to exhibit negative features

such as allele drop out, peak height imbalance, stochastic effects, allele drop in, etc. The dilution series were also amplified and analyzed using the AmpFlSTR Profiler Plus, AmpFlSTR COfiler, AmpFlSTR SGM Plus, and AmpFlSTR Identifiler systems following Applied Biosystems recommended protocol for each in order to compare the performance of the miniSTR system to the performance of the standard kits at the lower DNA quantity levels.

A study was conducted to test the ability of the miniSTR multiplex system to amplify DNA extracted from low copy number casework samples. Eleven DNA samples extracted from bone and tooth samples from forensic casework were supplied by the UNTHSC Missing Persons Laboratory. These samples were extracted and previously analyzed by the Missing Persons lab. Samples were selected that had previously generated incomplete DNA profiles using the AmpFlSTR Profiler Plus and AmpFlSTR COfiler systems. Each bone extract sample was amplified using the miniSTR multiplex system, in triplicate when possible, following both the manufacturer recommended protocol using 30 PCR cycles and the modified protocol using 32 PCR cycles. 10ul of each sample was used in the PCR reaction. For samples that contained less than 10ul sample volume, the entire sample volume was used in the PCR reaction. Genetic profiles were generated using capillary electrophoresis on the ABI 3130xl Genetic Analyzer instrument. The success of the miniSTR system were evaluated by comparing the profiles generated to those obtained with the AmpFlSTR Profiler Plus and AmpFlSTR COfiler systems.

B. Data Collected

The Quantifiler Human DNA Quantification Kit from Applied was used to determine the DNA quantity present in each of the buccal reference samples (PCIA extracts) as well as the Dilution Series stock samples, as well as any other DNA quantifications that are needed in the process of the study. The real-time PCR requirement of the Quantifiler procedure was conducted using the ABI Prism 7000 Sequence Detection System instrument and software. The DNA quantity values generated were used to determine the amount of DNA extract needed to prepare desired dilutions for the Concordance Study and the Dilution Study.

Genetic profiles were detected using the Applied Biosystems 3130xl Genetic Analyzer instrument and the GeneMapper ID v.3.2 software. The genetic profiles are visualized in the form of electropherograms. The GeneMapper ID software uses the signals detected for the Internal Lane Standard and Allelic Ladders to determine the allele size calls for each of the loci amplified by the multiplex system. For each sample, the following parameters were evaluated:

a. Allele size call: Indicates the number of short tandem repeats present in each of the two alleles detected at a genetic locus. Individuals who are homozygous at a particular locus will display a single allele peak, and therefore a single allele size call, for that locus. The allele sizes produced by the miniSTR system were compared to those produced using the standard STR typing kits to insure the reliability of this new system.

b. Peak heights: The height of each allele peak on the electropherogram is measured in Reflective Fluorescence Units (RFUs). These values are typically proportionate to the amount of template DNA added to the PCR reaction and were useful in evaluating the performance of the miniSTR system in the dilution study.

c. Peak height balance of heterozygous loci: A heterozygous locus will display two allele peaks on the electropherogram. The peak heights of each allele within each of these loci should be approximately the same. The ratio of the peak heights (RFU values) of the two alleles at a locus is typically greater than 70%. These ratios were important in evaluating the overall performance of the miniSTR system in each of the three studies.

d. Allelic drop-out: Occurs when one or both of the alleles at a locus are not present in the electropherogram for a particular sample. This could indicate that either the quality or the quantity of input template DNA was insufficient for the amplification system used. The presence or absence of allelic drop out was an important factor in evaluating the performance of the miniSTR system as well as the standard STR multiplex systems in the Dilution Study and the Bone Sample Study. The DNA quantity at which each of the STR multiplex system begins to display allelic drop out was compared to determine whether the miniSTR system was able to successfully amplify lower template DNA quantities as compared to the conventional multiplex systems.

e. Stochastic effects, primer blobs, spikes, pull-up: The electropherograms for each sample was examined for the presence of other features that aid in

determining the efficacy of the miniSTR system. Occurrences of these features could reveal issues with the primer design, multiplexing capabilities, and the amplification and/or analysis parameters used with the system and aid in determining steps that can be taken to further optimize the system.

C. Materials and Methods

Amplification and quantification of samples with of the AmpFlSTR Profiler Plus, AmpFlSTR COfiler, AmpFlSTR SGM Plus, AmpFlSTR Identifiler systems, and Quantifiler Human DNA Quantification Kit from Applied Biosystems were carried out using the manufacturer's recommended protocols. The PCIA extraction method was carried out using the protocol validated by the UNTHSC DNA Identity Laboratory.

Applied Biosystems provided all of the reagents required for the amplification of the miniSTR multiplex system. The components provided by Applied Biosystems are shown in Table 5-1.

Reagent	Contents	Quantity	Storage
MiniSTR 5X Primer Set	Forward and reverse primers to amplify human DNA target	1 tube, 0.55 mL	2 to 8 °C
MiniSTR PCR Reaction Mix (2.5X)	$MgCl_2$, dNTPs, bovine serum albumin, and AmpliTaq Gold DNA polymerase in buffer with 0.05% sodium azide	1 tube, 1.1 mL/tube	2 to 8 °C
MiniSTR Allelic ladder	Allelic ladder containing amplified alleles	1 tube, 25 uL	2 to 8 °C
Female DNA 9947A	0.10 ng/uL human female cell line DNA in 0.05% sodium azide and buffer	1 tube, 0.15 mL	2 to 8 °C

Table 5-1 *The amplification components provided by Applied Biosystems for the miniSTR multiplex system.*

The fluorescent dye used with each primer pair for each locus included in the miniSTR Multiplex System is shown in Table 5-2:

Locus	Dye
D13S317	6-FAM
D7S820	6-FAM
AMELOGENIN	VIC
D2S1338	VIC
D21S11	VIC
D16S539	NED
D18S51	NED
CSF1PO	PET
FGA	PET

Table 5-2 *The fluorescent dye used for the detection of amplification products for each loci contained within the Applied Biosystems miniSTR multiplex system.*

The standard operating procedure for the amplification of samples with the miniSTR PCR Amplification Kit was as follows:

A. PCR Preparation:

1. Thaw the miniSTR reaction mix and primer set, then vortex for 3 seconds, Centrifuge the tubes briefly to remove any liquid from the caps.
2. For each sample to be amplified, combine 10uL of the 2.5X MiniSTR Reaction Mix with 5uL of the MiniSTR 5X Primer Set.
3. Mix thoroughly by vortexing at medium speed for 3 seconds. Centrifuge the tubes briefly to remove any liquid from cap.

4. Dispense 15uL of the PCR master mix into each reaction tube or well.
5. Add 10 uL of sample or control for a total of 1ng of DNA. The final reaction volume should be 25 uL. (The amount of DNA added for Dilution Study will be equal to amount needed to obtain desired DNA quantity, rather than the 1ng total).
6. It is recommended to centrifuge the plate or tubes at 3000 rpm for about 30 seconds in a tabletop centrifuge with plate holders to remove any bubbles.

B. PCR Amplification Conditions:

The parameters used for the GeneAmp PCR System 9700are shown in Table 5-3:

Thermal Cycler	Tube Type	Times and Temperatures for miniSTR kit					
		Initial Incubation Step	Cycle			Final Extension	Final Hold
			Denature	Anneal	Extend		
		HOLD	CYCLE			HOLD	HOLD
Silver 96-well GeneAmp PCR System 9700*	0.2mL MicroAmp Reaction Tubes with Caps	95 °C 11 min.	94 °C 20 sec.	59 °C 1:20 min.	72 °C 1 min.	60 °C 60 min.	4 °C forever

*GeneAmp PCR System 9700 used in 9600 emulation mode.

Table 5-3 *Applied Biosystems recommended amplification protocol utilizes 30 cycles, the alternative protocol utilized 32 cycles*

Electrophoresis Protocol:

Using the ABI PRISM 3130xl Genetic Analyzer using Data Collection software set up the ABI PRISM 3130xl Genetic Analyzer using POP-4 Performance Optimized Polymer and a 3100 Capillary array, 36cm.

Preparation of samples for electrophoresis:

1. Determine the number of sample injections required for analysis. Include one injection of allelic ladder per 16 sample injections in the calculations. Typically two additional injections are added to provide excess volume to account for loss that occurs during reagent transfers. Combine 8.3uL of Hi-Di formamide and 0.7uL of GeneScan-600 LIZ Internal Lane Size Standard per sample injection in a single microcentrifuge tube.
2. Vortex tube to mix, then spin briefly in a microcentrifuge.
3. Dispense 9uL of formamide/GeneScan-600 LIZ solution into each well of a MicroAmp Optical 96-well reaction plate.
4. Add 1.0uL of PCR product or allelic ladder per well.
5. Cover the reaction plate with the appropriate septa.
6. Briefly spin the reaction plate in a centrifuge to ensure that the contents of each well are mixed and collected at the bottom.
7. To denature the DNA, heat the reaction plate in a thermal cycler for 3 minutes at 95°C.
8. Place the reaction plate immediately on ice for 3 minutes.
9. Insert the 96-well plate into the plastic base provided with the instrument, prepare the plate assembly and place on the autosampler.
10. Complete the plate manager spreadsheet for the wells loaded. Select or create an Instrument Protocol wit the appropriate run module (HIDFragmenAnalysis36_POP4) and the correct dye set (G5). The run module for the 3130xl instrument should have an injection voltage of 3 kV and injection time of 10 seconds. Create a results group for storage of .fsa files for later analysis using GeneMapper ID v.3.2 (17).

VI. RESULTS AND DISCUSSION

A. Concordance Study

The results of the concordance study demonstrated that the miniSTR multiplex kit correctly typed all 75 of the reference samples. All allele calls were identical to those obtained using the standard AmpFlSTR typing kits (Profiler Plus, COfiler, SGM Plus, Identifiler). The allele peak heights and peak areas were comparable to those seen using the standard AmpFlSTR typing kits. The heterozygous peak height ratios between alleles at each of the nine loci displayed good balance. The average peak height ratios for each of the 9 loci in the miniSTR multiplex kit are listed in table 6-1.

The peak height ratios were above 70% for all heterozygous loci in both the FTA Blood and FTA buccal reference samples typed with the miniSTR multiplex kit. Due to problems with the Quantifiler System for the quantification of the organic extract reference samples, the quantity of template DNA used in the amplification procedure was significantly lower than the 1ng quantity that was estimated for these samples. Due to the lower template DNA input, the average peak height ratios for the organic extracts were lower than the average peak height ratios for the FTA samples. Out of the 182 heterozygous loci observed in the 25 organic samples, 32 had peak height ratios less than 70%, and an additional six loci had peak height ratios less than 50%. Lower peak height ratios are expected with low template DNA input and the overall average peak height ratios for each locus across all 25 organic samples were all above 70%.

Table 6-1 Average Peak Height Ratios								
FTA Blood Extracts								
D13S317	D7S820	Amelogenin	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
91%	89%	93%	89%	90%	91%	92%	87%	90%
FTA Buccal Extracts								
D13S317	D7S820	Amelogenin	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
91%	93%	95%	89%	93%	93%	90%	86%	90%
Organic Extracts								
D13S317	D7S820	Amelogenin	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
85%	80%	77%	81%	74%	84%	79%	84%	80%

Table 6-1 *Average peak height ratios by locus for each of the three reference sample types amplified with the MiniSTR Multiplex Kit. Allele peak height ratios are calculated by dividing the RFU value of the allele with the lower peak height by the RFU value of the allele with the higher peak height at each heterozygous locus.*

The electropherograms produced for samples amplified with the miniSTR multiplex kit did not display a higher incidence of stutter or stochastic effects than the standard AmpFlSTR typing kits. Figures 6-1 and 6-2 display the electropherograms for FTA Buccal Sample 7 typed with miniSTR and Identifiler. Loci found in both systems are circled in red on Figure 6-2 and demonstrate that both systems resulted in identical allele calls at all loci and that the peak heights and heterozygous peak height ratios were similar for both systems.

There were artifacts observed on the electropherograms produced by the miniSTR multiplex kit. These artifacts were the result of primer impurities and were observed in all samples amplified with the miniSTR multiplex kit. This multiplex system is still in the development stage, and these primer impurities will be eliminated before the kits are released commercially. Artifacts resulting from primer impurities can be observed in the 100-110 base pair region of the VIC dye channel on the electropherograms produced by version 3 of the multiplex; and in the 120-125 base pair range of the 6-FAM dye channel on the electropherograms produced by version 6 of the multiplex. For version 6 of the miniSTR multiplex system, the primer pairs for the D2S1338 and D16S539 loci were modified, resulting in a shift in the electrophoretic mobility of the amplicons produced by these primers. The allelic ladders for the miniSTR multiplex were not modified to account for the new primers, therefore the Genemapper ID software produced allele calls at these loci that were either off ladder or one repeat unit off. For the sensitivity study performed with version 6, the alleles observed at these loci were interpreted by the analysts and reported with the known allele values if they appeared in the correct location

based on the base pair size. For the bone sample study, the alleles at these loci were reported based on the designation assigned by the software since the profiles of the samples were not known.

The recommended protocol provided by Applied Biosystems with the miniSTR multiplex system did not specify the analysis parameters. Initially, a peak detection threshold of 150 RFUs was chosen for the analysis of the Concordance Study reference samples. The results from these samples did not exhibit increased stutter peak heights or an elevated baseline, therefore the peak detection threshold was lowered to 50 RFUs for future studies. At a 50 RFU threshold, additional interpretable allele data was observed in dilution samples containing very small quantities of DNA as well as for the bone samples. Further validation studies will be needed in order to determine the optimal peak detection threshold for use with the final version of the miniSTR kit.

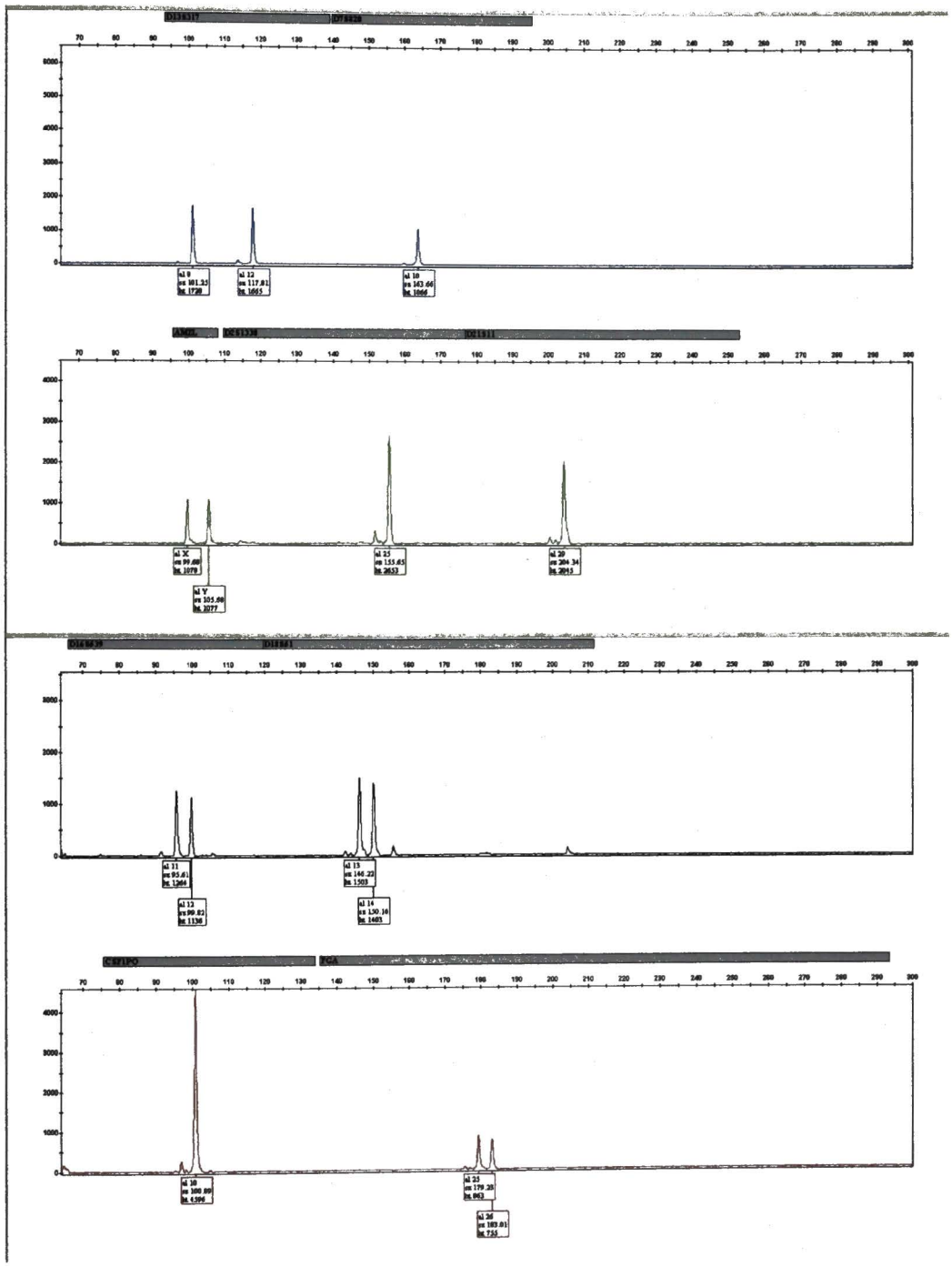

Figure 6-1 *Electropherogram for FTA Buccal Sample 7 amplified with the miniSTR multiplex system version 3.*

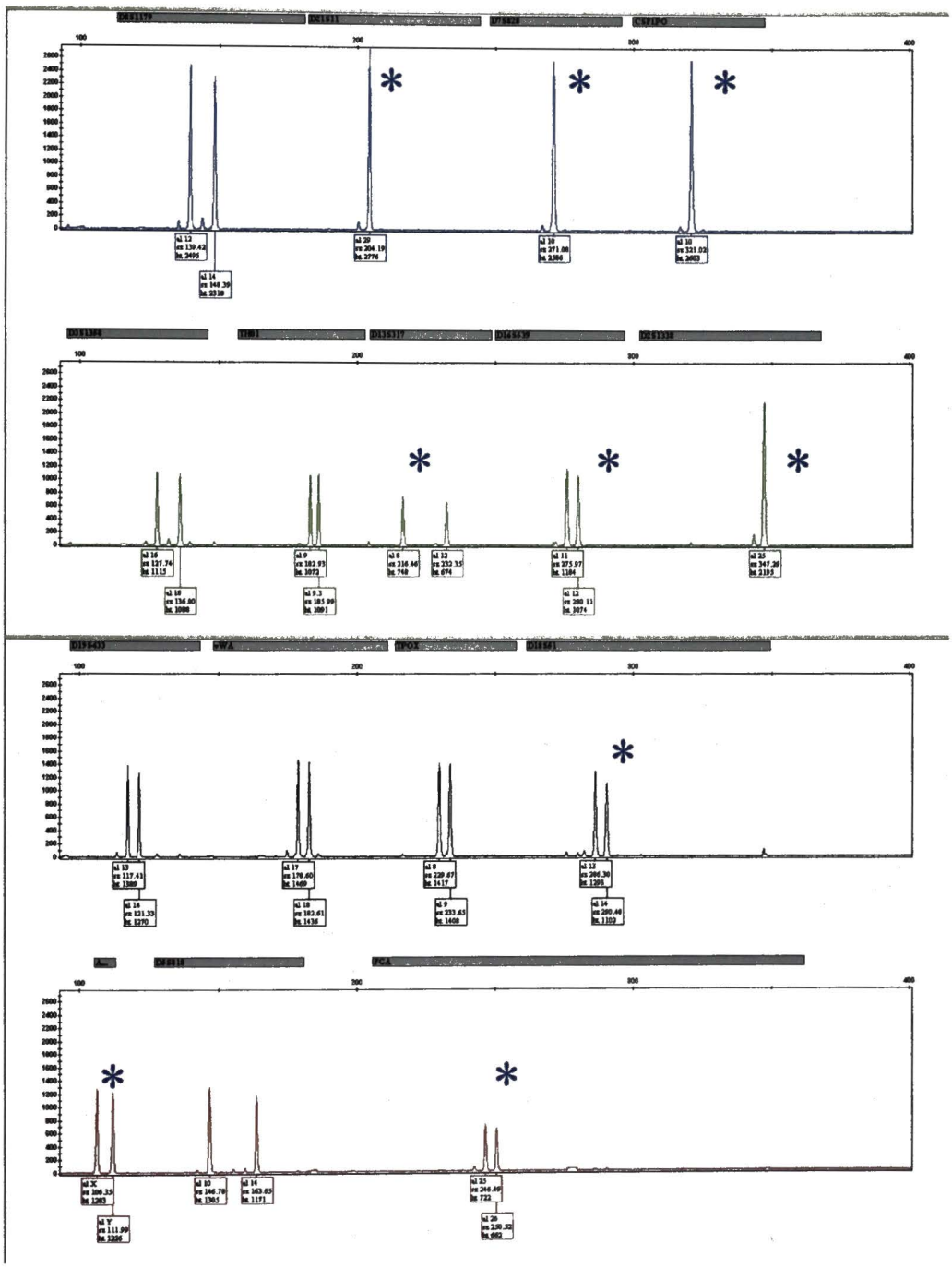

Figure 6-2 *Electropherogram for FTA Buccal Sample 7 amplified with the Identifiler multiplex system. Loci concordant with the miniSTR multiplex system are indicated with a blue asterisk.*

B. Dilution/Sensitivity Study

For the dilution/sensitivity study, serial dilutions were prepared from three DNA samples with known STR profiles. The DNA sample from individual XGA was heterozygous at all nine loci included in the miniSTR multiplex system (Amelogenin, CSF1PO, D13S317, D16S539, D18S51, D21S11, D2S1338, D7S820, and FGA) and therefore contained 18 alleles in the miniSTR profile. The DNA sample from individual XGA also contained 20 alleles for the ten loci included in the Profiler Plus multiplex system (Amelogenin, D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820); 13 alleles for the seven loci included in the COfiler multiplex system (Amelogenin, D3S1358, D16S539, TH01, TPOX, CSF1PO, and D7S820); 22 alleles for the eleven loci included in the SGM Plus multiplex kit (Amelogenin, D3S1358, vWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S488, TH01, and FGA); and 31 alleles for the 16 loci included in the Identifiler multiplex system (Amelogenin, D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S488, vWA, TPOX, D18S51, D5S818, and FGA). DNA sample 9947A was purchased from Promega Corporation and exhibited 15 alleles for the nine loci included in the miniSTR multiplex system, 15 alleles for the ten loci included in the Profiler Plus multiplex system, 12 alleles for the seven loci included in the COfiler multiplex system, 19 alleles for the eleven loci included in the SGM Plus multiplex system, and 26 alleles for the 16 loci included in the Identifiler multiplex system. The DNA sample from individual AJE contained 16 alleles for the nine loci included in the miniSTR multiplex system, 17 alleles for the 10 loci included in the Profiler Plus

multiplex system, 11 alleles for the seven loci included in the COfiler kit, 20 alleles for the eleven loci included in the SGM Plus multiplex system, and 27 alleles for the 16 loci included in the Identifiler multiplex system. A dilution series was prepared for each of the three DNA samples and amplified using each of the typing systems in an attempt determine at which DNA quantity each system would exhibit significant allelic drop out.

In part one of the dilution/sensitivity study, the DNA dilution samples were amplified using the miniSTR multiplex kit version 3 at Applied Biosystems recommendation of 30 PCR cycles. The DNA dilution samples were amplified using the four AmpFlSTR typing kits at the manufacturer's recommendation of 28 PCR cycles. The dilution series samples 0.2ng, 0.1ng, 0.05ng, 0.025ng, and 0.0125ng were initially amplified and analyzed one time using each typing system. After receiving version 6 of the miniSTR multiplex system, the dilution series prepared from DNA samples XGA and 9947A were run in triplicate using the manufacturer recommended 30 PCR cycles, and allele calls were made using the duplication rule. Under the duplication rule, an allele is only called if it appears in at least two of the three replicate profiles for each sample.

The results for part one of the dilution study in which each DNA dilution sample was amplified using each of the five typing systems following Applied Biosystems recommended number of PCR cycles is shown in Table 6-2. The data indicate that the miniSTR multiplex version 3 is more sensitive to lower DNA quantities than are the AmpFlSTR kits. The miniSTR multiplex system version 6 not only detected more alleles at the lower input DNA quantities, but additionally displayed higher allele peak heights and more balanced peaks at heterozygous loci (Figure 6-3 and Figure 6-4). The data also

show that the adjustments made in miniSTR multiplex version 6 increased the sensitivity of the miniSTR system and produced multiple allele calls at the 25pg (Figure 6-5) and 12.5pg DNA quantities as compared to version 3. Detailed results for each typing system used to amplify the dilution series samples following the manufacturer's recommended number of PCR cycles showing the allele calls and peak height values for each DNA dilution quantity are displayed in Tables 6-3, 6-4, 6-5, 6-6, 6-7, and 6-8.

Table 6-2 Numer of Alleles detected by All Systems-30 PCR Cycles						
	miniSTR v.3 30 cycles	**miniSTR v.6 30 cycles**	**Profiler Plus 28 cycles**	**Cofiler 28 cycles**	**SGM Plus 28 cycles**	**Identifiler 28 cylces**
XGA						
0.2 ng	18/18	18/18	20/20	13/13	21/22	30/31
0.1 ng	17/18	18/18	8/20	9/13	13/22	10/31
0.05 ng	10/18	16/18	0/20	1/13	0/22	1/31
0.025 ng	5/18	10/18	1/20	0/13	1/22	0/31
0.0125 ng	0/18	1/18	0/20	1/13	0/22	0/31
9947A						
0.2 ng	15/15	15/15	15/15	12/12	16/19	26/26
0.1 ng	15/15	15/15	15/15	11/12	19/19	25/26
0.05 ng	2/15	15/15	14/15	5/12	6/19	17/26
0.025 ng	0/15	15/15	8/15	5/12	4/19	7/26
0.0125 ng	5/15	12/15	2/15	0/12	1/19	0/26
AJE						
0.2 ng	16/16	N/A	17/17	11/11	19/20	18/27
0.1 ng	16/16	N/A	6/17	9/11	4/20	9/27
0.05 ng	13/16	N/A	1/17	4/11	1/20	1/27
0.025 ng	8/16	N/A	0/17	1/11	1/20	2/27
0.0125 ng	4/16	N/A	1/17	1/11	0/20	0/27
COMBINED						
0.2 ng	49/49 (100%)	33/33 (100%)	52/52 (100%)	36/36 (100%)	56/63 (89%)	74/84 (88%)
0.1 ng	48/49 (98%)	33/33 (100%)	29/52 (56%)	29/36 (81%)	36/63 (57%)	44/84 (52%)
0.05 ng	25/49 (51%)	31/33 (94%)	15/52 (29%)	10/36 (28%)	7/63 (11%)	19/84 (23%)
0.025 ng	13/49 (27%)	25/33 (76%)	9/52 (17%)	6/36 (17%)	6/63 (10%)	9/84 (11%)
0.0125 ng	9/49 (18%)	13/33 (39%)	3/52 (6%)	2/36 (6%)	1/63 (2%)	0/84 (0%)

Table 6-2 *Number of alleles detected divided by the number of known alleles for each typing system at each DNA dilution quantity. The COMBINED portion of the table shows the total number of alleles detected divided by the total number of alleles for all of the DNA extracts typed by each system with percentages of known alleles called.*

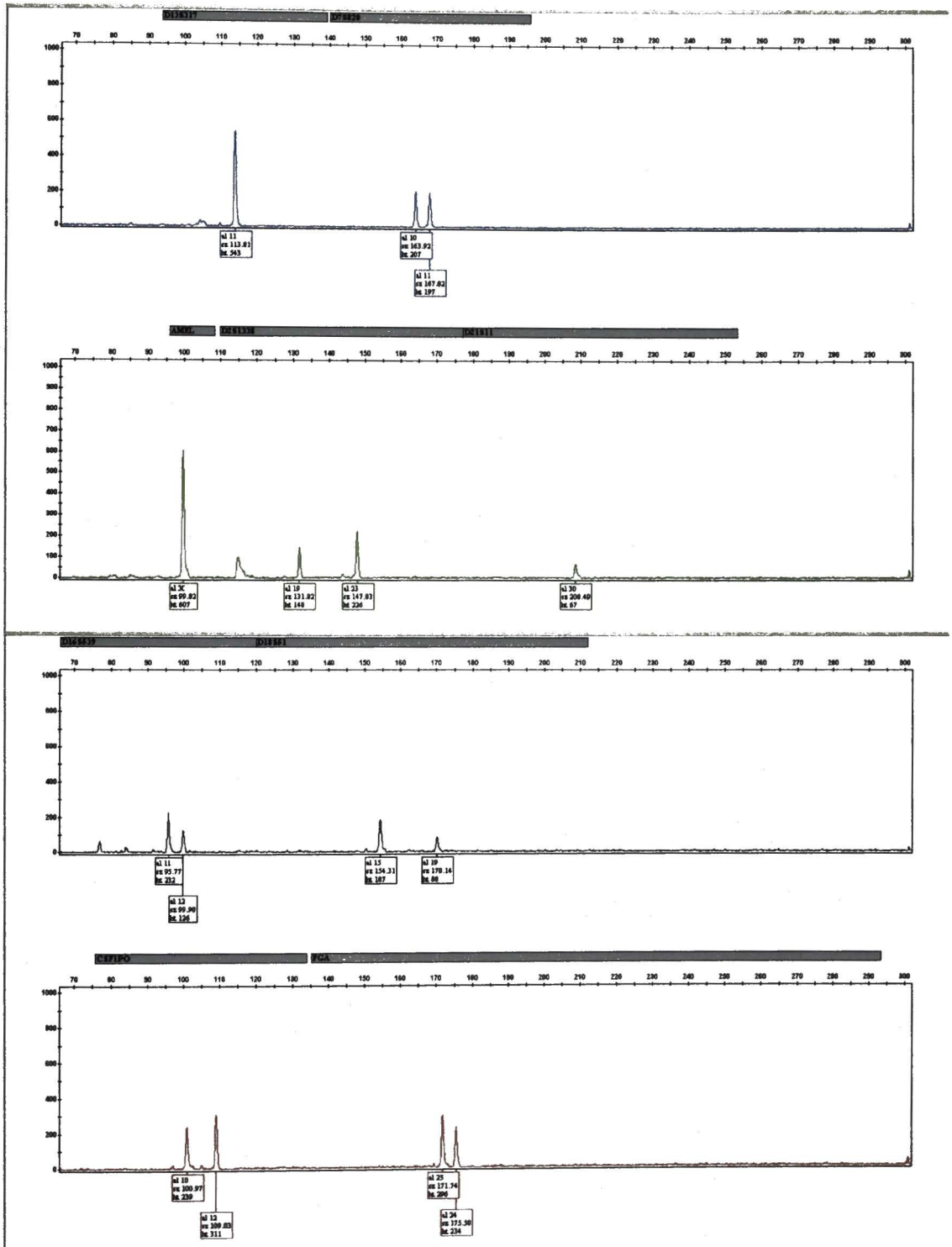

Figure 6-3 *Electropherogram for the 0.1ng dilution of DNA sample 9947A amplified with the miniSTR multiplex version 3 using 30 PCR cycles.*

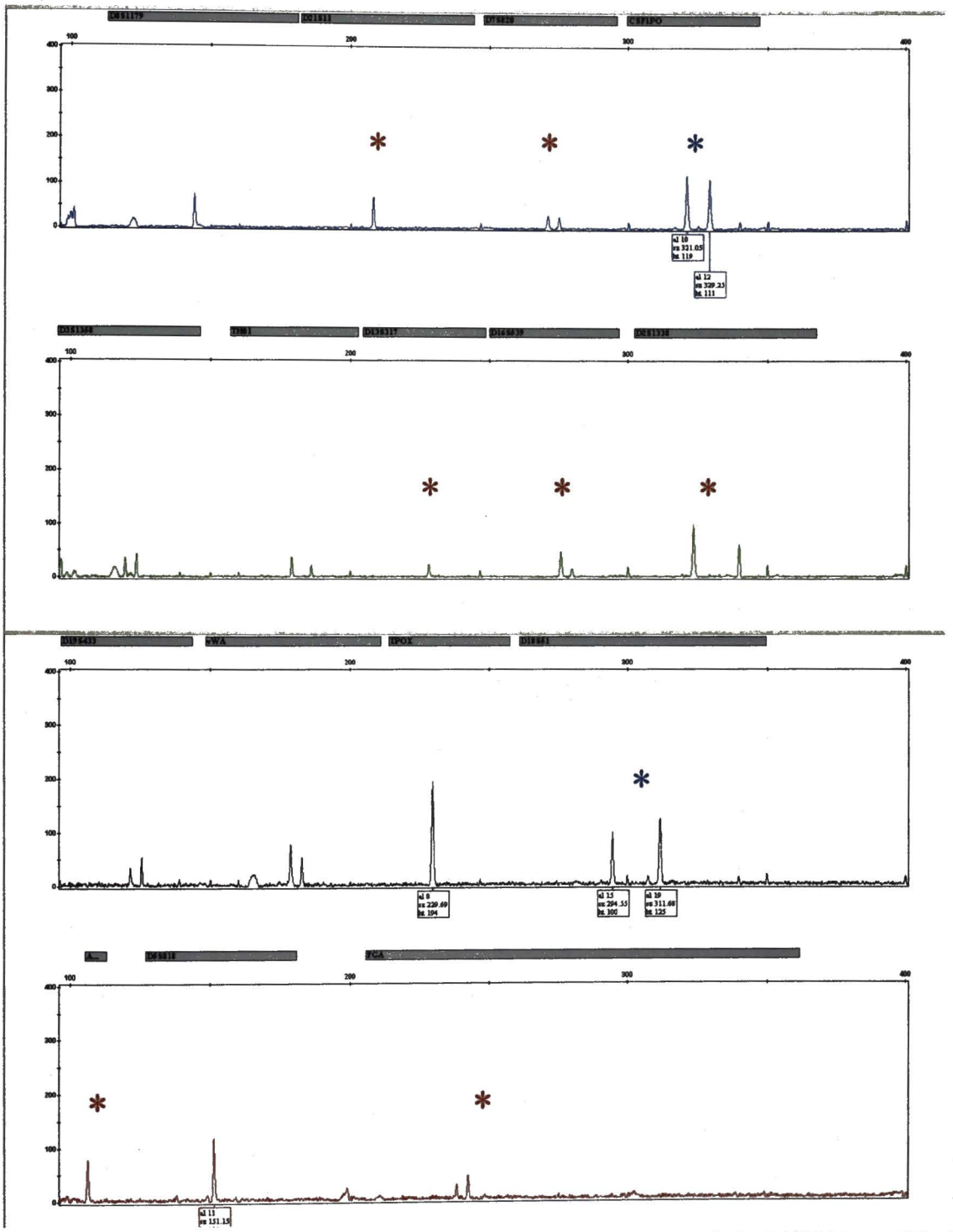

Figure 6-4 *Electropherogram depicting the results from 0.1ng of 9947A DNA amplified with the Identifiler multiplex system at 28 PCR cycles. Loci concordant with the miniSTR system are indicated by an asterisk. Loci with red asterisk exhibited allelic dropout in the Identifiler results but were correctly typed by the miniSTR multiplex v. 3.*

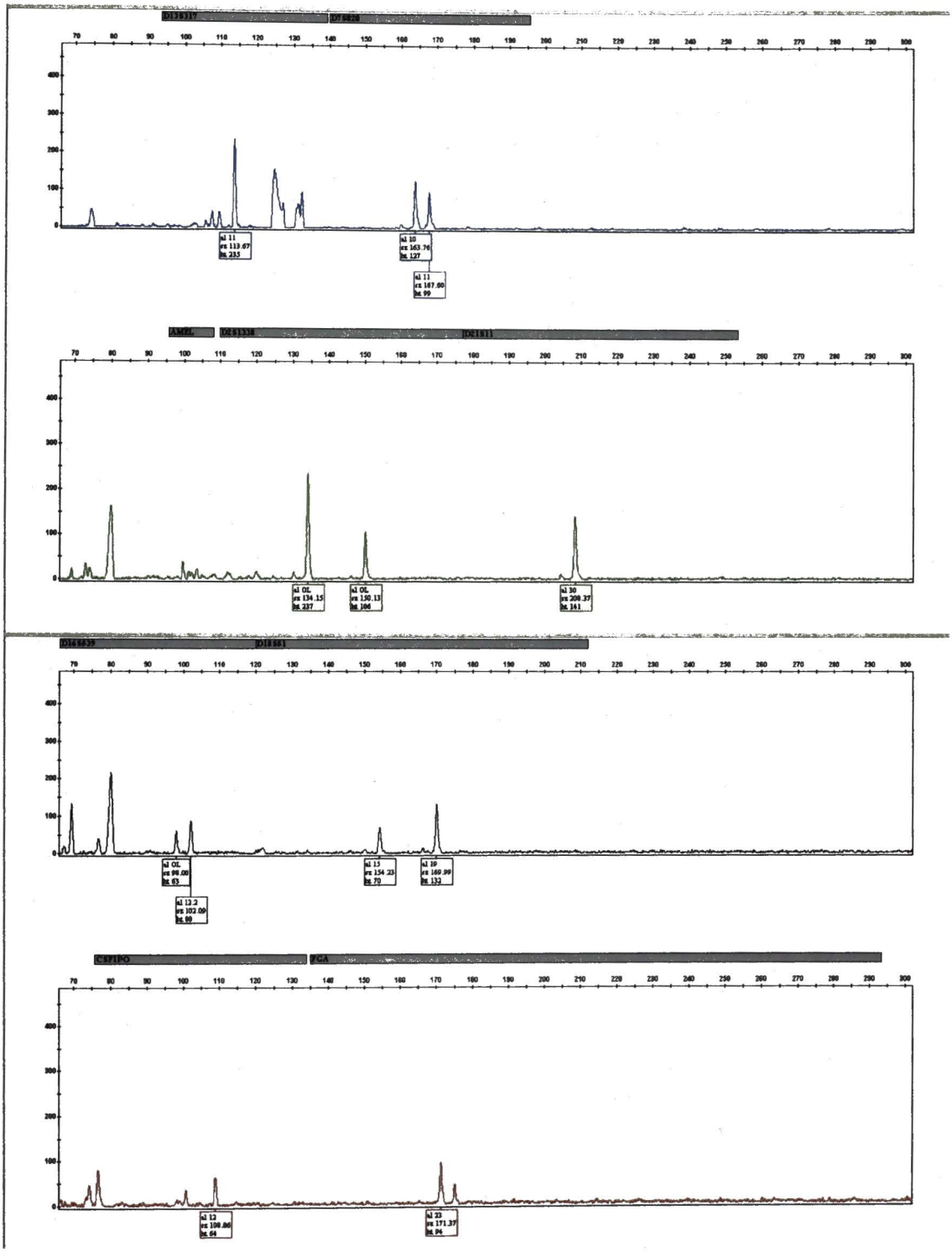

Figure 6-5 *Electropherogram depicting the results from 0.025ng of 9947A DNA amplified with miniSTR multiplex version 6. 12 of the 15 known alleles were observed with the peak detection threshold set at 50 RFUs.*

Table 6-3 Dilution/Sensitivity-MiniSTR Multiplex Kit Version 3-30 PCR Cycles																		
	AMEL		CSF1PO		D13S317		D16S539		D18S51		D21S11		D2S1338		D7S820		FGA	
XGA	X	Y	11	12	9	14	12	13	13	16	29	31	19	20	10	11	20	23
1 ng	7379	5943	6745	6401	7480	7608	4682	4423	6034	6587	5986	5550	5761	7205	7319	6238	4629	4683
0.5 ng	869	631	871	815	1082	1348	870	819	550	620	347	349	533	989	1035	1083	830	435
0.2 ng	407	279	470	425	339	817	273	344	278	222	129	173	477	437	259	491	457	673
0.1 ng	255	331	54	178	570	225	206	144	91	266	58	67	-	152	142	-	188	247
0.05 ng	235	-	-	-	93	65	144	102	-	-	-	-	116	156	123	-	98	105
0.025 ng	-	-	-	82	145	-	69	-	-	-	-	-	55	-	-	-	-	99
0.0125 ng	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AMEL		CSF1PO		D13S317		D16S539		D18S51		D21S11		D2S1338		D7S820		FGA	
9947A	X		10	12	11		11	12	15	19	30		19	23	10	11	23	24
1 ng	1908		1090	1132	2829		1054	1006	929	872	1087		1043	857	884	741	1276	1007
0.5 ng	2344		2442	1900	3254		1497	664	1003	763	1450		973	851	710	720	903	1264
0.2 ng	1196		1034	621	1481		521	525	512	368	594		436	365	362	494	670	532
0.1 ng	607		239	311	543		232	126	187	88	67		148	226	207	197	296	234
0.05 ng	112		-	-	-		-	-	-	-	-		-	-	-	-	-	63
0.025 ng	-		-	-	-		-	-	-	-	-		-	-	-	-	-	-
0.0125 ng	-		-	77	116		-	-	54	-	-		-	-	60	-	72	-
	AMEL		CSF1PO		D13S317		D16S539		D18S51		D21S11		D2S1338		D7S820		FGA	
AJE	X	Y	11		11	12	11	12	13	15	30	31.2	19	20	9		19	22
1 ng	1526	1756	4725		2229	2274	1883	1778	1687	1439	1341	1191	2328	1985	3707		1790	1710
0.5 ng	574	570	1442		729	792	407	642	478	459	219	284	668	763	1023		722	630
0.2 ng	212	337	377		522	777	340	297	231	133	87	65	662	363	542		279	238
0.1 ng	196	148	562		296	618	202	195	101	74	60	79	256	149	310		178	120
0.05 ng	168	75	183		158	114	72	109	75	121	-	-	77	124	211		-	107
0.025 ng	61	-	213		99	-	-	-	78	64	-	-	126	-	104		-	62
0.0125 ng	-	-	-		-	-	74	-	-	-	-	-	-	64	50		-	-
	Peak Height in RFUs																	

Table 6-4 Dilution/Sensitivity-Profiler Plus Kit-30 PCR Cycles																				
	AMEL		D3S1358		vWA		FGA		D8S1179		D21S11		D18S51		D5S818		D13S317		D7S820	
XGA	X	Y	16	17	17	18	20	23	13	14	29	31	13	16	10	11	9	14	10	11
0.2 ng	231	294	350	351	124	255	428	446	228	242	238	281	318	378	298	242	327	157	251	174
0.1 ng	105	184	118	109	114	-	115	149	-	-	-	-	-	-	-	-	163	-	-	-
0.05 ng	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.025 ng	-	-	-	106	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.0125 ng	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AMEL		D3S1358		vWA		FGA		D8S1179		D21S11		D18S51		D5S818		D13S317		D7S820	
9947A	X		14	15	17	18	23	24	13		30		15	19	11		11		10	11
0.2 ng	735		686	612	614	445	820	725	938		995		351	443	794		1076		513	382
0.1 ng	390		325	398	297	196	422	342	411		613		302	277	567		313		195	111
0.05 ng	257		152	223	217	138	259	319	229		271		175	173	218		151		-	113
0.025 ng	-		109	-	174	-	125	-	128		117		154	-	122		103		-	-
0.0125 ng	-		-	-	-	-	-	-	117		-		-	-	108		-		-	-
	AMEL		D3S1358		vWA		FGA		D8S1179		D21S11		D18S51		D5S818		D13S317		D7S820	
AJE	X	Y	15	16	14		19	22	13		30	31.2	13	15	11	12	11	12	9	
0.2 ng	245	148	333	327	385		350	302	324		184	190	246	315	214	155	197	202	477	
0.1 ng	-	-	-	104	176		163	-	237		-	158	-	-	-	-	-	-	133	
0.05 ng	-	-	-	-	-		-	-	-		-	-	-	-	-	-	-	-	109	
0.025 ng	-	-	-	-	-		-	-	-		-	-	-	-	-	-	-	-	-	
0.0125 ng	-	-	-	-	-		105	-	-		-	-	-	-	-	-	-	-	-	
	Peak Height in RFUs																			

Table 6-5 Dilution/Sensitivity-COfiler Kit-30 PCR Cycles													
	AMEL		D3S1358		D16S539		TH01		TPOX	CSF1PO		D7S820	
XGA	X	Y	16	17	12	13	6	9	8	11	12	10	11
0.2 ng	264	196	409	424	401	446	213	184	688	242	255	231	224
0.1 ng	127	180	192	-	116	-	103	116	208	149	123	-	-
0.05 ng	-	-	-	-	-	-	-	-	-	-	-	-	-
0.025 ng	-	-	-	-	-	-	134	-	-	-	-	-	-
0.0125 ng	-	-	-	-	-	-	-	-	-	-	-	-	-
	AMEL		D3S1358		D16S539		TH01		TPOX	CSF1PO		D7S820	
9947A	X		14	15	11	12	8	9.3	8	10	12	10	11
0.2 ng	615		481	400	336	306	224	147	344	209	342	170	212
0.1 ng	787		563	360	421	245	235		591	243	340	399	145
0.05 ng	222		-	217	220	236	-	-	136	-	-	-	-
0.025 ng	-		137	149	-	-	-	-	129	-	113	115	-
0.0125 ng	-		-	-	-	-	-	-	-	-	-	-	-
	AMEL		D3S1358		D16S539		TH01		TPOX	CSF1PO		D7S820	
AJE	X	Y	15	16	11	12	8	9.3	8	11		9	
0.2 ng	335	192	300	308	452	352	154	191	367	457		323	
0.1 ng	-	-	170	142	102	206	262	133	113	174		152	
0.05 ng	-	-	-	107	193	-	-	-	118	179		-	
0.025 ng	-	-	100	-	-	-	-	-	-	-		-	
0.0125 ng	-	-	-	-	-	-	-	-	-	119		-	
	Peak Height in RFUs												

Table 6-6 Dilution/Sensitivity-SGM Plus Kit-30 PCR Cycles

	AMEL		D3S1358		vWA		D16S539		D2S1338		D8S1179		D21S11		D18S51		D19S433		TH01		FGA	
XGA	**X**	**Y**	**16**	**17**	**17**	**18**	**12**	**13**	**19**	**20**	**13**	**14**	**29**	**31**	**13**	**16**	**14**	**15**	**6**	**9**	**20**	**23**
0.2 ng	177	280	238	238	135	187	430	232	192	157	156	256	113	130	162	337	249	241	172	169	-	249
0.1 ng	154	-	-	117	105	-	152	104	125	-	256	-	164	142	275	106	-	115	-	120	-	-
0.05 ng	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.025 ng	-	-	-	-	-	-	-	106	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.0125 ng	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	AMEL		D3S1358		vWA		D16S539		D2S1338		D8S1179		D21S11		D18S51		D19S433		TH01		FGA	
9947A	**X**		**14**	**15**	**17**	**18**	**11**	**12**	**19**	**23**	**13**		**30**		**15**	**19**	**14**	**15**	**8**	**9.3**	**23**	**24**
0.2 ng	393		157	245	199	305	174	323	-	191	361		526		161	217	300	164	146	-	188	-
0.1 ng	475		379	201	320	305	243	214	182	234	337		621		430	237	189	190	134	147	243	250
0.05 ng	178		-	168	-	-	218	-	-	-	-		-		112	-	-	111	-	-	-	100
0.025 ng	261		-	142	-	-	-	-	-	-	227		-		-	143	-	-	-	-	-	-
0.0125 ng	-		-	-	-	-	-	-	-	-	-		116		-	-	-	-	-	-	-	-

	AMEL		D3S1358		vWA		D16S539		D2S1338		D8S1179		D21S11		D18S51		D19S433		TH01		FGA	
AJE	**X**	**Y**	**15**	**16**	**14**		**11**	**12**	**19**	**20**	**13**		**30**	**31.2**	**13**	**15**	**14**	**16**	**8**	**9.3**	**19**	**22**
0.2 ng	211	211	266	333	319		176	398	156	284	220		325	196	264	351	-	134	138	190	144	171
0.1 ng	-	-	-	143	153		-	148	-	-	-		-	-	-	-	-	-	111	-	-	-
0.05 ng	-	-	-	-	-		-	-	-	-	-	-	-	-	126	-	-	-	-	-	-	-
0.025 ng	-	-	-	-	-		-	-	-	-	108	-	-	-	-	-	-	-	-	-	-	-
0.0125 ng	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Peak Height in RFUs

Table 6-7 Dilution/Sensitivity-Identifiler Kit-30 PCR Cycles																
	AMEL		**D8S1179**		**D21S11**		**D7S820**		**CSF1PO**		**D3S1358**		**TH01**		**D13S317**	
XGA	**X**	**Y**	**13**	**14**	**29**	**31**	**10**	**11**	**11**	**12**	**16**	**17**	**6**	**9**	**9**	**14**
0.2 ng	201	131	379	419	228	267	246	496	227	144	257	223	185	217	129	155
0.1 ng	-	-	-	-	-	103	171	-	-	127	104	102	-	-	133	-
0.05 ng	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.025 ng	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.0125 ng	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AMEL		**D8S1179**		**D21S11**		**D7S820**		**CSF1PO**		**D3S1358**		**TH01**		**D13S317**	
9947A	**X**		**13**		**30**		**10**	**11**	**10**	**12**	**14**	**15**	**8**	**9.3**	**11**	
0.2 ng	362		810		558		393	310	540	504	293	145	182	148	262	
0.1 ng	232		627		532		236	125	286	254	118	-	292	129	229	
0.05 ng	127		191		272		104	194	-	103	-	-	-	-	172	
0.025 ng	-		-		-		-	-	145	121	-	-	-	-	-	
0.0125 ng	-		-		-		-	-	-	-	-	-	-	-	-	
	AMEL		**D8S1179**		**D21S11**		**D7S820**		**CSF1PO**		**D3S1358**		**TH01**		**D13S317**	
AJE	**X**	**Y**	**13**		**30**	**31.2**	**9**		**11**		**15**	**16**	**8**	**9.3**	**11**	**12**
0.2 ng	-	-	138		-	-	301		470		-	113	298	233	153	172
0.1 ng	-	-	158		-	116	193		-		-	-	-	203	-	-
0.05 ng	-	-	-		-	-	-		-		-	-	-	-	-	-
0.025 ng	-	-	-		-	-	-		180		-	-	-	-	-	-
0.0125 ng	-	-	-		-	-	-		-		-	-	-	-	-	-
	Peak Height in RFUs															

Table 6-7 Dilution/Sensitivity-Identifiler Kit-30 PCR Cycles															
	D16S539		D2S1338		D19S433		VWA		TPOX	D18S51		D5S818		FGA	
XGA	12	13	19	20	14	15	17	18	8	13	16	10	11	20	23
0.2 ng	216	-	389	192	271	148	120	211	688	298	285	326	190	163	142
0.1 ng	110	120	-	-	-	-	-	-	147	-	-	-	114	-	-
0.05 ng	-	-	-	102	-	-	-	-	-	-	-	-	-	-	-
0.025 ng	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.0125 ng	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	D16S539		D2S1338		D19S433		VWA		TPOX	D18S51		D5S818		FGA	
9947A	11	12	19	23	14	15	17	18	8	15	19	11		23	24
0.2 ng	418	208	149	359	224	193	282	190	490	347	212	370		166	165
0.1 ng	226	269	100	142	187	313	296	171	468	337	429	270		214	213
0.05 ng	109	124	114	-	133	141	209	-	117	177	132	127		-	-
0.025 ng	-	-	114	-	-	-	-	-	199	112	131	117		-	-
0.0125 ng	-	-	-	-	-	-	-	-	-	-	-	-		-	-
	D16S539		D2S1338		D19S433		VWA		TPOX	D18S51		D5S818		FGA	
AJE	11	12	19	20	14	16	14		8	13	15	11	12	19	22
0.2 ng	181	129	181	171	-	-	252		226	139	-	118	-	130	116
0.1 ng	-	-	109	104	-	-	172		136	117	-	-	-	-	-
0.05 ng	-	-	-	-	-	-	-		140	-	-	-	-	-	-
0.025 ng	-	-	-	-	-	-	-		118	-	-	-	-	-	-
0.0125 ng	-	-	-	-	-	-	-		-	-	-	-	-	-	-
Peak Height in RFUs															

Table 6-8 Dilution/Sensitivity-MiniSTR Multiplex Kit Version 6-30 PCR Cycles																	
	AMEL		**CSF1PO**		**D13S317**		**D16S539**		**D18S51**		**D21S11**		**D2S1338**		**D7S820**		**FGA**	
XGA	**X**	**Y**	**11**	**12**	**9**	**14**	**12**	**13**	**13**	**16**	**29**	**31**	**19**	**20**	**10**	**11**	**20**	**23**
0.2 ng	147	152	372	417	1065	1203	324	257	223	278	526	677	852	810	644	479	579	587
0.1 ng	134	104	206	162	363	544	191	185	154	106	355	108	344	381	217	214	319	405
0.05 ng	-	-	136	92	290	196	115	75	111	98	359	187	198	197	96	100	215	119
0.025 ng	-	-	-	92	175	143	73	-	-	67	132	110	80	143	154	-	-	91
0.0125 ng	-	-	-	-	-	-	81	-	-	-	-	57	-	-	-	-	-	-
	AMEL		**CSF1PO**		**D13S317**		**D16S539**		**D18S51**		**D21S11**		**D2S1338**		**D7S820**		**FGA**	
9947A	**X**		**10**	**12**	**11**		**11**	**12**	**15**	**19**	**30**		**19**	**23**	**10**	**11**	**23**	**24**
0.2 ng	711		904	956	4905		855	856	1097	1155	3013		1372	1269	1226	1003	1475	1201
0.1 ng	615		473	476	2330		563	582	522	519	1511		640	635	550	581	909	875
0.05 ng	115		229	152	1232		238	274	158	215	619		291	415	228	157	387	434
0.025 ng	68		74	101	532		131	434	123	158	325		255	141	153	162	168	81
0.0125 ng	59		87	81	312		-	110	106	93	188		88	127	-	-	132	163
	Peak Height in RFUs																	

In part two of the dilution/sensitivity studies, the DNA dilution samples were amplified using the miniSTR multiplex kit version 3 as well as each of the four AmpFlSTR typing kits using a modified amplification protocol of 32 PCR cycles. The 0.2ng, 0.1ng, 0.05ng, 0.025ng, and 0.0125ng quantity samples were each amplified and analyzed one time with each typing system. Results of the dilution series amplified using 32 PCR cycles with each typing system are displayed in table 6-9.

Table 6-9 Number of Alleles detected by All Systems-32 PCR Cycles					
	miniSTR v.3 32 cycles	**Profiler Plus 32cycles**	**Cofiler 32 cycles**	**SGM Plus 32 cycles**	**Identifiler 32 cylces**
XGA					
0.2 ng	18/18	20/20	13/13	22/22	31/31
0.1 ng	17/18	20/20	13/13	22/22	31/31
0.05 ng	18/18	18/20	13/13	20/22	27/31
0.025 ng	13/18	18/20	13/13	19/22	26/31
0.0125 ng	5/18	5/20	5/13	5/22	9/31
PRO-9947A					
0.2 ng	15/15	15/15	12/12	19/19	26/26
0.1 ng	15/15	15/15	12/12	19/19	26/26
0.05 ng	15/15	15/15	12/12	19/19	26/26
0.025 ng	15/15	15/15	11/12	19/19	21/26
0.0125 ng	9/15	14/15	8/12	12/19	21/26
AJE					
0.2 ng	16/16	17/17	11/11	20/20	27/27
0.1 ng	16/16	17/17	11/11	20/20	27/27
0.05 ng	16/16	16/17	10/11	18/20	27/27
0.025 ng	14/16	17/17	9/11	19/20	27/27
0.0125 ng	5/16	12/17	6/11	15/20	17/27
COMBINED					
0.2 ng	49/49 (100%)	52/52 (100%)	36/36 (100%)	63/63 (100%)	84/84 (100%)
0.1 ng	48/49 (98%)	52/52 (100%)	36/36 (100%)	63/63 (100%)	84/84 (100%)
0.05 ng	49/49 (100%)	49/52 (94%)	35/36 (97%)	59/63 (94%)	80/84 (95%)
0.025 ng	42/49 (86%)	50/52 (96%)	33/36 (92%)	59/63 (94%)	74/84 (88%)
0.0125 ng	19/49 (39%)	31/52 (60%)	19/36 (53%)	32/63 (51%)	47/84 (56%)

Table 6-9 *Number of alleles detected divided by the number of known alleles for each typing system at each DNA dilution quantity amplified at 32 PCR cycles. The COMBINED portion of the table shows the total number of alleles detected divided by the total number of alleles for all of the DNA extracts typed by each system with percentages of known alleles detected.*

Results for the part two of the dilution/sensitivity study in which DNA dilution samples were amplified using 32 PCR cycles indicate that the AmpFlSTR multiplex systems were able to detect more alleles at DNA quantities below 50pg than compared to the miniSTR multiplex v.3. However, the results for the Profiler Plus, COfiler, SGM Plus and Identifiler systems also displayed a large amount of stutter peaks, which exceeded 15% in numerous instances. In addition a large number of aberrant alleles were detected, as well as numerous off ladder peaks. The heterozygous loci in these profiles also exhibited significant peak height imbalance. Since the STR profiles of the DNA samples used to prepare the dilution series were known, the presence of spurious alleles and aberrant peak height balance were easily detected. For samples with a previously unknown profile, the presence of stutter peaks, peaks resulting from primer dye impurities occurring within the range of known alleles at one or more of the loci, and imbalanced heterozygous peaks greatly complicated the interpretation of the DNA typing results. The presence of these additional peaks made it difficult to determine whether the sample contained a DNA mixture possibly caused by adventitious transfer or sporadic contamination. Even though the electropherogram data produced by the miniSTR multiplex system did not result in as many allele calls at the lower amounts of input DNA, the results did not exhibit increased stutter peak heights or spurious allele calls. The only alleles interpreted that were not part of the known profile were attributed to artifacts from primer impurities seen in all samples amplified with the miniSTR kits. At 32 PCR cycles the miniSTR multiplex displayed an increased number of imbalanced peaks at heterozygous loci in comparison to the results obtained when 30 PCR cycles

were used. Examples of the electropherograms produced by the miniSTR multiplex and the AmpFlSTR multiplexes can be seen in Figures 6-6 and 6-7. Detailed results for each typing system used to amplify the dilution series samples using 32 PCR cycles are displayed in Tables 6-10, 6-11, 6-12, 6-13, and 6-14.

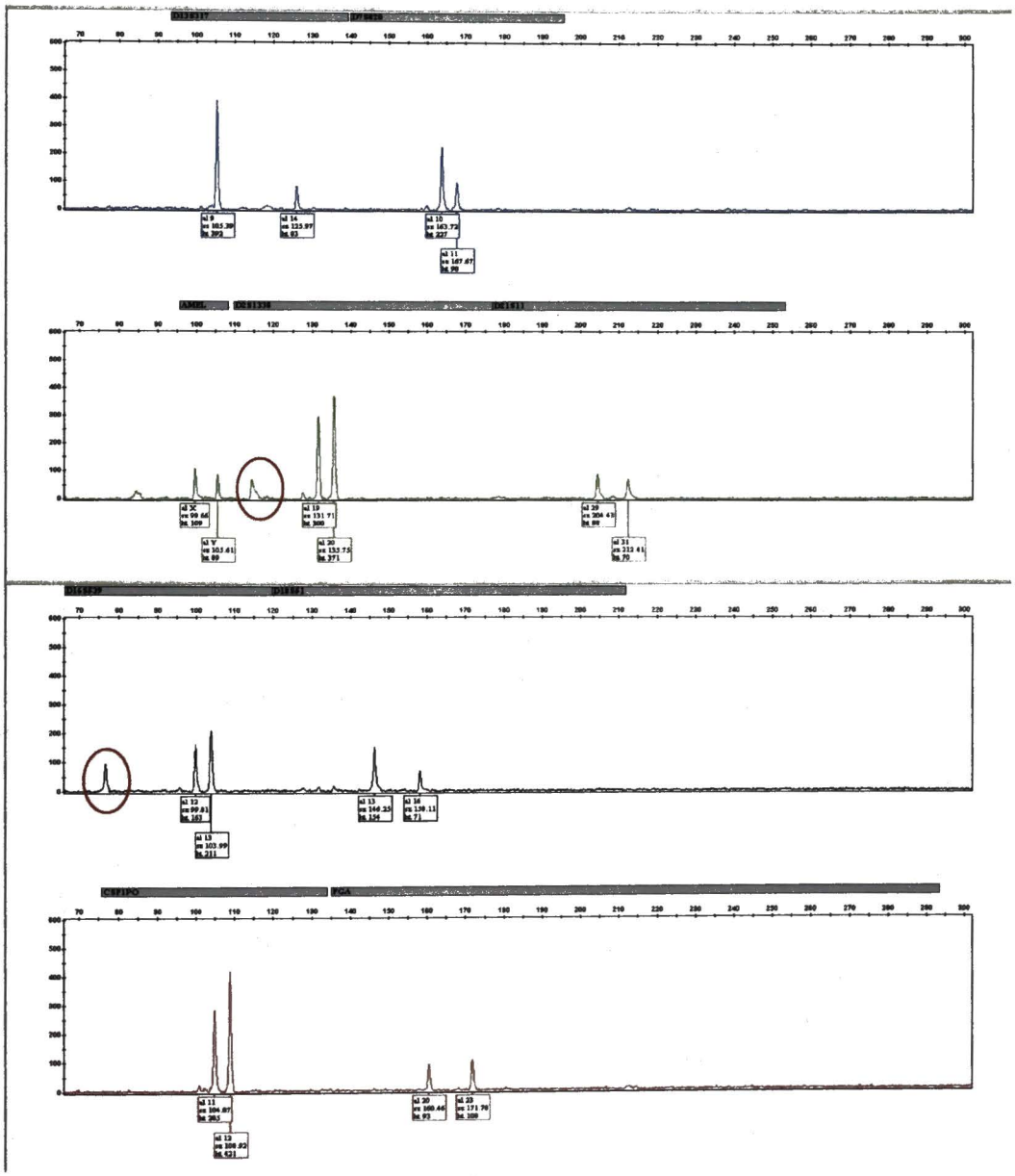

Figure 6-6 *Electropherogram for the 0.05ng of XGA DNA amplified with miniSTR multiplex version 3 at 32 PCR cycles. Peaks circled in red indicate artifacts caused by known primer purification issues.*

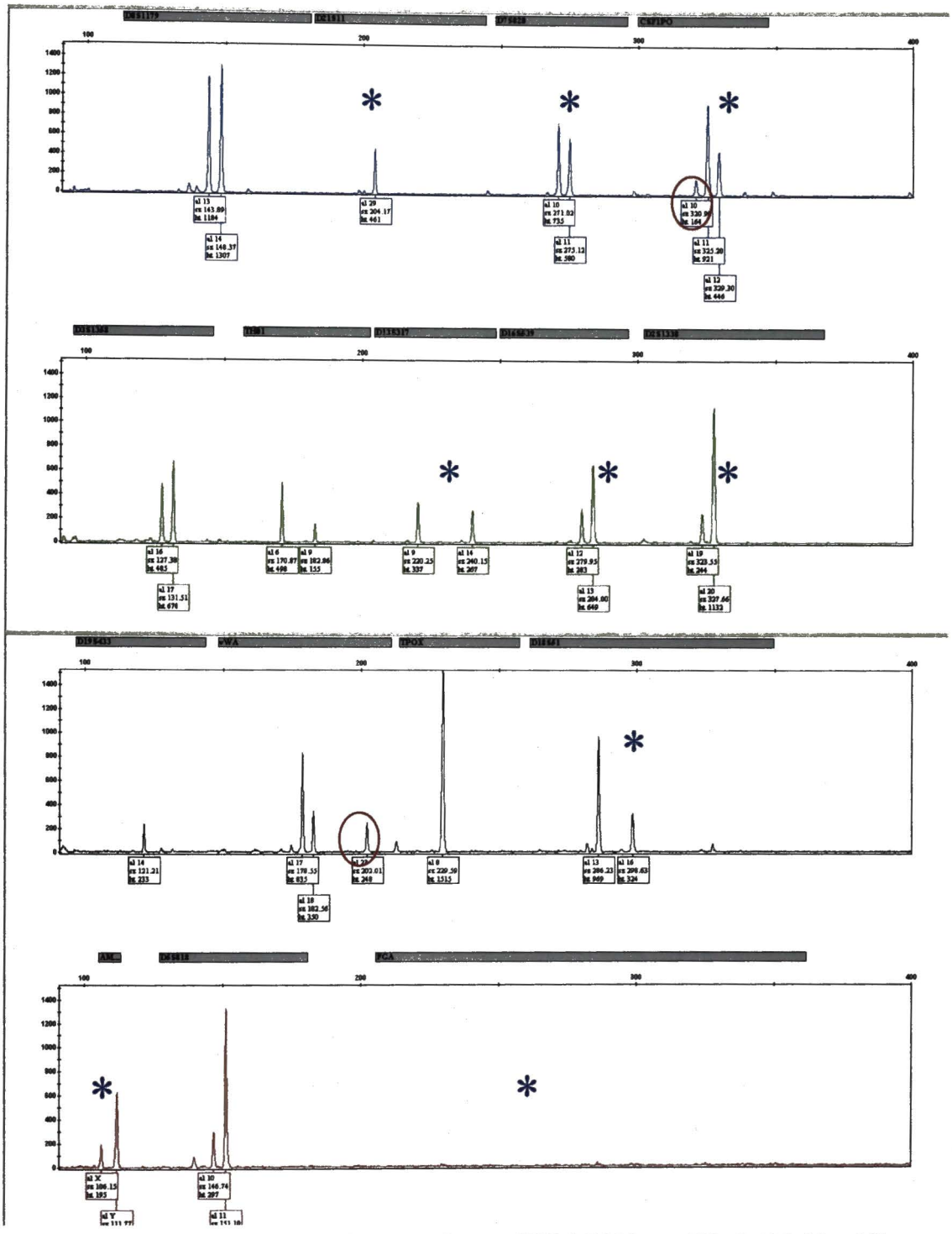

Figure 6-7 *Electropherogram for the 0.05ng of XGA DNA amplified with Identifiler multiplex at 32 PCR cycles. Loci concordant with the miniSTR multiplex system are indicated by an asterisk. Alleles circled in red indicate alleles not present in the known profile of XGA. Also note the significant peak height imbalance seen at some of the heterozygous loci.*

Table 6-10 Dilution/Sensitivity-MiniSTR Multiplex Kit Version 3-32 PCR Cycles																		
	AMEL		**CSF1PO**		**D13S317**		**D16S539**		**D18S51**		**D21S11**		**D2S1338**		**D7S820**		**FGA**	
XGA	**X**	**Y**	**11**	**12**	**9**	**14**	**12**	**13**	**13**	**16**	**29**	**31**	**19**	**20**	**10**	**11**	**20**	**23**
0.2 ng	602	687	2065	2084	1078	831	349	398	797	883	775	248	926	912	520	535	930	657
0.1 ng	274	85	388	204	557	316	404	688	218	214	398	230	614	572	124	256	-	519
0.05 ng	109	89	285	421	392	83	163	211	154	71	88	70	300	371	227	98	93	108
0.025 ng	102	68	307	94	397	57	235		147	143	-	-	240	75	143	-	-	173
0.0125 ng	90	-	-	68	-	-	-	-	-	-	-	-	85	67	-	-	152	-
	AMEL		**CSF1PO**		**D13S317**		**D16S539**		**D18S51**		**D21S11**		**D2S1338**		**D7S820**		**FGA**	
9947A	**X**		**10**	**12**	**11**		**11**	**12**	**15**	**19**	**30**		**19**	**23**	**10**	**11**	**23**	**24**
1 ng	9450		7322	7758	9283		7261	3279	9012	8902	8667		5391	5558	3161	2780	5996	5675
0.5 ng	8858		7545	7839	8998		5534	2922	6421	4439	9183		3308	3979	3397	2969	5464	4697
0.2 ng	4910		5655	4797	7273		2047	2649	1674	2264	5529		1882	1302	1122	1371	2566	1411
0.1 ng	2795		1591	996	3968		1375	1309	926	1195	2157		1212	997	1006	606	1194	1045
0.05 ng	678		920	998	1697		557	620	256	280	394		432	399	172	296	283	184
0.025 ng	559		901	265	883		373	396	201	179	216		224	181	245	321	354	800
0.0125 ng	422		352	67	390		451	103	-	157	484		336	165	213	-	113	81
	AMEL		**CSF1PO**		**D13S317**		**D16S539**		**D18S51**		**D21S11**		**D2S1338**		**D7S820**		**FGA**	
AJE	**X**	**Y**	**11**		**11**	**12**	**11**	**12**	**13**	**15**	**30**	**31.2**	**19**	**20**	**9**		**19**	**22**
0.2 ng	700	576	1890		1191	1342	789	336	942	413	329	192	1105	905	1437		389	688
0.1 ng	601	382	1276		1040	676	382	717	441	478	566	127	799	399	1024		441	478
0.05 ng	216	164	690		65	279	105	155	66	58	263	78	264	75	243		273	275
0.025 ng	350	104	625		364	307	204	-	141	152	94	-	367	220	114		125	103
0.0125 ng	215	-	273		-	-	65	-	-	-	-	-	-	153	-		-	57
	Peak Height in RFUs																	

Table 6-11 Dilution/Sensitivity-Profiler Plus Kit-32 PCR Cycles																				
	AMEL		**D3S1358**		**vWA**		**FGA**		**D8S1179**		**D21S11**		**D18S51**		**D5S818**		**D13S317**		**D7S820**	
XGA	**X**	**Y**	**16**	**17**	**17**	**18**	**20**	**23**	**13**	**14**	**29**	**31**	**13**	**16**	**10**	**11**	**9**	**14**	**10**	**11**
0.2 ng	1767	2456	2995	2395	5925	1449	2032	2696	3188	3183	3207	2221	1313	2322	2202	2040	3623	1948	1218	1673
0.1 ng	628	2311	1116	468	1344	1733	1258	836	1211	843	941	1791	1187	922	1999	1851	467	1546	927	610
0.05 ng	250	500	688	1677	354	269	498	698	-	806	987	601	1116	664	1029	384	811	748	265	-
0.025 ng	497	-	952	939	700	311	1037	587	938	546	405	594	363	1713	319	565	757	909	730	-
0.0125 ng	-	222	-	-	154	205	-	-	-	-	150	-	-	-	-	-	150	-	-	-
	AMEL		**D3S1358**		**vWA**		**FGA**		**D8S1179**		**D21S11**		**D18S51**		**D5S818**		**D13S317**		**D7S820**	
9947A	**X**		**14**	**15**	**17**	**18**	**23**	**24**	**13**		**30**		**15**	**19**	**11**		**11**		**10**	**11**
0.2 ng	7032		4694	3763	4640	5795	3914	3751	6485		7281		2430	1914	7369		7543		2268	2004
0.1 ng	5154		3014	1768	4274	1729	4205	2969	7240		5116		2740	2051	5124		4063		1328	1132
0.05 ng	3079		1106	1170	1749	906	2788	2801	3174		3653		2116	1995	2064		905		673	915
0.025 ng	1730		1197	1187	565	1465	962	1813	1506		2527		1244	1129	2001		2046		645	1022
0.0125 ng	678		373	583	764	516	993	425	983		661		1146	719	1546		489		-	665
	AMEL		**D3S1358**		**vWA**		**FGA**		**D8S1179**		**D21S11**		**D18S51**		**D5S818**		**D13S317**		**D7S820**	
AJE	**X**	**Y**	**15**	**16**	**14**		**19**	**22**	**13**		**30**	**31.2**	**13**	**15**	**11**	**12**	**11**	**12**	**9**	
0.2 ng	1953	2580	2553	2401	4466		3319	2465	5343		2139	2143	2980	1061	2859	1331	1390	2694	2811	
0.1 ng	861	1195	1493	746	2294		545	1158	2185		1212	1188	743	646	1439	737	570	1190	1035	
0.05 ng	630	665	314	364	1641		935	864	875		408	574	749	928	734	825	932	-	714	
0.025 ng	180	240	483	1074	351		1500	1971	1216		592	174	873	265	631	257	1347	514	708	
0.0125 ng	-	444	215		1389		1043	477	672		346	345	401	-	589	-	-	679	435	
	Peak Height in RFUs																			

Table 6-12 Dilution/Sensitivity-Cofiler Kit-32 PCR Cycles													
	AMEL		D3S1358		D16S539		TH01		TPOX	CSF1PO		D7S820	
XGA	X	Y	16	17	12	13	6	9	8	11	12	10	11
0.2 ng	3691	1790	2109	3717	4844	3220	2395	4119	4939	2022	2613	2217	834
0.1 ng	914	1105	2583	1579	1577	2314	2176	882	2803	950	628	827	658
0.05 ng	847	589	222	1343	803	1132	434	196	956	655	276	246	228
0.025 ng	339	352	448	684	766	279	648	698	716	741	418	194	199
0.0125 ng	205	182		162					360		223		
	AMEL		D3S1358		D16S539		TH01		TPOX	CSF1PO		D7S820	
9947A	X		14	15	11	12	8	9.3	8	10	12	10	11
0.2 ng	6714		7197	4851	7379	6966	4277	3255	7165	6952	5084	3558	3079
0.1 ng	5486		5395	4069	4305	5641	2382	2034	5009	2055	3574	2626	2015
0.05 ng	4050		1843	2138	1494	2342	678	392	1761	1581	684	1603	774
0.025 ng	1510		2082	1739	3119	593	610		1653	882	918	474	266
0.0125 ng	680				625	378	210		766	493		686	871
	AMEL		D3S1358		D16S539		TH01		TPOX	CSF1PO		D7S820	
AJE	X	Y	15	16	11	12	8	9.3	8	11		9	
0.2 ng	4110	1790	4726	2296	3449	3546	3311	1638	4678	5556		3604	
0.1 ng	1952	1151	1972	601	1098	890	2686	1108	2174	2285		1951	
0.05 ng	1080		3096	1844	1280	1781	731	2080	770	1452		1725	
0.025 ng	210	297	1356	587	1379		499		362	819		1018	
0.0125 ng			334	665	1114			1034		953		868	
	Peak Height in RFUs												

Table 6-13 Dilution/Sensitivity-SGM Plus Kit-32 PCR Cycles																				
	AMEL		D3S1358		vWA		D16S539		D2S1338		D8S1179		D21S11		D18S51		D19S433		TH01		FGA	
XGA	X	Y	16	17	17	18	12	13	19	20	13	14	29	31	13	16	14	15	6	9	20	23
0.2 ng	3348	1700	3086	2190	2798	1996	1849	2861	1755	1003	1665	2287	2624	2674	955	1178	1476	1559	1941	1544	2431	1424
0.1 ng	1161	1398	1482	1803	1642	752	1589	629	375	1024	2381	1267	1801	247	977	821	1893	1156	621	1210	511	952
0.05 ng	1159	301	985	1106	446	695	1090	964	1242	-	768	417	895	371	1145	-	993	795	1028	725	581	463
0.025 ng	432	458	677	163	475	460	396	-	511	177	-	556	-	378	894	466	446	340	412	176	218	447
0.0125 ng	-	-	-	-	-	-	-	-	-	248	-	-	-	427	209	796	-	-	-	-	-	252
	AMEL		D3S1358		vWA		D16S539		D2S1338		D8S1179		D21S11		D18S51		D19S433		TH01		FGA	
9947A	X		14	15	17	18	11	12	19	23	13		30		15	19	14	15	8	9.3	23	24
0.2 ng	6528		5991	6761	5519	5512	7401	6750	3941	2257	6619		7333		4490	4503	3978	4806	4988	3080	3636	2531
0.1 ng	7168		2831	5073	4530	2972	3555	2899	1599	1805	3902		6595		2816	2937	2741	1859	1533	1132	2900	2270
0.05 ng	3583		2372	3006	1917	1988	2233	1952	506	1260	1631		2379		733	1100	1252	949	722	841	1399	773
0.025 ng	1397		581	360	1233	817	1460	1275	198	548	2721		2057		1116	1046	1049	1168	450	981	753	1461
0.0125 ng	959		598	329	912	-	176	-	-	-	1730		274		159	329	300	253	-	-	444	-
	AMEL		D3S1358		vWA		D16S539		D2S1338		D8S1179		D21S11		D18S51		D19S433		TH01		FGA	
AJE	X	Y	15	16	14		11	12	19	20	13		30	31.2	13	15	14	16	8	9.3	19	22
0.2 ng	2513	2171	2803	1637	3417		2219	2429	1362	1629	4561		1529	1361	1516	1116	1500	2045	845	859	980	1992
0.1 ng	652	1085	940	2352	963		1269	1815	2027	1219	2501		307	1055	1198	1268	1597	791	1437	1270	1092	439
0.05 ng	349	160	1376	-	2029		1248	859	788	155	1273		576	326	1422	1425	463	480	881	238	-	806
0.025 ng	299	465	786	491	702		197	401	1228	333	650		687	687	570	955	742	191	-	399	1064	170
0.0125 ng	-	527	704	832	1025		680	1016	415	-	475		524	990	220	300	-	-	-	435	493	260
	Peak Height in RFUs																					

Table 6-14a Dilution/Sensitivity-Identifiler-32 PCR Cycles																
	AMEL		**D8S1179**		**D21S11**		**D7S820**		**CSF1PO**		**D3S1358**		**TH01**		**D13S317**	
XGA	**X**	**Y**	**13**	**14**	**29**	**31**	**10**	**11**	**11**	**12**	**16**	**17**	**6**	**9**	**9**	**14**
0.2 ng	2482	2791	3580	3202	2692	4161	3880	1869	1976	1207	1613	1857	2404	4109	1136	1553
0.1 ng	593	1304	2772	2024	1645	1363	746	2036	2347	2471	2304	760	3339	2015	911	2322
0.05 ng	195	636	1184	1307	461	-	735	580	921	446	485	678	498	155	337	267
0.025 ng	253	835	411	552	610	731	573	184	935	611	361	547	1549	235	221	249
0.0125 ng	-	200	418	497	160	-	-	-	399	-	-	-	-	388	-	388
	AMEL		**D8S1179**		**D21S11**		**D7S820**		**CSF1PO**		**D3S1358**		**TH01**		**D13S317**	
9947A	**X**		**13**		**30**		**10**	**11**	**10**	**12**	**14**	**15**	**8**	**9.3**	**11**	
0.2 ng	7271		9216		6548		2193	2234	3026	3612	3109	2611	2303	3180	3979	
0.1 ng	5048		7686		2630		1452	888	2516	1218	3888	2500	1046	942	1693	
0.05 ng	1596		4810		3190		943	1911	1293	1315	846	610	962	929	2348	
0.025 ng	907		413		1220		-	595	-	-	368	510	-	587	553	
0.0125 ng	466		657		201		-	394	279	152	-	347	222	280	-	
	AMEL		**D8S1179**		**D21S11**		**D7S820**		**CSF1PO**		**D3S1358**		**TH01**		**D13S317**	
AJE	**X**	**Y**	**13**		**30**	**31.2**	**9**		**11**		**15**	**16**	**8**	**9.3**	**11**	**12**
0.2 ng	1219	2004	6573		1365	2489	5404		4562		1499	878	3142	2268	1483	1523
0.1 ng	1017	721	2357		660	1140	1450		2763		1223	363	1838	875	704	871
0.05 ng	250	358	3241		231	401	1949		1125		426	666	631	834	400	150
0.025 ng	500	348	1352		328	153	978		777		284	493	745	409	175	178
0.0125 ng	242	-	1830		-	-	800		970		-	-	-	865	376	647
	Peak Height in RFUs															

Table 6-14b Dilution/Sensitivity-Identifiler-32 PCR Cycles																
	D16S539		**D2S1338**		**D19S433**		**VWA**		**TPOX**	**D18S51**		**D5S818**		**FGA**	
XGA	**12**	**13**	**19**	**20**	**14**	**15**	**17**	**18**	**8**	**13**	**16**	**10**	**11**	**20**	**23**
0.2 ng	2093	1847	2501	3216	2622	1759	1802	2549	6402	2284	2512	3077	3414	901	2215
0.1 ng	1733	1901	3110	592	1872	1677	1709	1363	2465	1508	829	1156	2168	1449	757
0.05 ng	283	649	244	1132	233	-	835	350	1515	969	324	297	1329	-	-
0.025 ng	227	235	192	-	337	-	397	-	1045	-	164	664	495	-	425
0.0125 ng	-	-	-	399	-	-	-	-	-	338	-	-	-	-	-
	D16S539		**D2S1338**		**D19S433**		**VWA**		**TPOX**	**D18S51**		**D5S818**		**FGA**	
9947A	**11**	**12**	**19**	**23**	**14**	**15**	**17**	**18**	**8**	**15**	**19**	**11**		**23**	**24**
0.2 ng	2479	2147	2612	1817	3951	4038	3715	4178	8594	2823	1821	5882		1923	1453
0.1 ng	1186	1780	709	1270	2626	2077	1719	1093	3092	1420	1005	5298		840	1076
0.05 ng	583	1409	1326	721	1165	1132	869	584	2416	1635	817	1339		426	428
0.025 ng	477	490	617	540	1014	731	417	171	1478	466	476	820		-	308
0.0125 ng	179	364	-	201	756	271	390	237	496	351	618	643		-	270
	D16S539		**D2S1338**		**D19S433**		**VWA**		**TPOX**	**D18S51**		**D5S818**		**FGA**	
AJE	**11**	**12**	**19**	**20**	**14**	**16**	**14**		**8**	**13**	**15**	**11**	**12**	**19**	**22**
0.2 ng	358	1525	1286	1265	2067	2457	5048		5174	1270	1579	4619	2606	1685	1168
0.1 ng	352	322	1884	815	1237	1214	1069		1751	241	846	742	584	478	554
0.05 ng	844	767	837	340	1129	247	1358		2451	703	1435	488	574	329	716
0.025 ng	171	948	476	1562	421	499	1238		493	267	562	388	447	416	415
0.0125 ng	-	469	-	352	266	266	634		158	758	-	230	412	575	-
	Peak Height in RFUs														

The DNA dilution samples used for studies with version 3 of the miniSTR kit had been consumed. Therefore, new serial dilutions were prepared from the DNA sample from individual XGA as well as from control DNA 9948 purchased from Promega Corporation for additional studies. These new dilution samples in DNA quantities of 1ng, 0.25ng, 0.125ng, 0.063ng, 0.032ng, and 0.016ng were amplified one time using version 6 of the miniSTR multiplex system at 30 PCR cycles as well as one time at 32 PCR cycles. Results indicate that version 6 of the miniSTR multiplex was more sensitive with low amounts of input DNA when both 30 and 32 PCR cycles are used. There were only slightly more alleles observed when 32 PCR cycles were used as when 30 PCR cycles were used. When 30 PCR cycles were used, full profiles were obtained at 63pg of input DNA for both of the new dilution samples., The electropherograms generated were easily interpreted with no aberrant stutter peaks or spurious allele calls other than those due to artifacts caused by known primer impurities. When 32 PCR cycles were used, there were slightly more interpretable alleles, and a full profile was observed for sample 9948 at 16pg of input DNA. The electropherograms for the new dilution samples amplified at 32 PCR cycles with version 6 of the miniSTR exhibited a large amount of stutter and aberrant allele calls, making the interpretation of the results for DNA quantities above 63pg difficult. Overall, the results from the dilution series samples amplified at 30 PCR cycles provided clean profiles that were easier to interpret. Results of the new dilution series amplified using the miniSTR multiplex system at 30 and 32 PCR cycles are displayed in tables 6-15, 6-16, and 6-17.

Table 6-15 Number of Alleles detected by MiniSTR Version 6-30 PCR Cycles		
	30 cycles	**32 cycles**
XGA		
0.25 ng	18/18	18/18
0.125 ng	18/18	18/18
0.063 ng	18/18	18/18
0.032ng	15/18	16/18
0.016 ng	11/18	12/18
9948		
0.25 ng	15/15	15/15
0.125 ng	15/15	15/15
0.063 ng	15/15	14/15
0.032ng	14/15	15/15
0.016 ng	15/15	15/15
COMBINED		
0.25 ng	33/33 (100%)	33/33 (100%)
0.125 ng	33/33 (100%)	33/33 (100%)
0.063 ng	33/33 (100%)	32/33 (97%)
0.032ng	29/33 (88%)	31/33 (94%)
0.016 ng	26/33 (79%)	27/33 (82%)

Table 6-15 *The number of alleles detected divided by the number of known alleles at each DNA dilution quantity amplified at 30 and 32 PCR cycles with miniSTR multiplex system v.6. The COMBINED portion of the table shows the total number of alleles detected divided by the total number of alleles for all of the DNA extracts typed by each system with percentages of known alleles detected.*

Table 6-16 Dilution/Sensitivity-MiniSTR Multiplex Kit Version 6-30 PCR Cycles																		
	AMEL		**CSF1PO**		**D13S317**		**D16S539**		**D18S51**		**D21S11**		**D2S1338**		**D7S820**		**FGA**	
XGA	**X**	**Y**	**11**	**12**	**9**	**14**	**13**	**14**	**13**	**16**	**29**	**31**	**19**	**20**	**10**	**11**	**20**	**23**
1 ng	1945	1682	3177	3565	6888	6537	3579	3668	2770	3070	6685	4917	6512	6044	4176	4519	4806	4860
0.25 ng	520	677	756	847	2243	1087	1351	1260	842	695	1241	1251	1360	1202	1170	1001	1026	777
0.125 ng	244	224	362	339	841	999	644	741	261	334	753	418	745	480	426	652	637	613
0.063 ng	165	215	598	316	472	1101	665	422	294	446	757	685	809	774	860	508	562	403
0.032ng	-	-	86	-	169	299	55	82	75	257	366	213	115	207	166	182	225	94
0.016 ng	-	-	-	73	109	175	141	125	50	70	-	122	-	136	-	-	101	154
	AMEL		**CSF1PO**		**D13S317**		**D16S539**		**D18S51**		**D21S11**		**D2S1338**		**D7S820**		**FGA**	
9948	**X**	**Y**	**10**	**11**	**11**		**12**		**15**	**18**	**29**	**30**	**23**		**11**		**24**	**26**
1 ng	2401	1899	4430	4388	9421		8261		4801	4177	7640	7663	7942		8257		5308	4979
0.25 ng	514	526	1091	941	4462		2006		937	900	2251	1543	2062		2197		2161	1432
0.125 ng	360	300	479	667	2109		972		407	553	827	895	1505		1021		633	619
0.063 ng	298	163	527	314	2326		922		278	403	1203	741	1102		754		762	678
0.032ng	-	81	169	341	580		328		57	147	516	411	247		244		99	187
0.016 ng	72	90	209	80	307		216		94	64	197	122	60		136		87	88
	Peak Height in RFUs																	

Table 6-17 Dilution/Sensitivity-MiniSTR Multiplex Kit Version 6-32 PCR Cycles																		
	AMEL		**CSF1PO**		**D13S317**		**D16S539**		**D18S51**		**D21S11**		**D2S1338**		**D7S820**		**FGA**	
XGA	**X**	**Y**	**11**	**12**	**9**	**14**	**13**	**14**	**13**	**16**	**29**	**31**	**19**	**20**	**10**	**11**	**20**	**23**
1 ng	3255	3445	5517	4839	8416	7994	4734	4009	7019	6007	8695	7574	7984	8009	7798	6406	6798	5326
0.25 ng	565	848	1858	1137	4733	3481	2354	1691	1809	2063	3159	2953	3162	3831	3627	1743	3222	2910
0.125 ng	404	562	853	930	2464	1006	908	974	1689	887	2034	2936	1887	2244	1030	1457	1151	1874
0.063 ng	331	281	650	352	2315	1306	890	983	516	1028	1414	1414	1184	912	652	1012	961	660
0.032ng	210	151	248	-	764	-	218	736	174	169	290	327	1026	1253	1082	521	810	609
0.016 ng	152	169	239	-	-	-	224	252	461	65	440	401	-	-	191	155	125	-
	AMEL		**CSF1PO**		**D13S317**		**D16S539**		**D18S51**		**D21S11**		**D2S1338**		**D7S820**		**FGA**	
9948	**X**	**Y**	**10**	**11**	**11**		**12**		**15**	**18**	**29**	**30**	**23**		**11**		**24**	**26**
1 ng	5630	5210	7035	6344	8759		7839		8087	8409	8959	9105	8849		9039		7098	7295
0.25 ng	1368	1322	4264	3383	9240		7520		3080	2872	7587	7133	7778		6536		5169	4520
0.125 ng	1167	615	2808	1911	7257		6944		1446	1851	2730	3739	5579		5574		3025	2581
0.063 ng	271	269	708	859	3326		2414		1168	741	2445	-	3334		2137		1468	1757
0.032ng	114	201	685	218	1559		488		201	149	693	368	1296		966		829	687
0.016 ng	91	63	369	393	213		387		441	283	619	226	301		293		302	537
	Peak Height in RFUs																	

C. Bone Sample Study

DNA extracts from two bone samples and one tooth sample were provided by the Missing Persons Laboratory at UNTHSC. Each sample was typed using the miniSTR multiplex system version 3 at 30 PCR cycles. Each of these samples had been previously typed using Profiler Plus and COfiler at 32 PCR cycles by the Missing Persons Laboratory.

For bone sample F2442.1, Profiler Plus/COfiler typing obtained alleles at seven of fourteen loci for this sample, with drop out of the Amelogenin, D13, D7, D18, FGA, D8, and D5 loci. Amplification with the miniSTR multiplex kit v.3 produced a partial profile with alleles obtained at eight of the nine loci, providing alleles at five additional loci not obtained in the profiles from Profiler Plus and COfiler (including the D2S138 locus).

For bone sample F2719.2BC, Profiler Plus/COfiler typing obtained alleles at seven of fourteen loci for this sample, with drop out of D21, D13, D7, D16, CSF, TH01, and TPOX loci. Amplification with the miniSTR multiplex kit v.3 obtained alleles at all nine loci (Figure 6-8), providing alleles at six additional loci not obtained in the profiles from Profiler Plus and COfiler (including the D2S138 locus).

For tooth sample F2908.3ABC, Profiler Plus/COfiler typing produced a partial profile with alleles observed at seven of the 14 loci, with drop out of the D7, D21, D16, D18, CSF1PO, TPOX, and TH01 loci. This sample contained a sufficient volume to perform amplification with the miniSTR multiplex kit v.3 at both 30 and 32 PCR cycles. Amplification at 30 PCR cycles resulted in observation of only four alleles (Figure 6-9). Amplification at 32 PCR cycles produced a partial profile with alleles obtained at six of

the nine loci (Figure 6-10). There were three alleles observed at the D18S51 locus. Amplification with the miniSTR system at 32 PCR cycles provided alleles at four additional loci not obtained in the profiles from Profiler Plus and COfiler.

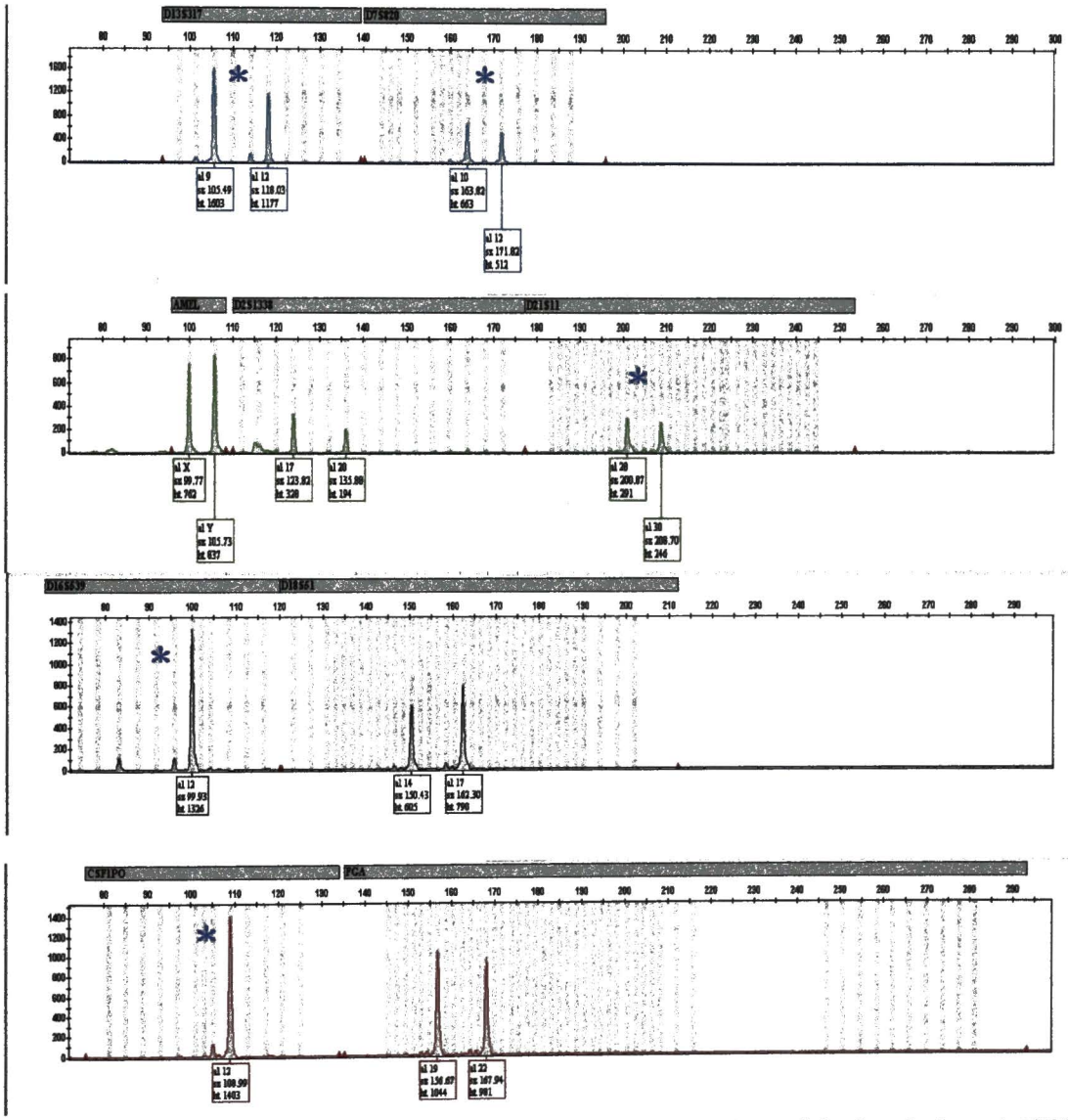

Figure 6-8 *Electropherogram for bone sample F2719.2BC amplified with the miniSTR multiplex system version 3 at 30 PCR cycles. Alleles were not observed at loci designated with an asterisk when the sample was amplified with the Profiler Plus and COfiler multiplex systems.*

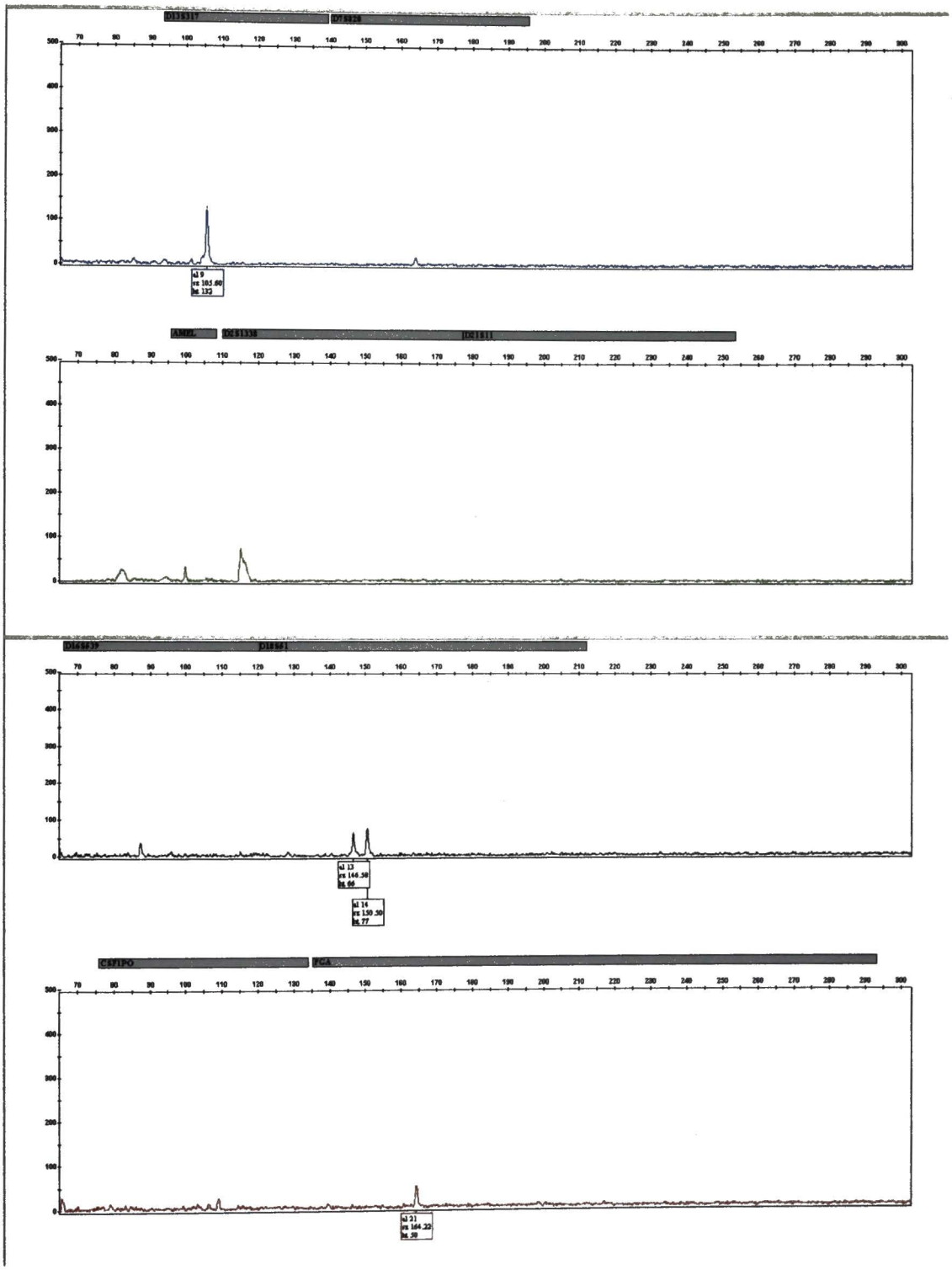

Figure 6-9 *Showing the electropherogram for bone sample F2908.3ABC amplified with the miniSTR multiplex system version 3 at 30 PCR cycles.*

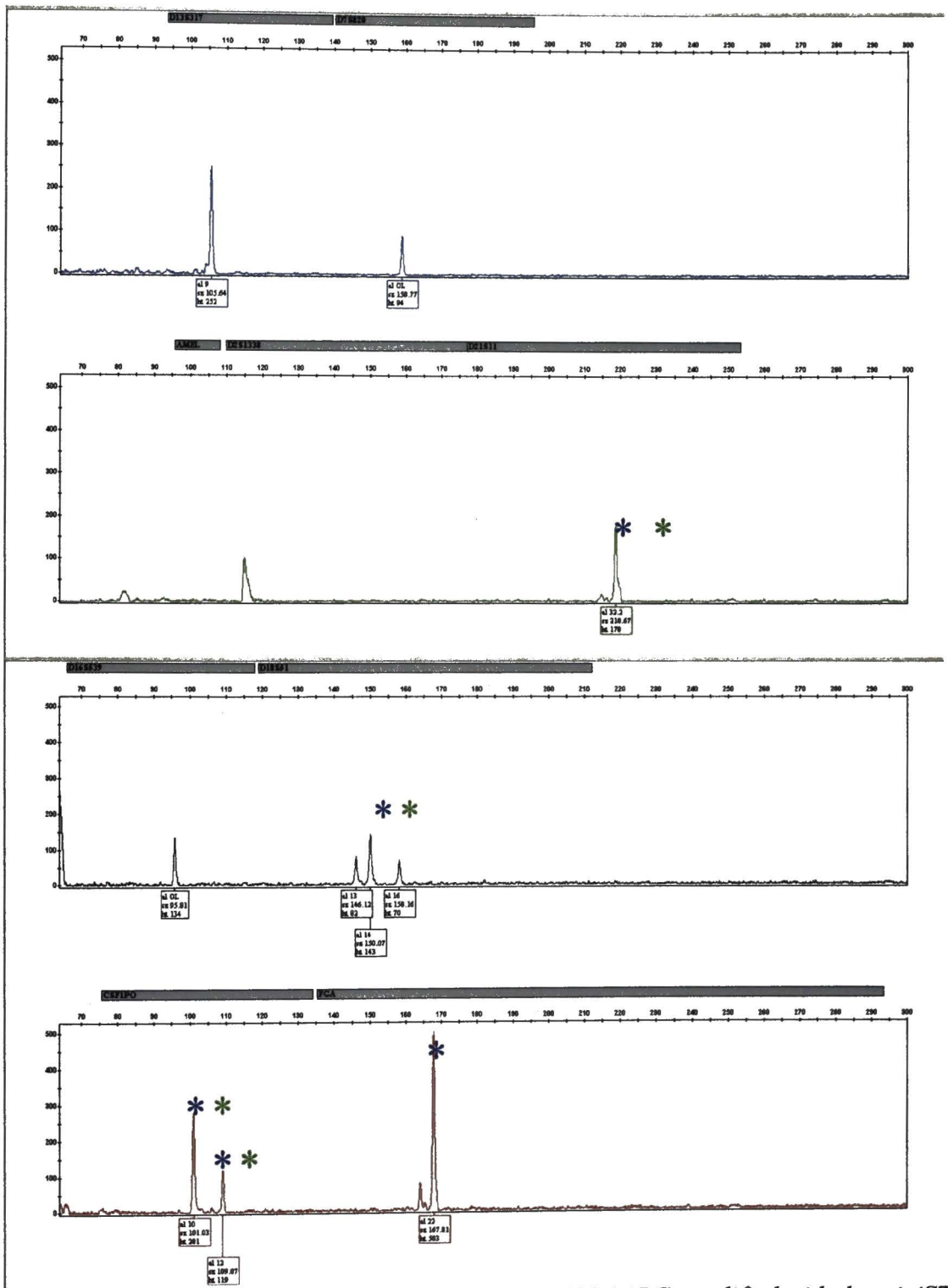

Figure 6-10 *Electropherogram for bone sample F2908.3ABC amplified with the miniSTR multiplex system v. 3 at 32 PCR cycles. Alleles designated with a blue asterisk were not observed when amplified at 30 cycles with the miniSTR multiplex. Alleles designated with a green asterisk were not observed using the Profiler Plus and COfiler multiplex systems.*

Overall, more alleles were obtained by amplification with the miniSTR multiplex kit v.3 than by Profiler Plus and COfiler for the bone and tooth samples analyzed. The results obtained using the Profiler Plus and COfiler typing systems at 32 PCR cycles produced relatively small peaks that were difficult to interpret potentially due to increased stochastic effects. The results displayed in the electropherograms produced by typing with the miniSTR multiplex system were much easier to interpret, with greater allele peak heights, better heterozygous peak height balance, and fewer stochastic effects. Increasing the number of PCR cycles to 32 when amplifying the bone and tooth samples with the miniSTR multiplex resulted in more interpretable alleles as compared to 30 PCR cycles. Allele calls for the bone and tooth samples typed using the miniSTR multiplex v.3 and Profiler Plus/COfiler are displayed in Table 6-18.

Table 6-18 Bone Samples 1																					
	Bone Extract F2442.1																				
	AMEL		D13S317		D7S820		D2S1338		D21S11		D16S539		D18S51				CSF1PO		FGA		
Profiler/Cofiler			8				N/A	N/A	30	32.2		12					9	10			
MiniSTR 30 cycles	X		8	12			15	18	30	32.2	11	12	12	18			9	10	23		
	Bone Extract F2719.1BC																				
	AMEL		D13S317		D7S820		D2S1338		D21S11		D16S539		D18S51				CSF1PO		FGA		
Profiler/Cofiler	X	Y					N/A	N/A					14	17					19	22	
MiniSTR 30 cycles	X	Y	9	12	10	12	17	20	28	30		12	14	17			12		19	22	
	Bone Extract F2908.3ABC																				
	AMEL		D13S317		D7S820		D2S1338		D21S11		D16S539		D18S51				CSF1PO		FGA		
Profiler/Cofiler	X		9				N/A	N/A											21	22	
MiniSTR 30 cycles			9										13	14					21		
MiniSTR 32 cycles			9						32.2		11		13	14	16		10	12		22	

Table 6-18 *Alleles detected for bone and tooth samples typed with the miniSTR multiplex kit version 3, as well as the allele calls obtained by Profiler Plus and COfiler typing performed by the UNTHSC Missing Persons Laboratory.*

After receiving version 6 of the miniSTR multiplex system, eight additional bone samples were obtained from the UNTHSC Missing Persons Laboratory. These samples had also been previously typed using Profiler Plus and COfiler kits at 32 PCR cycles. DNA samples were amplified using the miniSTR multiplex kit version 6 at 30 PCR cycles, and some selected samples were also amplified at 32 PCR cycles.

For bone sample F2281.2AB Profiler/COfiler typing produced a nearly complete profile with alleles observed at 12 of the 14 loci with drop out of the D16 and CSF1PO loci. When this sample was amplified with the miniSTR multiplex kit v.6 using 30 PCR cycles, a complete nine-locus profile was produced. The miniSTR system detected alleles as two additional loci not obtained by Profiler Plus or COfiler.

Amplification of bone sample F2852.2D with Profiler Plus and COfiler produced a partial profile with alleles observed at 10 of the 14 loci. The miniSTR multiplex system produced a full nine-locus profile at both 30 and 32 PCR cycles. Three alleles were observed at the CSF1PO locus for both the miniSTR and COfiler systems. The three alleles detected at the CSF1PO locus with COfiler had similar peak heights, where as the CSF1PO locus in the miniSTR showed two alleles with high, balanced peaks and the third allele with a very low peak height. Similar results were obtained at both 30 and 32 PCR cycles. The Profiler/COfiler data and the miniSTR data all indicate a DNA mixture in this sample, with 3 or 4 alleles detected at multiple loci. The electropherograms for the miniSTR typing made the determination of the major and minor contributor easier to determine since the peak heights between pairs of alleles at a given locus showed more consistent balance.

Amplification of DNA from bone sample F2418.2B with miniSTR multiplex kit v.6 produced identical profiles when amplified at both 30 and 32 PCR cycles. Multiple alleles were detected at all nine loci. This sample appeared to be an equal mixture of two DNA contributions which resulted in peak height balance between the multiple alleles at each locus. The Profiler Plus/COfiler typing produced alleles at the Amelogenin locus only for bone sample F2418.2B.

For bone sample 2419.3AB, Profiler Plus/COfiler typing produced only three alleles across all loci. MiniSTR amplification at 30 PCR cycles produced a full nine-locus profile. MiniSTR typing provided alleles at seven additional loci not observed in the results of the Profiler Plus and COfiler results.

For bone sample 2419.2BCD, no alleles were obtained for this sample by Profiler Plus and COfiler. MiniSTR typing at 30 PCR cycles produced a partial profile with 8 alleles observed (Figure 6-10). When this sample was amplified using the miniSTR multiplex at 32 PCR cycles, alleles were observed at all nine loci, with two alleles present at seven of the loci (Figure 6-11).

Bone samples F2421.1A, F2554.2A, F2713.1A did not produce interpretable results when amplified with the miniSTR multiplex v.6 at 30 PCR cycles, or by Profiler Plus/COfiler typing. Sample F2421.1A did produce a partial profile with seven total alleles observed when amplified with the miniSTR multiplex system at 32 PCR cycles.

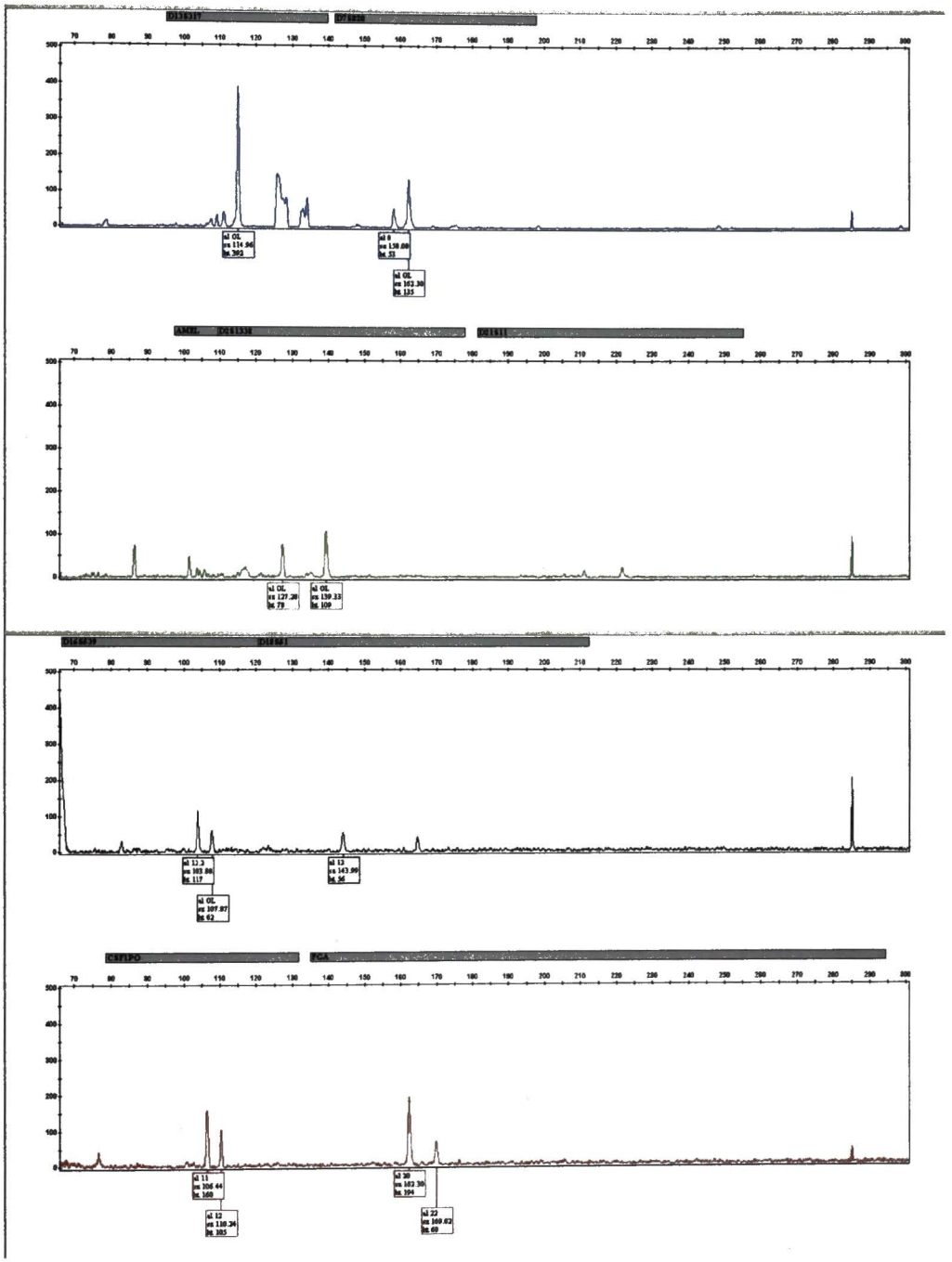

Figure 6-11 *Electropherogram for bone sample F2419.2BCD amplified with the miniSTR multiplex system version 6 at 30 PCR cycles.*

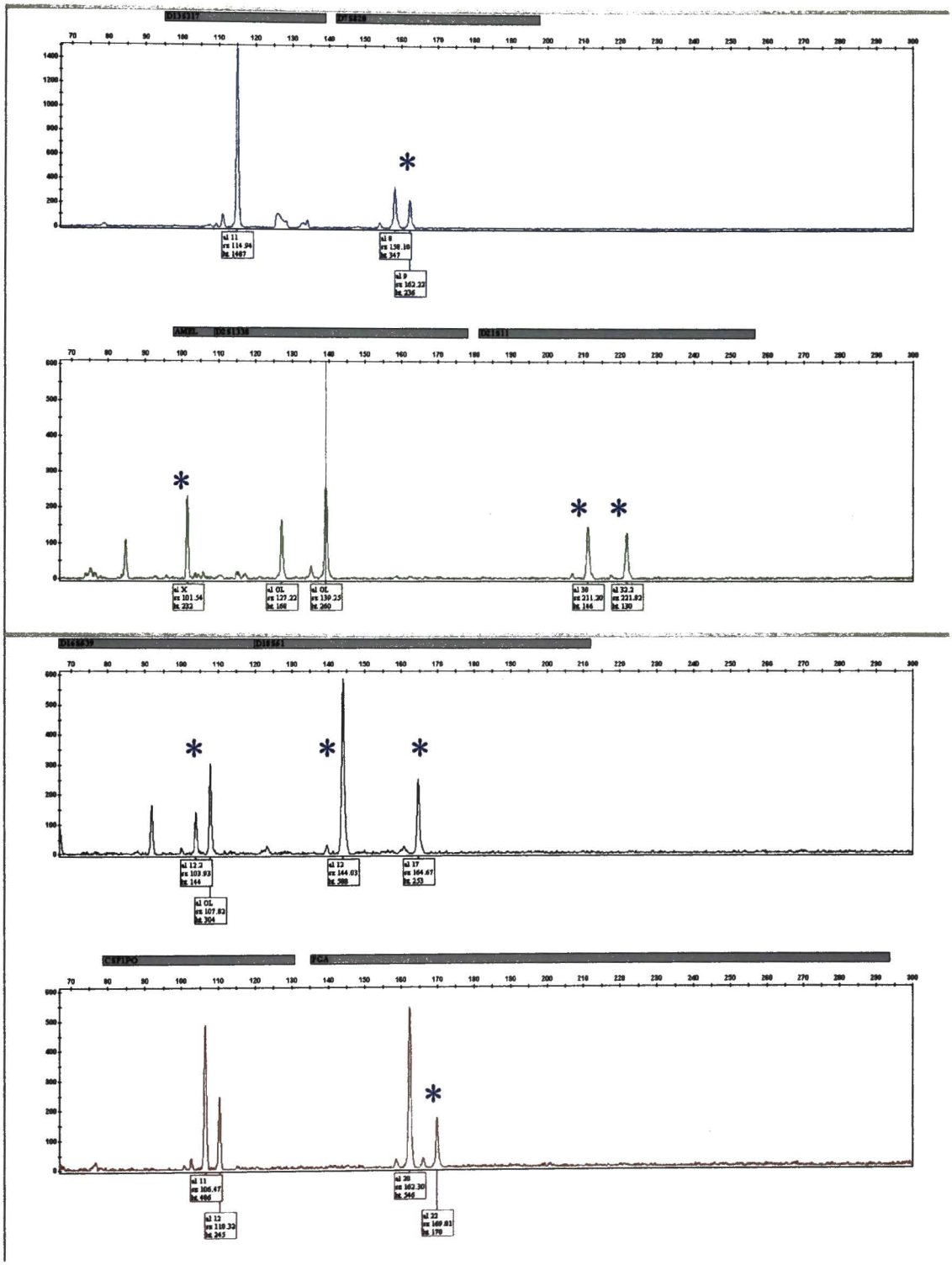

Figure 6-12 *Electropherogram for bone sample F2419.2BCD amplified with the miniSTR multiplex system version 6 at 32 PCR cycles. Alleles designated with a blue asterisk were not observed when the sample was amplified with the miniSTR multiplex at 30 PCR cycles.*

Overall, the miniSTR kit displayed a greater ability to produce interpretable genetic profiles from low copy number and/or degraded DNA samples. In addition, the quality of the profiles produced with the miniSTR system made them easier to interpret in comparison with profile generated with the Profiler Plus/COfiler typing systems. The miniSTR multiplex kit v.6 produced profiles at all nine loci for five of the eight bone samples. In comparison, none of these bone samples provide typing results at each of the loci contained within the Profiler Plus/COfiler kits. In some cases where the miniSTR multiplex system did not produce interpretable results when 30 PCR cycles were used, amplification at 32 PCR cycles did result in additional interpretable alleles. Allele calls for the bone samples typed using the miniSTR multiplex v.6 and Profiler Plus/COfiler are displayed in Table 6-19.

Table 6-19 Bone Samples 2

Bone Extract F2281.2AB

	AMEL	D13S317	D7S820	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
Profiler/Cofiler	X	11 14	11	N/A N/A	31 34.2		14 17		24 25
MiniSTR 30 cycles	X	11 14	11	OL OL	31 34.2	10 11	14 17	11 12	24 25

Bone Extract F2852.2D

	AMEL	D13S317	D7S820	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
Profiler/Cofiler	X Y		10 11	N/A N/A		9 10 13		10 11 13	
MiniSTR 30 cycles	X	11	10 11	OL OL	29 30	10 13	12 15	10 11	22 26
MiniSTR 32 cycles	X	11	10 11	OL OL	29 30	OL 10 13	11 15	10 11	22 26

Bone Extract F2419.3AB

	AMEL	D13S317	D7S820	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
Profiler/Cofiler	X			N/A N/A					20
MiniSTR 30 cycles	X	11	8	OL OL	30 32.2	13 14	11 17	11 12	20 22

Bone Extract F2419.2BCD

	AMEL	D13S317	D7S820	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
Profiler/Cofiler				N/A N/A					
MiniSTR 30 cycles		11	9	OL OL		13		11 12	20
MiniSTR 32 cycles	X	11	8 9	OL OL	30 32.2	12 13	11 17	11 12	20 22

Bone Extract F2421.1A

	AMEL	D13S317	D7S820	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
Profiler/Cofiler	X			N/A N/A					
MiniSTR 30 cycles									
MiniSTR 32 cycles		12	10	OL	29 30		18	12	

Bone Extract F2554.2A

	AMEL	D13S317	D7S820	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
Profiler/Cofiler	X			N/A N/A					
MiniSTR 30 cycles				OL					

Bone Extract F2713.1A

	AMEL	D13S317	D7S820	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
Profiler/Cofiler				N/A N/A					
MiniSTR 30 cycles									

Bone Extract F2418.2B

	AMEL	D13S317	D7S820	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
Profiler/Cofiler	X Y			N/A N/A N/A					
MiniSTR 30 cycles	X Y	9 11 12 13	8 11 12	OL OL OL	29 30 31	12 13	12 14 15 17	10 11 12	21 23 24
MiniSTR 32 cycles	X Y	9 11 12 13	8 11 12	OL OL OL	29 30 31	12 13	12 14 15 17	10 11 12	21 23 24

Table 6-19 *Alleles detected for bone samples typed with the miniSTR multiplex kit version 6, as well as the allele calls obtained by Profiler Plus and COfiler typing performed by the UNTHSC Missing Persons Laboratory.*

VII. CONCLUSIONS

The miniSTR multiplex system evaluated in these studies showed very promising results. The system provided concordant results in comparison with the STR multiplex systems currently used in forensic DNA analysis. The miniSTR multiplex system generated more interpretable alleles with very low amounts of input DNA, providing greater sensitivity than the standard STR multiplexes. The results of the sensitivity study indicate that the miniSTR multiplex system works most efficiently when 30 PCR cycles are used to amplify a range of 200pg to 50pg of input DNA. The miniSTR multiplex system demonstrated the ability to amplify challenging casework samples, and in virtually all cases provided more complete STR profiles than those detected with standard multiplex systems. Prior to commercialization of this new system, Applied Biosystem must purify these new primers to remove the artifacts observed in the prototype kits and generate the appropriate allelic ladders. Once accomplished, the Applied Biosystems miniSTR multiplex system will provide a valuable tool for forensic scientists to obtain genetic data from degraded and low copy number casework samples.

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