5-2002

# DNA Extraction From Archived Slides: Analysis and Use in Current Forensic Identification

Jennifer Lee Millsaps
*University of Tennessee - Knoxville*

To the Graduate Council:

I am submitting herewith a thesis written by Jennifer Lee Millsaps entitled "DNA Extraction From Archived Slides: Analysis and Use in Current Forensic Identification." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Life Sciences.

Karla Matteson, Major Professor

We have read this thesis and recommend its acceptance:

Neil Quigley, Cymbeline Culiat

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:
I am submitting herewith a thesis written by Jennifer Lee Millsaps entitled "DNA extraction from archived slides: analysis and use in current forensic identification." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Life Sciences.

Karla Matteson
Major Professor

We have read this thesis
and recommend its acceptance:

Neil Quigley

Cymbeline Culiat

Acceptance for the Council:

Dr. Anne Mayhew
Vice Provost and Dean of Graduate Studies

(Original signatures are on file in the Graduate Student Services Office.)

# DNA extraction from archived slides: analysis and use in current forensic identification

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Jennifer Millsaps
May 2002

Acknowledgments

I would like to acknowledge several people who helped make this research and my degree possible. First I would like to thank my husband, Rob, for his constant patience and support. I would like to thank Dr. Karla Matteson, Dr. Neil Quigley, and Dr. Cymbeline Culiat for their willingness to serve on my thesis committee and the time spent in discussion and review of my thesis. I would also like to acknowledge the High Risk Clinic at the University of Tennessee Medical Center, Dr. Roger Hubbard at Molecular Pathology in Maryville, and especially Dr. Stephen Swanger for their generous donations of samples for this project. Finally, I would like to gratefully acknowledge Special Agents Kevin Helson, Kelvin Woodby, Regional Lab Supervisor Jerry Maine, and the other scientists at the Knoxville Regional Crime Laboratory of the Tennessee Bureau of Investigation for their assistance on the design and concepts of this project.

Abstract

Positive identification of human remains in missing persons cases can be difficult, especially when dental records or other information normally utilized are not available. Pap smears and other paraffin-embedded tissues taken and archived by medical professionals are a potential resource in the identification of remains in such cases. However, before this material can be useful to forensic scientists, an efficient DNA isolation and analysis protocol must be developed and properly validated. The protocol must work satisfactorily with fresh, archived, and possibly degraded samples when using the multiplexed short tandem repeat (STR) systems, the current standard in DNA identification. We have developed a protocol for the efficient removal of the coverslip from the slide, destaining, DNA extraction, and DNA quantification. DNA recovered by this protocol was amplified with the Profiler Plus and COfiler multiplex primer sets (Applied Biosystems), and analyzed by capillary electrophoresis. 55 Pap smear samples were tested, which ranged in age from 2 weeks to more than 6 years. The revised protocol reduces by several days the time required by other methods for coverslip removal. In addition, destaining was improved, which increased the number of genetic loci that could be typed. Although some allele dropout was observed in the older samples, all of the samples tested gave results which would identify the origin of the samples to between 1 in $10^3$ and 1 in $10^{19}$ individuals. This technique combines efficiency with availability, and demonstrates that DNA isolated from archived Pap smears can continue to be a useful tool in forensic cases.

Table of Contents


| | |
|---|---|
| I. Introduction | 1 |
| II. Materials and Methods | 10 |
| III. Results | 17 |
| IV. Discussion | 29 |
| References | 39 |
| Appendices | 40 |
| Appendix A | 41 |
| Appendix B | 66 |
| Vita | 69 |

List of Figures

A1 – Profiler Plus and COfiler loci 52
A2 – Comparison of D21 and D18 to D3 locus 53
A3 – D3, vWA, and FGA comparisons 54
A4 – Electropherograms (a-c) 55
A5 – Mixed sample 58
A6 – Loci typed vs. statistical exclusion rates (a-d) 61

List of Tables


| | |
|---|---|
| 1 – Profiler Plus and COfiler designations | 4 |
| 2 – ABI Prism 310 analysis parameters | 15 |
| 3 – Correlation and normality findings | 23 |
| 4 – T-test results | 24 |
| A1 – Sample extraction parameters and results | 41 |
| A2 – Sample set S-1 – S-9 | 43 |
| A3 – Sample set S-32 – S-41 | 44 |
| A4 – Sample set S-52 – S-56 | 46 |
| A5 – Sample set S-10 – S-19 | 47 |
| A6 – Sample set S-20 – S-31 | 48 |
| A7 – Thin Prep samples, unstained | 49 |
| A8 – Population statistics | 50 |

Nomenclature/Abbreviations

| Nomenclature | Abbreviation |
|---|---|
| Papanicolau | Pap |
| Human Leukocyte Antigen | HLA |
| Restriction fragment length polymorphism | RFLP |
| Short tandem repeat | STR |
| May Grunwald-Giemsa | MGG |
| Hematoxylin-Eosin | HE |
| Baecchi | Bae |
| Phenol/chloroform/isoamyl alcohol | PCI |
| Hydrochloric acid | HCl |
| Combined DNA Index System | CODIS |
| Base pairs | bp |
| Capillary electrophoresis | CE |
| Relative fluorescent units | rfu |
| micro | µ |
| Magnesium | Mg |
| Deionized water | $dH_2O$ |

## I. Introduction

The identification of human remains has long been a difficult task for investigators. Sometimes enough circumstantial evidence exists to support the probable identity of a specific person. In other cases, information such as dental records and other types of medical information have been useful in comparisons to teeth, bones and tissues of the victim. DNA analysis is another means of identification. While it generally takes much longer to identify a person by this process - several days of analysis and possibly months of waiting for that analysis from a laboratory - the genetic match that can be obtained is often unmistakable. Blood and saliva are among the most common sources for DNA, but they are not always available. Extraction of DNA from teeth, bone and hair roots have also become routine processes. Even hair shafts, once thought useless for DNA extraction, have been shown to yield mitochondrial DNA profiles that can be helpful in tracing family history [1].

For any of these methods to be useful with unidentified remains they must be compared to a DNA standard from the potential individual to make a positive identification. In some cases, it is possible to get this standard from personal articles belonging to the victim that may have blood, other bodily fluid stains, or even hair follicles. While it is common, this technique is not infallible because the item used may not bear the victim's DNA, but that of another person. In other cases, a comparison of DNA from family members is used to connect remains to a victim. This is also a difficult process, causing strain on families, and in some cases is impossible if family members are unknown or deceased. Therefore there is a need for an untainted standard, which can be collected by a professional, which creates minimal distress to the family. One such possible standard could be obtained from existing fixed pathological tissues taken during surgery or other medical procedures. An example of this type of resource is a Papanicolau (Pap) smear, taken during a gynecological examination. 'Pap smears' are routinely made on glass slides, which are stained histotopically, examined by medical professionals, and then are conventionally stored for approximately five years before

being discarded. As this material is collected by a medical professional under controlled conditions, it is a particularly good potential source of material for subsequent molecular genetic analysis.

In the last decade of forensic-related studies, several advances have been made in the extraction of DNA from fixed, paraffin-embedded tissues and stained smears. In 1991, Shibata, *et al.* [2], reported the use of formalin-fixed, paraffin-embedded tissues in the identification of individuals. These authors used primers specific for the human leukocyte antigen (HLA) DQ α region to demonstrate a paternal relationship between a deceased male and his girlfriend's daughter. In 1995, Roy and Reynolds [3] extracted and amplified DNA from pap smear, semen smear, and postcoital slides up to 17 months in age. They analyzed them using restriction fragment length polymorphism (RFLP) analysis with the Polymarker and HLA DQ α human identification kits (AmpliType), which were the standard methods of forensic identification at the time. The Polymarker kit amplified six genetic loci: HLA DQA1, low-density lipoprotein receptor, glycophorin A, hemoglobin G gamaglobin, D7S8, and group specific antigen. It also contained a DNA probe strip which could type five non-DQA1 loci, each of which contained two or three alleles. The HLA DQα kit also had a probe strip, which allowed for six DQA1 alleles to be typed, resulting in a total genotype number of 21 [4]. Of the Pap smear slides, 100% yielded sufficient DNA for amplification, while only 67% of the stained rape kit slides gave sufficient DNA for typing. None of the samples were quantified before PCR, and it was suggested by the authors that the unsuccessful slides may not have been suitable for DNA extraction and amplification in the first place [3].

In 1996, Dimo-Simonin, *et al.* [5], were able to identify short tandem repeats (STRs) and polymorphisms in DNA isolated from smears stained with either Papanicolaou (Pap), May-Grunwald-Giemsa (MGG), Hematoxylin-Eosin (HE), or Baecchi (Bae) stains. The particular STRs and polymorphisms used were HLA-DQA1, PolyMarker, Amelogenin (a gender identifier), and four short tandem repeat (STR) markers: HUMTH01 (183-201bp), HUMV-WFA31 (130-166bp), HUMF13B (169-189bp), and HUMFES/FPS(211-239bp). After the slides had been stored for a period of two weeks, it was found that all of the extracted DNA yielded similar results to their

respective blood standards, with the exception of the Baecchi stain test, in which the yield of DNA was significantly lower. Some possible explanations were given for these results. First of all, two of the staining reagents in the Baecchi stain (Fuchsin acid and methylene blue) were dissolved with 1% HCl which promoted the good contrast and detection of spermatozoa heads (red) in a nonbiological support (blue) [5]. Hydrochloric acid, along with other strong acids, causes depurination and strand scission or base modification in DNA. When the stain was made with water instead of acid, STR typing was possible [5]. Another problem encountered by these authors was nonspecific or contaminated PCR products during amplification of very small amounts of DNA or poor-quality DNA. Overall, these reports demonstrated that it was possible to obtain DNA from a chemically fixed source such as a cytological smear, and, with some exceptions, the more common types of staining techniques did not prevent DNA retrieval.

Recently, Sweet *et al.*. [6], described a case study in which DNA was extracted from the teeth of a skeleton and compared with DNA from a Pap smear to make a positive identification of the remains. In this case, the woman to whom the remains were believed to belong had no dental records available for comparison. However, the suspected victim had did have a Pap smear on file in a medical facility from approximately 5-6 years. These slides were soaked in xylene to remove the cover slips, and the DNA was extracted with Proteinase K buffer and phenol/chloroform/isoamyl alcohol (PCI). The identification system Profiler Plus, which contains nine STR loci and the gender identifier Amelogenin (see Table 1) was used in this case. Analysis of DNA obtained from the slides and from teeth permitted the positive identification of this victim [6].

There are many applications for which archived Pap smears can be used. Research has investigated their use in forensic identification as well as traditional pathology. Traditional pathological research involving archived tissue and slide samples has focused more on improving the techniques used for coverslip and media removal, destaining, and DNA extraction. The goal of these studies is to find ideal methods of storage that will allow increased recovery of DNA over longer periods of time. While not directly related to forensic analysis, these studies are applicable to forensic analysis

**Table 1. Profiler Plus and COfiler loci designations, location on chromosomes, size ranges, dyes used with the primers, and their shorthand designations.**

| | Locus Designation | Chromosome Location | Size Range (in bp) | Dye Label | Shorthand |
|---|---|---|---|---|---|
| Profiler Plus™ | | | | | |
| *** | D3S1358 | 3p | 114-142 | 5-FAM (blue) | D3 |
| | vWA | 12p12-pter | 157-197 | 5-FAM (blue) | vWA |
| | FGA | 4q28 | 219-267 | 5-FAM (blue) | FGA |
| *** | Amelogenin | X:p22.1-22.3 | 107 | JOE (green) | Amel |
| | | Y:p11.2 | 113 | | |
| | D8S1179 | 8 | 128-168 | JOE (green) | D8 |
| | D21S11 | 21 | 189-243 | JOE (green) | D21 |
| | D18S51 | 18q21.3 | 273-341 | JOE (green) | D18 |
| | D5S818 | 5q21-31 | 135-171 | NED (yellow) | D5 |
| | D13S317 | 13q22-31 | 206-234 | NED (yellow) | D13 |
| *** | D7S820 | 7q | 258-294 | NED (yellow) | D7 |
| Cofiler™ | | | | | |
| *** | D3S1358 | 3p | 114-142 | 5-FAM (blue) | D3 |
| | D16S539 | 16q24-qter | 234-274 | 5-FAM (blue) | D16 |
| *** | Amelogenin | X:p22.1-22.3 | 107 | JOE (green) | Amel |
| | | Y:p11.2 | 113 | | |
| | TH01 | 11p15.5 | 169-189 | JOE (green) | TH01 |
| | TPOX | 2p23-2per | 218-242 | JOE (green) | TPOX |
| | CSF1PO | 5q33.3-34 | 281-317 | JOE (green) | CSF |
| *** | D7S820 | 7q | 258-294 | NED (yellow) | D7 |

*** Denotes duplicated loci; Profiler and COfiler are products of Applied Biosystems

because similar methods and materials are being studied.

Coverslip removal methods have remained relatively unchanged: the slide must be soaked in xylene at room temperature for up to several days before the coverslip can be removed. In cases where the mounting medium, Canada balsam, a mounting media, was used, the slide could be boiled briefly to facilitate coverslip removal [7]. If the coverslip is plastic, acetone can be used to dissolve the coverslip. The only notable improvement in the xylene treatment was suggested by Poljak, *et al.*. [8], who reported that coverslips could be removed by incubating Pap smear slides in xylene for 2 hours at -30°C, then for 10 minutes at 37°C. While this treatment may indeed be much quicker, it requires that a laboratory be equipped with appropriate chemical fume hoods, because xylene is relatively volatile and is highly flammable. Therefore, this procedure might not be feasible in some laboratories.

The necessity of destaining Pap smear slides for optimal DNA extraction has been the subject of debate. Some authors [9], [10] have reported that components of the Papanicolau stain are inhibitors of the polymerase chain reaction, and must be removed or inactivated before adequate DNA amplifcation can occur. Chen *et al.* [9] showed that

there are specific PCR inhibitors present in the Papanicolau stain, specifically hematoxylin and aluminum sulfate. They were able to amplify DNA after implementing a destain procedure that involved the addition of 1% hydrochloric acid (HCl). They also found that initial attempts involving 0.1% HCl were unsuccessful in destaining the sample to a degree sufficient to support amplification by PCR. They further concluded that simply diluting the sample was not enough: it was necessary to use a phenol/chloroform/isoamyl alcohol extraction protocol to overcome inhibition.

Others have claimed that destaining is unnecessary. Roy and Reynolds reported that staining had no effect on the ability to type an individual: stained and unstained smears from the same person were equally typable [3]. Pavelic, *et al.* [10] used a simple coverslip removal and proteinase K digestion (in successive overnight procedures), followed by PCI extraction. Alhough residual dye remained, these authors claimed that it did not interfere with the PCR. Poljak and coworkers [7], [11] and Dimo-Simonin, *et al.* [5] utilized cold ethanol precipitations ranging in duration from 40 minutes to overnight following PCI extraction. Both groups of researchers reported successful results without destaining.

Methods of DNA extraction are equally varied. Most published procedures employ a 'digestion buffer' treatment, which contains Tris salts. In earlier reports [3], [5] a Chelex extraction was commonly employed. This is a chelating-resin suspension that can be added directly to the sample. Chelex is composed of styrene divinylbenzene copolymers containing paired iminodiacetate ions that act as chelating groups in binding polyvalent metal ions such as magnesium. When Mg is removed from the reaction, destructive DNA enzymes such as nucleases are inactivated and the DNA molecules are then protected [12]. This method is still used routinely for paraffin-embedded tissues, and involves a boiling step, which removes paraffin from the tissue. This is a critical step because paraffin is a PCR inhibitor [11]. Poljak, *et al.* [11] described three equally successful techniques: a salting-out procedure using NaCl, modification of a commercially available DNA extraction kit, and PCI extraction. The PCI extraction is by far the most commonly used DNA extraction method in this body of literature. Although many newer procedures have been developed in recent years, PCI extraction continues to

be used routinely, possibly because it offers an economical advantage over commercially available kits. The PCI method is used widely in other forensic labs, and for this reason it is the method used exclusively in this study for the extraction of DNA from test samples.

In a recent pathology study, Poljak *et al.* [11] investigated several different methods for extracting DNA from Pap smear slides that were up to 10 years in age. These techniques ranged from the traditional PCI extraction to a commercially available quick extraction protocol. These authors claimed that a DNA extraction efficiency of over 95% was possible with these slides, with little change in the efficiency occurring with time. The research was done using a single (or sometimes double) primer set, amplifying one (or two) loci at one time. Their results do not show multiple loci reproducibility from a single sample. This question is of paramount concern to forensic analysis, where the Profiler Plus/COfiler multiplexed systems are used for legal identification of an individual.

Also of interest is the relationship of time in storage to the usefulness of this analysis when using the Profiler Plus/COfiler PCR primers. Sweet *et al.* [6] extracted DNA from a single 6-year-old slide and used the Profiler Plus system for analysis. However, they reported that D7S820 and D18S51, two of the longer loci in the Profiler Plus system, could not be typed (allelic dropout). Because this was a single case study, it is unclear whether or not this dropout occurred because of age of the slide or the protocol used. Poljak *et al.*. amplified DNA from 10-year-old slides [11], but examined only a single locus. In a multiplexed system with multiple primers, it is unclear if an archived smear would be capable of producing typable alleles at all of the loci.

The work completed in this area of research over the last decade demonstrates that it is possible to extract DNA from cytological slides, under varying fixation and staining conditions. There are, however, several questions that remain unanswered. One concern is that none of the published reports are recent enough to have used both the Profiler Plus and COfiler systems (Applied Biosystems) together to identify an individual. These two primer sets collectively examine 13 different tetrameric STR loci and the gender-defining locus Amelogenin. These ‘thirteen core loci’ STRs represent the

standard loci for the Combined DNA Index System (CODIS) database, the national registry for DNA from convicted felons in the United States. The DNA intervals amplified at these loci range in size from 100 to approximately 325 base pairs in length (see Table 1, Figure A1). Because the size ranges of some amplicons among these loci overlap, three fluorescent dyes are used in the primer sets to distinguish the 13 different loci. A fourth fluorescent dye color, red, is used to label a set of sizing standards, which is comprised of 16 single-stranded fragments ranging from 35 to 500 bases in length. Once the sample is run on the capillary electrophoresis instrument, GeneScan software (Applied Biosystems) is used to size the different fragments from each locus by comparing them to the internal sizing standard, and assigns the alleles at each locus based on their size.

The use of these STR methods has increased over the last few years while analysis of RFLP methods has diminished. One reason for this shift in emphasis is that RFLP methods cannot accommodate low DNA quantities or poor quality DNA, as can STR methods. In addition, RFLP methods require radioactive or chemiluminescent probes, and can take days or weeks to yield results and thus high volume sample processing. In contrast, STR methods can yield results in just 1 to 2 days.

Several other issues such as the repeat sequence and the type of STRs suitable for identification purposes must be considered. It was found that tetranucleotide repeats have some basic advantages over di- or trinucleotide repeats [12]:

- A narrow allele size range that permits multiplexing and reduces allelic dropout resulting from preferential amplification of smaller alleles
- The capability of generating small PCR product sizes that facilitate recovery of information from degraded DNA specimens
- Reduced stutter product formation (compared to dinucleotide repeats) which aide in the interpretation of sample mixtures (Butler, p. 57)

For tetranucleotide repeats, there are specific selection criteria for selecting a suitable STR for identification purposes. Some differential characteristics include:

- High discriminating power, usually >0.9, with observed heterozygosity >70% (a high level of difference in alleles between unrelated individuals)

- Separate chromosomal locations, which ensure that closely linked loci are not chosen
- Robustness and reproducibility of results when multiplexed with other markers
- Low stutter characteristics and mutation rates
- Predicted length of alleles that fall within the range of 90 – 500 base pairs (bp) - these smaller sizes are more useful for analysis of degraded DNA samples

Today, the 'thirteen core loci' are used and accepted by forensic laboratories across the United States and in much of the world and serve as the backbone of the CODIS database. The primers have been tested to see if the primer pairs will work well in combination with each other during multiplex PCR conditions. The primers themselves are proprietary to the two major producers of multiplexed identification primer sets, Promega and Applied Biosystems. These thirteen loci have characteristics that make each one unique in terms of the number of alleles present, the type of repeat sequence, and the kinds of microvariants that have been observed (Butler p. 68).

Ideally, from this information, 13 loci and the Amelogenin locus can be typed. However, even an incomplete STR profile can be useful in determining the identity of an individual. Statistical analysis of even as few as three to five of the loci can make a profile accurate to 1 in 100,000 people. Forensic laboratories utilize both of these loci systems in casework involving human identification. Determining if archived Pap smears typed with Profiler Plus/COfiler would provide enough genetic information for these databases would be valuable for the forensic laboratory system.

Demonstrating that DNA extracted from archived cytological smears can reliably produce a Profiler Plus/COfiler DNA profile is essential for forensic use of this method, and a statistically significant number of samples must be tested. Standard protocols are a vital part of forensics work because of the nature of the analyses being performed: one lab must not deviate significantly from another in the manner in which it obtains DNA profiles. This requirement insures reproducibility and, more importantly, fewer disputes about protocols in legal proceedings. Because several techniques are described in the literature for various uses, determining which one would be the most effective in a

forensics laboratory setting will be an important first step. Various aspects of the protocol that vary in the literature, such as coverslip removal and destaining and its effects on DNA extraction should be addressed. Another aspect not addressed in the literature is the amount of starting material required for DNA analysis. For example, if it were possible to use material from only a portion of the slide, rather than consuming all of the material on the slide during the extraction/analysis procedure, it would allow the remaining smear material to be re-stained and re-fixed, for storage in the medical record. This may allow DNA samples to be taken from slides younger than 5 years that are being stored for medical use without destroying the entire sample in the process. Legally, this is an important consideration. Determination of the parameters that influence the recovery and analysis of DNA from Pap smear slides is the focus of this project. The results of this study should add significant new information about the utility of Pap smears in forensics analyses.

## II. Materials and Methods

The tissue materials used for this project included slides of varying age, which were obtained from three sources. Pap slides, approximately 6 years in age, were obtained from a local pathology laboratory. Laboratories are mandated by law in Tennessee to retain the slides for a period of five years before discarding them. Because they were scheduled to be discarded, they served as a convenient source of sample material of that age. Approximately 55 slides were tested from this group. Patient names and other identifying information were removed from the slides prior to being available for this project.

Fresh slides were taken from voluntary subjects in a private gynecological/obstetrics office at the University Medical Center in Knoxville. The subjects would be scheduled for routine, yearly examinations in which Pap smears are taken. Before the exam, the patient was informed of the nature of this project as well as their role in the research. If they chose to participate in the project, they were required to sign a consent form (see Appendix B). The medical staff at the time of the examination created a slide for this project in addition to the slide that was prepared for medical analysis. For comparison purposes, buccal swabs were also taken. An expedited review of this project for both the archived slides and the fresh samples was submitted to the Institutional Review Boards at the University of Tennessee, Knoxville and the University of Tennessee Medical Center, where it was approved (IRB number 2110. While eight sets of smears and buccal swabs were received, only four were tested due to time constraints of the project.

The remaining samples used in this project came from a second pathology laboratory. These slides were also new material, but were prepared by the Thin Prep method, as opposed to traditional Pap smears. These samples are the cellular material taken during an exam and stored in a methanol solution before they are placed onto slides for storage. Five of these vials were tested as part of this project. In all cases, all personal information which could link the slide to an individual was removed before it was made available for this project. An intermediate individual acted as a "filter," removing this information. Slides were numbered for tracking purposes only. In

addition, none of the profiles obtained from this research were added to any DNA database as stipulated on the consent forms.

To create a protocol for this extraction, existing literature on the subject and protocols established for similar procedures were used as guides. Initially, slides were taken in groups of 5 – 10 and immersed in xylene for coverslip removal. Slides were numbered as they were tested, beginning with S-1 to S-n. To test the method proposed by Poljak, *et al.*. [7], [11] some were immersed and stored at -20°C from 2 hours to overnight, then left at room temperature for several hours. Others were stored at room temperature overnight. Once the coverslip was removed (they fell off spontaneously or were removed using a spatula) the slides were re-immersed in xylene for 20-60 minutes to insure residual fixative was removed. Control slides and the Thin Prep slides had no coverslips, making this step unnecessary.

Once coverslips were removed, most slides were destained. After treatment with xylene, slides were immersed in three changes of absolute ethanol for one minute each change. The slides were then placed into one of two destaining solutions. Both contained 70% ethanol, but one contained 1% HCl while the other contained 0.2% HCl. Slides were destained for a period of 30 minutes. To determine if time was a factor, some slides were immersed in the 1% HCl destain for six minutes instead of thirty minutes. These variables represented a five-fold range in both the concentration in the solution and the effect of time spent in the destain. A control smear was also tested with the destaining solution to see the effects on new, unstained material. Once the slides were destained, they were rinsed 3 times with 95% ethanol for one minute each to remove residual HCl, then allowed to air-dry.

The amount of material removed from the slide was determined by two different methods. Initially, the amount removed was selected based on a region of the slide that had relatively little stain. This was decided on an individual sample basis, so the amount of material taken ranged from 90 $mm^2$ to 400 $mm^2$. Subsequently it was decided that removal of a more uniform amount of material would be beneficial, so the amount removed for most slides was 200 $mm^2$. The slides were marked, selecting a region 10 mm by 20 mm, then immersed in deionized $H_2O$ ($dH_2O$ just long enough to wet the

material (a few seconds), and a sterile cotton swab was used to remove the material from the marked area.

In addition, 5 Thin Prep samples were tested for comparison to the Pap smear controls. These samples are cellular material stored in a methanol solution before they are placed onto slides for storage. The samples tested here were received in this methanol suspension. Some of the liquid was dropped onto a slide and allowed to dry, then a region 200 $mm^2$ was removed from the slide and extracted in the same manner as the regular slides.

The portion of the swab containing the sample material was then cut off with a scalpel and placed into a microcentrifuge tube for extraction. General extraction buffer (10mM Tris, 100mM NaCl, 39mM DTT, 10mM EDTA, 2% SDS, pH 8.0) and 7.5μL Proteinase K (Sigma Chemical Co., St. Louis, MO; 14.3 units/mg, 10mg/mL) were added to the sample. A reagent blank was prepared to serve as a control to check for contamination during the extraction. The tube was vortexed for five to ten seconds to force the liquid into the cotton and incubated at 56°C for 2 – 24 hours. In most cases, incubations were performed overnight. Once the tube was centrifuged to force the condensate to the bottom of the tube, the liquid was transferred to a new tube and 300 μL of a mixture of phenol/chloroform/isoamyl alcohol (25:25:1 by volume) was added. The solution was vortexed for fifteen to twenty seconds to form a milky mixture. After centrifugation at 12,000 x g for 3 minutes, the aqueous (top) phase was carefully removed and added to a Microcon 100 microconcentrator unit (Millipore, Bedford, MA), to which 100 μL of TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) had been added. The microconcentrator unit was centrifuged at 500 x g for 10 minutes. The liquid was removed from the bottom of the tube and 200 μL TE buffer was added to the top of the concentrator and centrifuged at 500 x g for 10 minutes. TE buffer was added to bring the total volume to between 100 and 200 μL in the unit, which was then inverted into a new tube and re-centrifuged for 5 minutes to elute DNA from the matrix. At this point the sample could be stored, or quantified and amplified.

The QuantiBlot (Applied Biosystems) procedure was used for DNA quantitation. Approximately 5 μL of sample DNA was added to 150 μL of spotting solution (0.4 N

NaOH, 25 mM EDTA, 0.00008% Bromothymol Blue), and then pipetted into wells on a slot-blot apparatus containing a nylon membrane pre-wetted for 15-30 minutes with pre-wetting solution (0.4 N NaOH, 25 mM EDTA). Standards, known amounts of DNA, were prepared and added to the membrane in a similar manner. A vacuum was applied, and the samples were pulled into the membrane. The membrane was removed from the apparatus and placed into a tray containing 100 mL hybridization solution (5X SSPE, 0.5% w/v SDS) and 5 mL 30% $H_2O_2$. The tray was covered and placed in an oscillating water bath at 50˚C for 15 minutes. Care was taken to prevent the membrane from drying out between steps.

The hybridization solution was poured off and 30 mL of fresh hybridization solution and 20 μL higher primate-specific D17Z1 probe were added, and the tray was placed back into the water bath for 20 minutes. This solution was poured off and 100 mL of pre-warmed (56˚C) wash solution (1.5X SSPE, 0.5 w/v SDS) was added to remove excess probe. This was poured off and 30 mL fresh wash solution and 180 μL of the enzyme conjugate horseradish peroxidase streptavidin (HRP-SA) were added. The tray was again placed in the water bath for 10 minutes, after which the solution was poured off. Two 1-minute wash steps with the wash solution were done, and 100 mL fresh wash solution was added. This time, the tray was placed on an orbital shaker (100-125 rpm) for 15 minutes at room temperature.

Color development solution was prepared with 30 mL citrate buffer (0.1 M sodium citrate, pH 5.0), 1.5 mL Chromogen: TMB solution (3,3',5,5'–tetramethylbenzidine; Perkin-Elmer), and 30μL 3% $H_2O_2$. After the final 15 minute wash, the solution was poured off and the color development solution was added and incubated for a period of 20 to 30 minutes. When color development was complete, the intensity of each spot for each sample was compared be eye to the standard intensity of the blots. This procedure provided an estimate of the concentration of the original sample, so that approximately 1 ng of each DNA would be used for future amplification and then analysis.

Fluorometric readings were also obtained for the samples as a comparison to the QuantiBlot procedure. 2 mL of dye solution (Hoechst 33258) was added to a glass

cuvette, and the cuvette was placed in a Dyna Quant 2000 (Hoeffer, San Francisco) for calibration. Once the instrument was calibrated and set to zero, 2 μL of DNA sample was added, and a reading obtained. Comparisons between the QuantiBlot reading and the fluorometric reading per sample were made, but because the QuantiBlot was a more sensitive method and provided information down to 0.15 ng/μL, these results were used when computing the amount of template DNA that should be added to the PCR mix.

Amplification master mix consisting of AmpF/STR PCR Reaction Mix, AmpF/STR Profiler Plus or COfiler primer sets, and AmpliTaq Gold DNA Polymerase was prepared and 30 μL was aliquoted to each PCR tube. A nanogram of DNA solution and sufficient TE buffer was added to bring the total volume of solution to 50 μL. The tubes were placed in the thermal cycler (Perkin-Elmer GeneAmp PCR System 9600) to run under the following program:

| **PCR Step** | **Temperature in °C** | **Time** |
|---|---|---|
| **Initial Incubation Step** | 95 | 11 min. |
| **28 Cycles** | | |
| Denature | 94 | 1 min. |
| Anneal | 59 | 1 min. |
| Extend | 72 | 1 min. |
| **Final Extension** | 60 | 45 min. |
| **Final Step** | 4 | hold |

Positive and negative controls were prepared for both the Profiler Plus and COfiler sets. These were in addition to the reagent blank prepared earlier. These control reactions served as checks on the integrity of the extraction and PCR procedures, ensured that no contamination had occurred, and that the PCR had worked properly. After amplification, samples were stored at -20˚C until preparation for analysis.

PCR products were subjected to capillary electrophoresis (CE) in an Applied Biosystems 310 Genetic Analyzer to resolve PCR products as unique molecular species. 24 μL of deionized formamide and 1 μL of GeneScan-500 [ROX] sizing standard were added to each tube. 1.5 μL of PCR sample was added to the tube and mixed with the end of the pipet tip. A sample tube containing an allelic ladder was prepared in the same manner for both Profiler Plus and COfiler. The allelic ladders contained all of the loci for the respective primer set and all of the known alleles at each locus, and were used as reference standards to enable genetic typing of test samples. Before running samples on

the 310 Genetic Analyzer, they were incubated at 95˚C for 5 minutes to denature the DNA. The samples were immediately plunged into either ice or placed in a -20˚C freezer for 5 minutes to prevent DNA renaturation.

The AB 310 instrument was operated according to the supplied user instructions. Parameters for the run are listed in Table 2.

Electrophoresis results were analyzed using the Genotyper version 2.1 program (Applied Biosystems). Macros for the Profiler Plus and COfiler primer sets designed to align the peaks and assign alleles for each locus were used to generate genetic 'profiles' of each sample. During the analysis the peak height threshold was set at 150 relative fluorescent units (rfu) as a means of eliminating smaller peaks from analysis. This 150 rfu 'threshold' level is used throughout the United States. Some laboratories, however, will analyze peaks between 75 rfu and 150 rfu with some degree of caution. Because of this practice, the minimal threshold level in this study was re-set to 75 rfu to retrieve as many alleles as possible from the samples. Peaks called in this range were reviewed by experienced analysts to insure they were indeed true alleles and not background noise.

Once the loci and alleles had been ascertained for each sample, the alleles were analyzed for statistical population genetics exclusion. The number of times (frequency) an allele at a particular locus can be found in a particular population has been researched and established for each of these loci. The alleles at every locus in each sample were analyzed for their frequency in the population (based on the population research discussed above), and those frequencies were combined to give a total exclusion statistic for the sample. The results could be interpreted in several ways. One way is to state that as the number of loci increases and thus the number of alleles in a sample, the likelihood that the genetic profile is unique to an individual also increases. The second way is to state that as the number of loci and alleles increases for a sample, the likelihood that another person would have the same profile decreases. Statistical analysis was performed on the populations considered indigenous to this region (East Tennessee). Exclusion numbers for Caucasian, African American, Southeastern Hispanic, and Southwestern Hispanic were calculated based on their established allelic frequencies.

**Table 2. AB Prism 310 analysis parameters.**

| Module | Inj Secs. | Inj. kV | Run kV | Run °C | Run Time | Auto Anlz. | Analysis Parameters |
|---|---|---|---|---|---|---|---|
| GS STR POP 4 (1 mL) | 5 | 15.0 | 15.0 | 60 | 24 min | On | 3300/75/75-450 |

# III. Results

Samples were divided into groups during the course of this project so that different protocol modifications could be tested. Table A1 shows all of the samples and the parameters under which they were analyzed.

## Allelic Dropout Minimization

Results from all archived samples regardless of their destain treatment showed signs of alleleic dropout and differential amplification. Allelic dropout is the loss of alleles at a particular locus, while differential amplification results from higher amplification of one or more loci over other loci within the multiplex set. In contrast, preferential amplification results in greater amplification of one allele over another within the same locus.

Allelic dropout is common for older samples in which a degree of DNA degradation has occurred. Various modifications of the basic protocol were tested to determine which ones minimized allelic dropout, particularly at larger loci, and also increased the peak heights of the alleles above the 150 rfu threshold.

### Limiting allelic dropout: destain changes

Results for the peak heights of each allele that was typed from the first group of samples (S-1 – S-9) as well as the total number of loci typed in each sample are presented in Table A2. This group was subjected to a 1% HCl destain treatment for 30 minutes. The average number of loci obtained from this first set of samples (S-1 – S-9) was 9.7 (of 9 samples) out of a possible 13 (excluding Amelogenin). Samples showed relatively high peaks in the smaller loci, but as the locus amplicon size increased the allele heights decreased sharply, frequently resulting in the complete loss of signal.

Preliminary results suggested that a protocol modification might address the questions of differential amplification and allelic dropout. Concerns were raised that the destaining procedure might be further damaging the already degraded DNA. Hydrochloric acid, a component of the destain, is known to cause strand scission [5] and purine deamination in DNA.

Two parameters of the destaining procedure were tested to determine if destaining could be performed with less detrimental effect on the DNA. The test parameter was the amount of time that the slides were treated with destain. The second parameter tested was the concentration of HCl in the destain itself. A five-fold difference in each parameter was to determine if a change from the original protocol was warranted. Two slides were tested: 0.2% HCl destain for 30 minutes, and 1% HCl destain for 6 minutes. When results were compared for the two new destain procedures, it was found that the DNA from slides destained with the 0.2% HCl solution for 30 minutes performed better overall than the 1% HCl slides that were destained for 6 minutes. In addition, it also appeared that this method might be preferable to using the protocol that called for 1% HCl destain for 30 minutes. Group S-32 – S-51, which was treated with 0.2% HCl for 30 minutes showed improved results (Table A3). The average number of loci typed for this set was 9.63. This was similar to the set S-1 – S-9, which had been treated for the same amount of time but with the stronger 1% HCl destain. However, the peak heights for the 0.2% HCl destain treated group were much higher overall and were above the 150 threshold level.

The literature presents differing views regarding the need for destaining slides prior to extraction. Therefore, 5 slides were tested with no destaining procedure (S-52 – S-56). Though there were only 5 samples tested, the DNA from the unstained slides performed extremely well when compared to the other destaining procedures (Table A4). While one of the samples only had 4 loci (S-56), 3 of 5 gave complete profiles (13 loci) and one gave 9 loci. Of the three complete profiles, all alleles were higher than 150 rfu. This was not the case for the destained samples: of the samples treated by any destaining method that gave full profiles, none showed all alleles above 150 rfu.

Limiting allelic dropout: quantity of DNA in PCR reaction

Another possibility for the cause of allelic dropout was that insufficient template DNA was added to the PCR reaction. The results for set (S-10 – S-19), where the amount of DNA solution was increased to 14 μL can be seen in Table A5. This volume of DNA used was equal for each of these samples, ignoring the QuantiBlot measurements

obtained for each sample. The average number of loci typed from this group was 8.4, which was lower than the set S-1 – S-9 (9.0 loci typed), for which efforts were made to come close to 1 ng of DNA according to QuantiBlot measurements. It is also much lower than the set S-32 – S-51 (9.6 loci typed), where a weaker destain procedure was used. While the smaller loci peaks are stronger than the first group, there is no relative change in the peak heights of larger loci, and, as can be seen by the lower average, it is possible that additional loci that might have been typed were lost. It appears that increasing the amount of DNA added to PCR within the 1% HCl treated slides group also increased the problem of preferential amplification of the samples. These results indicate that DNA amount is not limiting in these amplifications

**Control slides and Thin Prep samples**

Control slides comprising samples less than two weeks old, and which were unstained, were compared to archived six-year-old stained smears. Of interest was how the allele peak heights of the larger loci would compare to the alleles from archived slides, which showed sharp signal intensity declines and allele dropout in larger loci. Buccal swabs from the same individuals were used to show that the protocol could match two different types of samples from the same individual. One of the unstained control Pap slides was treated with the 1% HCl destain for 6 minutes to see if there would be any change in peak heights from its buccal swab match or a significant difference between its peak heights and those of other control unstained (and untreated) Pap slides. The remaining control Pap slides and buccal swabs were inserted in the protocol at the point of extraction in Proteinase K buffer overnight and went through the rest of the extraction process in parallel with the other samples.

The loci, peak heights, and amplicon lengths obtained from control samples are presented in Table A6. There was no difference between the buccal swab samples and the Pap smears from the same individual, and all pairs matched. The peak heights were even across all loci, which linked the allelic dropout in the archived slides to age and destaining. The slide treated with 1% HCl destain for 6 minutes showed some decrease in peak heights as the allele lengths increased. However, there was not as large a drop as

was seen with the archived slides, suggesting that while the destain may have some effect, the degradation over time is probably the largest factor in the dropout of alleles in archived samples.

Of the Thin Prep samples, 2 of the 5 samples showed no profile at all (Table A7). The other three provided profiles comparable to results obtained from the control samples, in that all of them gave a full profile. The peak heights of the alleles were constant throughout the samples, which was also similar to the controls. The two that did not produce a profile most likely failed because of the method by which samples were tested. If the cellular suspension was not suspended sufficiently before it was placed onto the slide, it is possible that no cellular material was present.

## Comparisons between new and archived slides

Additional comparisons can be made between the samples treated with different destaining procedures. However, while there are approximately 20 samples in each of the destain groups, they are not always useful for certain analyses. Homozygous alleles, for example, are difficult to compare to heterozygous alleles. It is not simply a matter of dividing the peak height in half to compare to a heterozygous allele. Technically this would be possible if no degradation was present because an equal amount of DNA would be present for both homozygous and heterozygous alleles from the same sample. However, even with ideal samples this cannot be assumed, therefore for these analyses heterozygous alleles will be used most often.

### Smaller and larger loci comparisons

One comparison that can be made between new and archived slides is to look at the peak heights of the smaller loci versus the peak heights of the larger loci on a percentage basis. This can demonstrate the amount of degradation that has occurred in the archived samples. To do this, samples within the different destain and control groups were analyzed at the D3, D18 and/or D21 loci. The peak heights of heterozygous alleles were averaged within each locus. Then, the average of the larger locus (either D18 or D21) was divided by the average of the smaller D3 locus, and the percentage of the

average heights was obtained. For the D21/D3 loci comparison, there were approximately 5-7 samples from the two destain groups that were suitable for this analysis (i.e., had heterozygous alleles at all three loci), and 2-3 from the non-destained group and the control group. As can be seen by Figure A2, there were fewer samples to compare at the D18 locus: either the samples did not have heterozygous alleles, or there was no D18 locus information available. Generally the latter applied, especially for the 1% HCl destain group. A trend emerges as the different destaining procedures are compared: the 1% HCl–destained samples had the lowest percentages, while the stained (non-destained) samples performed the best of the archived material.

A similar analysis shows the trend of peak heights for the different destain and control groups as the loci increase in size. Figure A3 displays averages of samples from each destain group at the D3, vWA, and FGA loci, all of which are labeled with the 5-FAM (blue) fluorescent dye in the Profiler Plus primer set. As with the previous Figure, the samples chosen were heterozygous at all loci: averages for the two alleles in each sample were obtained, and then averaged across the sample set to represent a particular destain treatment group. As the loci increased in size, the control and Thin Prep groups remained relatively unchanged in terms of average peak heights. While there is a slight decrease, overall the height of alleles at the FGA locus were similar to the heights at the D3 locus. In contrast, the heights of the alleles in the archived samples decreased as loci size increased. This was true for stained samples and for all destained samples, regardless of the destaining technique used. However, while allele signal for all of the archived samples did exhibit decreased peak height over time, there was a negative correlation between [HCl] used and peak height.

Correlation between the number of loci typed and amount of DNA in PCR reaction: destain groups

While some options are available during CE analysis to improve the peak heights of a sample, other factors were found that could help increase peak height. One approach was to decrease the amount of HCl in the destain, or possibly not employ any destain treatment. A second approach was to increase the amount of DNA that was used for PCR

analysis. While increasing the amount of DNA in PCR was problematic for the 1% HCl destained samples, as was shown by the decreased number of loci typed for S-10 – S-19 (8.4 versus 9.0 in other 1% HCl destained samples), this was not the case for the 0.2% HCl stained samples. This comparison was shown in a correlation study between the amount of DNA in the PCR reaction and the number of loci that were produced per sample. This analysis was completed using the Proc Corr procedure in the SAS statistical analysis software package (SAS Institute, North Carolina). The Pearson correlation coefficient, ρ, ranges on a scale from -1 to +1. As the association increases, ρ will approach either +1 or -1, depending on the positive or negative nature of the correlation. A value closer to zero means that no correlation exists.

When this test was performed on the various destain groups, it was found that no correlation between amount of DNA in PCR and number of loci per sample existed for the 1% HCl destained samples. However there was a statistically moderately strong correlation between the amount of DNA added and the number of loci typed for the 0.2% HCl group. Quantitated DNA values obtained from both QuantiBlot and fluorometric measurements were correlated against the number of loci typed per sample from both the 1% HCl and 0.2% HCl destain groups. The results from all four data sets appear in Table 3. As shown, ρ (rho) for the 1% HCl group is very low (0.049 and 0.075 for QuantiBlot and fluorometric analysis, respectively), while in the 0.2% HCl group, it is much higher and suggests a moderate to strong correlation (0.706 and 0.407). In addition, the probability (*p*-value) that quantity of DNA and number of loci are not correlated is very high for the 1% HCl group, but extremely low for the 0.2% HCl group, which supports the Pearson correlation findings. When a test for correlation between the QuantiBlot and fluorometric readings for each sample was performed, no correlation could be found (data not shown). These correlation studies were not performed for the controls, Thin Prep, and stained archived smears because of the small sample numbers from those groups.

Normality of the data was also assessed for these four data sets as shown in Table 3. The *p*-values for skewness, kurtosis, and the omnibus are the probability that the data is normal. With an $\alpha$=0.05, normality cannot be rejected because the *p*-values are all

**Table 3. Correlation and Normality findings for the comparisons of quantitated DNA in PCR reactions and the number of loci typed per sample.**

| | Correlation | | Normality - Loci | | | Normality - Nanograms of DNA | | |
|---|---|---|---|---|---|---|---|---|
| | Pearson Correlation Coefficient ρ | *p*- value | Skewness *p* -value | Kurtosis *p* -value | Overall Omnibus *p* - value | Skewness *p* -value | Kurtosis *p* -value | Overall Omnibus *p* - value |
| **1% HCl destain** | | | | | | | | |
| QuantiBlot analysis | 0.04921 | 0.8463 | 0.3894 | 0.2136 | 0.3186 | 0.3016 | 0.0024 | 0.0058 |
| Flurometric analysis | 0.07548 | 0.7588 | 0.2927 | 0.2378 | 0.2864 | 0.0019 | 0.0518 | 0.0012 |
| **0.2% HCl destain** | | | | | | | | |
| QuantiBlot analysis | 0.70623 | 0.0007 | 0.1305 | 0.354 | 0.2074 | 0.8605 | 0 | 0.0002 |
| Flurometric analysis | 0.40688 | 0.0672 | 0.145 | 0.0953 | 0.0861 | 0.0001 | 0.0004 | 0 |

greater than 0.05. Therefore, these data must be accepted as being distributed normally.

Number of loci typed above and below 150 rfu: destain groups

All of the archived samples decreased in allele height as loci sizes increased, and in many cases the larger loci were not typed at all. In some cases, it was clear that additional loci could have been typed from the sample if the parameters were changed. These loci were visible and easily distinguishable from background noise, but they were below 75 rfu. This is the lowest cut-off point used by forensic laboratories in the U.S. Most laboratories employ a lower threshold of 150 rfu, but some labs will occasionally call peaks that appear to be true alleles if they have amplitudes between 75 and 150 rfu. Figure A4 is one example in which three of the alleles in a mixed sample appeared above the 75 rfu threshold, but one was below and therefore was not called by the analysis software.

Concerning the importance of peak heights above threshold levels, another analysis that can be useful is to compare the general productivity of the two destain groups as it relates to the total number of loci per sample, as well as the number of loci each produced above the threshold of 150 rfu (subtracting out loci between 75 and 150 loci). A t-test conducted on the total number of loci typed for the 1% and 0.2% HCl treatment groups showed that they were similar in terms of the actual number of loci they typed. However, a second t-test performed on the two groups after loci under 150 rfu had been subtracted out from the total count revealed that the groups were dissimilar, and that the 0.2% HCl group had a higher overall range of loci typed when compared to the 1% HCl group. These results are listed in Table 4.

**Table 4. T-test results of a comparison between the 1% and 0.2% HCl destain groups.**

| | T-test results | | Confidence Intervals | | |
|---|---|---|---|---|---|
| | Prob>F' | Pooled equal variances *p* -value | Lower CL Mean | Mean | Upper CL Mean |
| **Loci** | | | | | |
| **Total Loci typed** | 0.5165 | 0.5597 | | | |
| 1% HCl | | | 7.4758 | 9 | 10.524 |
| 0.2% HCl | | | 8.0027 | 9.6364 | 11.27 |
| **Loci above 150** | 0.1142 | 0.0095 | | | |
| 1% HCl | | | 3.2107 | 4.6316 | 6.0524 |
| 0.2% HCl | | | 5.9191 | 7.8182 | 9.7173 |

The loci data sets for these groups were found to be normally distributed as shown earlier. The prob > F' measurement indicates that the variances of the destain groups for both the total number of loci typed and the loci above 150 rfu categories are equal ($\alpha$ =0.05). From this measurement, the Pooled equal variances *p*-value can be used to show if there is a difference between the two groups. At an $\alpha$=0.05, the total loci groups are accepted as similar, because $p$ = 0.5597. However, in the loci above 150 group, $p$ = 0.0095, indicating that the probability that the two groups are similar is less than 0.01. This is reflected in the confidence intervals, where the means are clearly different in the second group but more similar in the first.

## Multiple profiles

In some samples, multiple alleles at each locus were found. This occurs when more than one person's DNA is present in the sample. When mixed samples were encountered, such as in sample S-1 from Table A2, information from the additional alleles was obtained. However, for comparison purposes between mixed and single samples, these extra alleles were not considered in final statistical analysis. In most cases, the alleles could be assigned to either the major or the minor DNA contributor. The major contributor is the person whose DNA profile is represented by the stronger alleles (higher peaks) at each locus. The minor contributor is the person whose DNA profile is represented by the weaker (lower alleles) at each locus in the mixture. Figure A5 shows some examples of these mixtures. In all cases, the minor contributor was a male, as was evident by the Y peak in the Amelogenin locus.

Without proper controls, determining which alleles belonged to the major or minor contributor was not possible, especially in larger loci where all alleles appeared to be approximately the same height (in rfu). Alleles were selected as major or minor contributors on an individual basis so that analyses could be performed on these samples. At smaller loci, a sufficient difference in peak heights of alleles helped to determine major and minor contributor alleles. Stutter is a sample artifact caused by overloading DNA in PCR and subsequent problems with the enzyme *Taq* polymerase as it creates products 1 tetramer shorter than the desired product. This stutter appears as a much smaller peak in a position n-4 bases shorter than the actual allele. Percentages of stutter for each locus ((% of stutter peak/% of allele peak) x 100) have been established so that peaks that appear above these percentages will not be discounted as stutter. Smaller peaks found at the shorter loci could not always be ruled out as stutter. In a similar manner, some stutter peaks could not be ruled out as actual alleles because of the lack of controls. Because of these issues, any peaks that were questionable as stutter or alleles were kept as alleles. In other cases where three or four alleles were all of similar height, especially at the larger loci, the two highest alleles were 'called' as major contributor, while remaining alleles were left to the minor contributor profile. These decisions on major and minor contributors were not intended to separate out individual profiles as a forensic laboratory would, but were done solely to analyze these mixed samples alongside single profile samples in the same destain group. Only a comparative sample from the individual(s) could correctly identify all of the alleles.

Of the 46 tested archived slides, 9 were mixed samples for a total of almost 20%. Laboratory personnel who might have had contact with these slides have identification profiles on record, and all were ruled out as potential donors. Extraction protocols which can separate out sperm from other cellular material could remove the secondary profile, but this was not done in this project.

## Final protocol suggestions

### Coverslip removal

For most of the samples, the best method for removal of coverslips was to place the slides in xylene at room temperature overnight, then to facilitate the removal as needed with a spatula. This is in contrast to the method proposed by Poljak [12], who placed the slips in xylene for 2 hours at -30°C and then into a 37°C bath for 10 minutes. This latter method was not feasible for our laboratory due to safety concerns, and we were unable to modify it successfully.

### Destaining

Preliminary results indicate that it is best not to destain slides at all. These results suggest that any destain applied to a sample should contain a low HCl concentration (e.g., 0.2% or lower). The correlation studies showed that increasing HCl concentration has a detrimental effect on peak heights, and may have caused additional loci to be untypable through allele dropout.

### Slide sampling

Once the slides had been destained (as well as slides not destained), the next step was to select an area of the slide from which to remove material for analysis. This area was selected on an individual basis for the first round of samples, based on a region that had little stain remaining relative to the rest of the slide. The amount of stain remaining and the area harvested varied from slide to slide. The area that was harvested was measured so that comparisons could be made between slides. In later slide sets a constant area of sample was harvested, generally an area 200 $mm^2$. This area represented less than one-quarter of the total material found on most slides, and was adequate for DNA extraction.

### DNA quantitation

Though the QuantiBlot measurement had been made of DNA concentrations, and more than 1 ng of DNA was added to the reaction, concerns existed that this quantitation method might not give an accurate concentration measurement because it could not detect degraded DNA. This is due to the size of the probe used for the procedure, which is slightly over 40 bases in length. While it does detect the presence of primate DNA, it

cannot determine the fragment size beyond 40 bases. This means that QuantiBlot has no way of determining the number of target fragments available for the amplification of larger loci, particularly fragments 200 bases and longer.

When correlation studies were performed between QuantiBlot measurements and fluorometric measurements for the same sample, no correlation was found (results not shown). In general, the fluorescent measurements were higher than the QuantiBlot measurements, and in some cases much higher. Since neither measurement was an accurate description of how much DNA was present or would be required for adequate PCR amplifcation, the QuantiBlot measurement should probably be considered superior to the fluorometric measurement because it does measure the amount of human DNA as opposed to all DNA. QuantiBlot is commonly utilized quantitation method in forensics laboratories because of its specificity to human (primate) DNA, and might be easier to incorporate into this protocol.

The amount of DNA solution added to a 50 μL PCR reaction ranged from 1 μL to 14 μL. Neither QuantiBlot nor fluorometric methods gave accurate measurements of the actual amount of DNA. In general, the amount required to give the most typable number of loci greater than what would normally be used for conventional samples such as blood standards. Often 2 to 5 times more DNA (equivalent to 4 – 10 ng) from archived slides had to be used in PCR amplifications than is required from blood or other fresh forensic samples (the target amount is 1 ng).

## Statistical Analysis

The statistical exclusion analysis of all the samples was calculated for four of the largest ethnic backgrounds in East Tennessee. The results are listed in Table A8. The exclusion ranged from 1 in 1000 to 1 in $10^{19}$, depending on the alleles that were typed for each locus and the number of loci that were typed for each sample. As a general rule, as the number of loci that were typed increased, so did the exclusion rate. This was true for all destain groups and controls, regardless of ethnic group as can be seen in Figure A6(a-d).

The mixed samples, which were statistically analyzed using a different equation, showed lower numbers overall. This means that they were not as effective in identifying a particular profile as unique. However, the numbers ranged from $1.4 \times 10^6$ to $1.9 \times 10^{12}$, so at worst these samples still produced profiles that were specific to 1 in every 1,000,000 people.

# IV. Discussion

A major goal of this project was to develop a protocol for the recovery of DNA from archived Pap smear slides that could be used successfully to identify individuals – in particular, missing persons – in forensics cases. A related goal was to determine if these samples would amplify and produce profiles effectively with multiplexed primer sets. Several issues arose in the course of the achievement of these goals, which warrant further discussion.

## Protocol Suggestions

The protocol developed in this project represents a minor, but significant, improvement on the standard extraction protocol used for other types of forensic samples as it relates to degraded Pap smear slides. Removal of the cover slip is one procedure that is not routinely necessary for other types of forensic samples, however it can be done with little effort. Though previous reports suggested that coverslip removal could be done in a matter of a few hours [11] the final step of immersion in xylene at 37°C is often not feasible because a suitable ventilation hood is required. While this step would be preferable to the overnight procedure used in the present research, the overnight immersion in xylene at room temperature was not inconvenient. Coverslip removal was facilitated in this study with the aid of a small spatula. Very little source material was removed during this process. Alternatively, it was observed that coverslips fell off spontaneously within 48 hours of immersion.

The destaining procedure was evaluated at both the standard 1% HCl level and the alternate 0.2% HCl level. Some authors had suggested that 1% HCl destaining was necessary in order to amplify Papanicolau-stained smears [9]. All samples tested with less than 1% HCl or no destain at all, produced a DNA profile. In the case of the 0.2% HCl destain, samples were shown to produce numbers of loci with higher peaks than the 1% HCl destained group. Had additional samples been tested without any destaining procedure, it is possible that they would have performed even better than the 0.2% HCl destained samples. Since staining may vary widely from sample to sample, some procedure to minimally destain the slide may still be necessary. However, if the amount of sample permits more than one test it may be best to attempt an extraction with no

destaining procedure. If initial results are poor, it would be possible to return to the slide and destain a portion with a weaker solution such as the 0.2% HCl destain reagent. Ultimately it was found that the 1% HCl was more detrimental to the DNA than inhibition caused by remaining Papanicolau stain, and would not be recommended for archival DNA extraction.

The amount of cellular material needed for DNA extraction from Pap smear slides was in most cases minimal. To ensure that satisfactory results were obtained, most samples required a 200 $mm^2$ region from the slide. In most cases this is a reasonable amount of material to take from most slides and usually yielded enough DNA for PCR analysis. This area represented, in most cases, less than one-fourth (<25%) of the total slide material. If half of the material was needed to preserve for the medical history, then at least another quarter would be available for additional analysis. In addition, if the slide did not have to be destained for analysis, once a sample had been removed the coverslip could be replaced. This practice would help preserve the remaining sample for subsequent medical or forensic analysis.

The Proteinase K buffer and PCI extractions employed in this study were simple and similar to existing protocols. For convenience, the Proteinase K extraction was performed overnight. Some literature and protocols suggest that this step could be done in as little as 2-4 hours. The PCI extraction was unchanged from the method used most widely, and took less than 2 hours to perform even for a large number of samples (>20).

The QuantiBlot and the fluorometric quantitation methods were useful in that they served as a check for the presence of DNA prior to the expensive step of PCR amplification and electrophoretic analysis. However, for the actual quantitation of DNA neither method proved to be particularly useful. Though correlations could be shown for the amount of DNA added to PCR and the number of loci that were typed for at least one of the destain methods, neither quantitation method gave entirely reliable prediction of PCR yields.

The largest problem is that the DNA was in a degraded state on the archived slides. Calculations from these types of quantitation methods are used to add a specific amount of DNA to a PCR reaction to achieve optimal results. For new samples, this

amount is generally around 1ng of template DNA. In degraded samples, this amount cannot be quantified. The probe used in QuantiBlot is approximately 40 bases in length. This is smaller than all of the loci typed in either Profiler Plus or COfiler, which means that for degraded DNA nothing is really known about the state or presence of DNA fragments larger than 40 bases. Though QuantiBlot or fluorometric analysis may suggest for example that 1 ng of template DNA is present, this does not mean that 1 ng of completely typable DNA is present. This is especially true of alleles requiring larger DNA fragments for successful amplification. This means that in order to achieve usable results from degraded DNA samples, a fundamentally different method by which DNA is quantified prior to extraction must be used. Analysts should be willing to use a quantitated measurement as an initial guide only, and then increase the amount suggested by as many as 2 to 5 times what should be added for standard samples. In our hands we found a direct linear correlation between the amount of DNA added and the number of loci typed (for the 0.2% HCl destain samples). However, it is possible that a plateau effect might be reached beyond which no more loci could be typed from a particular sample. A larger sample size with multiple trials on each sample may answer this question.

Amplification by polymerase chain reaction was performed with a standard program used for many other types of samples and standards. There were no problems with this program, and in most cases ample signal was achieved to identify alleles during capillary electrophoresis. In most forensic laboratories, the minimal threshold peak height at which alleles are called is between 75 and 150 rfu. There were some instances where alleles were not called because they were just below 75 rfu threshold. In other cases, alleles might have been above the 150 rfu threshold had they been amplified a few more cycles.

Two steps could be taken to increase the signal strength of these alleles. The first would be to increase the number of cycles in the PCR amplification program. With this increase, however, comes a new set of problems. As cycle number increases, so does the risk for contamination. This is especially true as the cycle number approaches 40 and over, requiring significant measures to control contamination from the analyst and other

airborne sources. Not all laboratories are equipped to handle contamination issues of that magnitude, or have the space to dedicate to a 'clean room'. Another problem is that the peak heights for the smaller loci are already at a near-maximum level. Increasing the amplification cycles would more than likely drive the peaks of smaller loci up, but make little relative change to the larger alleles. Amplification of smaller alleles occurs much more quickly because they are shorter and do not require the time that larger alleles need for amplification. If the peaks of shorter alleles go off-scale, the profile would need to be re-run because it will not be approved and will have problems in court proceedings. It is for both these reasons that increasing the cycle number might not necessarily be the best option for increasing peak heights.

The second option is to increase the injection time of the sample as it is analyzed by the CE instrument. The default injection time currently used by most analysts is 5 seconds. This means that the capillary takes sample from the sample tube for a period of 5 seconds at a constant rate. A certain amount of material will be taken through the polymer for analysis. Increasing the injection time of the sample means that more material will be run through the polymer, increasing the strength of the signal. This holds some of the same risks as increasing the cycle number during thermal cycling. There is a possibility that the shorter loci will have off-scale peaks because the material was overloaded onto the capillary. However, unlike PCR, if the increased injection time does not work, then the sample can be re-injected for a shorter period of time. A PCR product that became contaminated or overloaded due to increased cycling would have to be re-amplified. This would use additional sample and would be very time-consuming. Because the run time for one sample on a CE instrument using the specifications outlined in Table 2 would take approximately 30 minutes, this is much preferable to several hours of re-amplification by PCR.

Of some interest is the fact that almost 20% of the archived slides tested were mixed with a male profile. The source of this second profile is most likely varied to a certain degree. The list of possible contributors includes medical personnel, laboratory technicians, a 'significant other', and male analyst donor. As all of the profiles of analysts who had any contact with the samples were available, they were immediately

ruled out. The amount of the secondary profile present in these samples would be much less if it were a result of routine handling in the clinical laboratory. Though impossible to confirm, the most likely contributor is a 'significant other'.

Even with this rather large number of mixed profiles, there would be no problem with identification of a missing individual. In such mixtures, all alleles would be reported and a match could be obtained by identifying the two alleles belonging to a single individual. Statistical analysis of these mixtures still ranged from 1 in 1,000,000 to 1 in $10^{12}$, meaning that the probability of finding the same two profiles in two other individuals is still extremely low. If there is a question, there are protocols that exist where the male sperm DNA is separated out from the rest of the sample, thereby separating the two samples. If the second profile is truly from a significant other this protocol should work, or at least minimize the second profile to a point that major and minor contributors could be identified. If the second profile is from another source, statistical analysis of the profile isolated from the mixture as it matches remains of a missing person might still be conclusive enough to identify the person. This would be especially true if additional circumstances were available that could help point to that particular individual.

Exclusion analysis of the samples in general was useful in showing how well they could identify a person given the profile they presented. This range was from 1 in 1000 to 1 in $10^{19}$, meaning that the odds of finding another individual with that profile were at best 1 in every 1000. These numbers were calculated based on populations indigenous to East Tennessee. These included Caucasian, African American, Southeastern Hispanic, and Southwestern Hispanic. This means that even a sample that only produced 2 loci of 13 could still identify an individual out of 1000 others.

## CODIS Database

Databases such as CODIS require 10 of 13 loci to be present before the profile can be placed at the national database. The database is set up on three levels: national, state, and local. National level requires a larger number of loci because searching profiles with only a few loci would yield unacceptably long search times. The number of

hits would also increase, as would the number of potential errors and the servers would become sluggish with additional workload. At the state and local levels, however, no such limit is currently in place. This means that profiles could be added and searched as needed. This is still useful for missing persons cases, because generally the person missing is also local and there is no need to search a national database, just at the local or possibly state level would be all that was required.

A national missing persons database is currently available in the United States. CODIS [13] was originally designed to not only handle the profiles of convicted felons and forensic unknowns, but missing person profiles as well. The problem, however is at the level of the laboratory, where the majority of laboratories are busy working on criminal cases, not necessarily missing persons cases. Spain has taken this step, however, and has a working missing persons database that serves that country [14]. They have been able to make a number of identifications based on information obtained from that database, and have plans to expand it in the future. Other nations are following suit, implementing databases of their own.

## Ethical Issues

Several ethical issues are raised when methods of DNA profiling and national databases are discussed. One such issue is the use of material taken for medical purposes to be used for another purpose other than its original intent. This would be the case for Pap smears, used for medical tests but could be used for identification in a forensics case. The patient only gave consent for the material to be used for a medical exam and no other purpose.

If Pap smears are to be used for forensic identification, it is hoped that a family member would be able to give this consent in the patient's absence. However, this is not always possible. Other types of materials, such as dental records, are also used for identification. In many cases consent cannot be obtained for the use of these records either. Nevertheless, dental records are still used frequently for forensic identification. One might assume that there would be an implied consent: if a person were missing, she would want investigators to use every means available to them and that would include

medical slides. However, if Pap smears and other such materials are used in the future for identification, the better solution might be for the person to be informed of this possibility prior to any procedure and their consent obtained at that time.

The second issue of great concern ethically is the acquisition of DNA profiles that will be placed in some form of a national database. There is a tendency to confuse a database like CODIS with a national database created solely for mass identification. The purpose of CODIS is to identify individuals, but in the context of connecting them to crimes or missing persons. People whose profiles are in this database are already linked to a crime in some form, either as convicted felons, victims, or another similar way. Profiles of the population at large are not placed into CODIS; neither is its purpose simply to have a person's profile on record like a social security number. In addition, access to this database is restricted to law enforcement personnel who use it in solving crimes. The purpose of this research is not to support any form of a mass identification DNA database. It is simply to provide another method by which people already linked to a crime in some manner might be identified so that justice could be served.

## Future Work and Goals

This project could be expanded in several directions, all of which would enhance the understanding of this field of research. One area would be to continue the study of the effects of destain on stained archival DNA samples. It would be beneficial to extract DNA from the same slides where one side had been treated with a destain and the other with no destain. This would more clearly show the effects of destain on the samples. Also, a greater sample number would help provide additional statistical information that was not available in this project.

Another direction that would be helpful would be to extract material from a slide and repeat the process two or three times. This would show reproducibility of the extraction process, and that the gain or loss of allelic information was not a random occurrence. It was shown in this project that the protocol used was able to retrieve and type DNA from archived slides. The number of slides – approximately 55 – is a rather large number statistically. However, while the protocol may work for DNA extraction,

showing that it can reliably produce the same results from the same sample would be beneficial information. When a CODIS match is discovered, both of the analysts who have the matched evidence and subject standard must re-extract DNA from the standard and show that the match is positive and was not an error. Showing that the protocol will give the same results more than once would ensure that this would not be a problem for analysts who might need to perform this procedure.

Though the Profiler Plus and COfiler amplification kits are the current standard for DNA identification, there is a new kit available that might soon replace them. Identifiler (Applied Biosystems) has recently been released, and it contains fifteen loci: the thirteen covered by the two current kits plus two additional loci. Both of these loci are smaller, between 100 and 200 base pairs. This might be helpful for degraded DNA samples such as the archived Pap smear material in this study because there is a chance they will amplify enough to be seen where larger loci are not available to amplify. If these loci did amplify, their alleles can be added into the statistical analysis, which would further isolate a particular profile as unique.

One potentially promising area in which this research might be expanded is degraded DNA repair. Imyanitov, *et al.* [15] showed that non-denatured DNA template with single-strand nicks caused by degradation could be partially restored by filling in the nicks in the DNA polymerase reaction. Though their work involved paraffin-embedded tissue, it could be done with this type of material as well. After the tissue was deparaffinized, partial digestion with Proteinase K was performed. Then the tissue was partially solubilized in a PCR-like mixture containing Tris buffer, $MgCl_2$, Triton X-100, and all four dNTPs for 60 minutes at 55°C. Next, *Taq* polymerase was added and the DNA polymerase reaction was carried out for 20 minutes at 72°C. This restores the DNA, which can then be isolated by protein digestion with Proteinase K for several hours and then is suitable for PCR. This increased the signal from DNA fragments ranging from 100 to 270 bases in length, which covers the lengths of the majority of the loci used in identification. This procedure may prove to be useful in restoring some of the larger loci that were lost to degradation over time.

## Concluding Remarks

In the past, Pap smears have proven to be useful for obtaining DNA for basic pathological analysis and older forms of forensic identification. With the current use of multiplexed primer sets that amplify multiple loci of varying lengths, archived samples have again proven to be useful sources of DNA for identification. Because of degradation over time and from fixation, working with these samples can be more difficult than fresh samples ordinarily used. However, with some additional steps and alterations to the protocol this can be done and can yield a significant amount of information. If other forms of identifying an individual are unavailable, DNA can be retrieved from these archived samples that will aid in that process. The entire sample is not consumed during analysis, which means that much of the material can be preserved for a medical record or used for multiple analyses if required. While samples which contained a second profile were encountered, additional extractions could be performed which may isolate the second profile. If additional extractions were not possible, statistical analysis would at least make an identification still possible. All of the samples tested yielded profiles with varying numbers of loci, but none were completely negative. Destaining had an effect on the quality of the profile that could be obtained, so it is not recommended unless absolutely necessary. Overall, archived samples are still valid sources of DNA for identifying an individual using the current Profiler Plus and COfiler primer sets, and should be considered for use in missing persons cases when other evidence is not available.

# References

## References


1. Baker, L.E., W.F. McCormick, and K.J. Matteson, *A silica-based mitochondrial DNA extraction method applied to forensic hair shafts and teeth.* J Forensic Sci, 2001. **46**(1): p. 126-30.
2. Shibata, D., M. Kurosu, and T.T. Noguchi, *Fixed human tissues: a resource for the identification of individuals.* J Forensic Sci, 1991. **36**(4): p. 1204-12.
3. Roy, R. and R. Reynolds, *AmpliType PM and HLA DQ alpha typing from pap smear, semen smear, and postcoital slides.* J Forensic Sci, 1995. **40**(2): p. 266-9.
4. Saiki, R.K., et al., *Genetic analysis of amplified DNA with immobilized sequence-specific oligonucleotide probes.* Proc Natl Acad Sci U S A, 1989. **86**(16): p. 6230-4.
5. Dimo-Simonin, N., F. Grange, and C. Brandt-Casadevall, *PCR-based forensic testing of DNA from stained cytological smears.* J Forensic Sci, 1997. **42**(3): p. 506-9.
6. Sweet, D., D. Hildebrand, and D. Phillips, *Identification of a skeleton using DNA from teeth and a PAP smear.* J Forensic Sci, 1999. **44**(3): p. 630-3.
7. Poljak, M. and J. Barlic, *Rapid, simple method of extracting DNA from archival Papanicolaou-stained cervical smears.* Acta Cytol, 1996. **40**(2): p. 374-5.
8. Poljak, M., K. Seme, and J. Barlic, *Processing of long-stored archival Papanicolaou-stained cytological smears.* Br J Cancer, 1996. **74**(9): p. 1508-9.
9. Chen, J.T., M.A. Lane, and D.P. Clark, *Inhibitors of the polymerase chain reaction in Papanicolaou stain. Removal with a simple destaining procedure.* Acta Cytol, 1996. **40**(5): p. 873-7.
10. Pavelic, J., et al., *PCR amplification of DNA from archival specimens. A methodological approach.* Neoplasma, 1996. **43**(2): p. 75-81.
11. Poljak, M., K. Seme, and N. Gale, *Rapid extraction of DNA from archival clinical specimens: our experiences.* Pflugers Arch, 2000. **439**(3 Suppl): p. R42-4.
12. Butler, J.M., *Forensic DNA Typing: Biology and technology behind STR markers.* 2001, New York: Academic Press.
13. Baechtel, F.S., et al., *Tracking the violent criminal offender through DNA typing profiles - a national database system concept.* Exs, 1991. **58**: p. 356-60.
14. Lorente, J.A., et al., *Identification of missing persons: the Spanish "Phoenix" program.* Croat Med J, 2001. **42**(3): p. 267-70.
15. Imyanitov, E.N., et al., *Partial restoration of degraded DNA from archival paraffin-embedded tissues.* Biotechniques, 2001. **31**(5): p. 1000, 1002.

# Appendices

# Appendix A

**Appendix A, Table A1. Sample extraction parameters and results. Coverage is the relative thickness of the material on the slide (L = light, M = medium, H = heavy); Amount is the amount of material removed from the slide in millimeters; Destain is the treatment the slide received and the length of time in destain. Final Volume is the final elution volume of the sample after PCI extraction. QB and fluorometric results are the amount of DNA in ng/µL quantified by each method while PCR Volume is the amount of DNA solution added to a 50 µL PCR reaction. The number of loci is the total number of loci obtained for a sample, while Alleles below/above 150 define the number of alleles above/below the 150 rfu threshold. The mixture designates those samples that were mixed profiles (Y=yes) or single profiles (N=no).**

| Samples | Coverage | Amount (in $mm^2$) | Destain | Final Vol. | QB results (ng/mL) | fluoro results (ng/mL) | PCR Vol (in mL) | ng for PCR - QB | ng for PCR - fluoro. | # Loci | Alleles below 150 | Total alleles above 150 | Mixture? |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S-1 | M | 81 | 1%/30min | 100mL | 0.25 | 4 | 6 | 1.5 | 24 | 13 | 3 | 10 | Y |
| S-2 | M | 111 | 1%/30min | 100mL | 0.25 | 2 | 6 | 1.5 | 12 | 11 | 9 | 2 | N |
| S-3 | L-M | 168 | 1%/30min | 100mL | 2 | 14 | 1 | 2 | 14 | 12 | 5 | 7 | N |
| S-4 | H | 209 | 1%/30min | 100mL | 1.25 | 14 | 2 | 2.5 | 28 | 4 | 4 | 0 | N |
| S-5 | L | 91 | 1%/30min | 100mL | 2 | 10 | 1 | 2 | 10 | 12 | 4 | 8 | N |
| S-6 | M | 117 | 1%/30min | 100mL | 2 | 9 | 1 | 2 | 9 | 6 | 3 | 3 | N |
| S-7 | H | 164 | 1%/30min | 100mL | 2 | 17 | 1 | 2 | 17 | 11 | 5 | 6 | N |
| S-8 | M | 272 | 1%/30min | 100mL | 0.8 | 4 | 2 | 1.6 | 8 | 8 | 4 | 4 | N |
| S-9 | M | 407 | 1%/30min | 100mL | 1 | 5 | 2 | 2 | 10 | 10 | 5 | 5 | N |
| S-10 | M | 90 | 1%/30min | 100mL | 2 | 7 | 14 | 28 | 98 | 10 | 7 | 3 | N |
| S-11 | M | 222 | 1%/30min | 100mL | 2 | 6 | 14 | 28 | 84 | 9 | 6 | 3 | N |
| S-12 | M | 315 | 1%/30min | 100mL | 2 | 23 | 14 | 28 | 322 | 12 | 2 | 10 | N |
| S-13 | M | 214 | 1%/30min | 100mL | 1 | 6 | 14 | 14 | 84 | 5 | 3 | 2 | N |
| S-14 | L | 234 | 1%/30min | 100mL | 2 | 6 | 14 | 28 | 84 | 9 | 5 | 4 | N |
| S-15 | L | 400 | 1%/30min | 100mL | 2 | 5 | 14 | 28 | 70 | 13 | 5 | 8 | N |
| S-16 | L | 289 | 1%/30min | 100mL | 2 | 15 | 14 | 28 | 210 | 11 | 11 | 0 | Y |
| S-17 | L | 272 | 1%/30min | 100mL | 2 | 23 | 14 | 28 | 322 | 7 | 2 | 5 | N |
| S-18 | L | 103.5 | 1%/30min | 100mL | 0.8 | 3 | 14 | 11.2 | 42 | 5 | 0 | 5 | N |
| S-19 | L | 150 | 1%/30min | 100mL | 0.6025 | 3 | 14 | 8.435 | 42 | 3 | 0 | 3 | N |
| S-20 | L | 200 | 0.2%/30min | 100mL | 0.5 | 3 | 5 | 2.5 | 15 | 10 | 8 | 2 | Y |
| S-21 | M | 200 | 0.2%/30min | 100mL | 2 | 9 | 5 | 10 | 45 | 13 | 1 | 12 | N |
| S-22 | L | 200 | 1%/6min | 100mL | 0.5 | 7 | 5 | 2.5 | 35 | 6 | 3 | 3 | N |
| S-23 | M | 200 | 1%/6min | 100mL | 0.5 | 3 | 5 | 2.5 | 15 | 6 | 5 | 1 | N |
| S-24 | C | 200 | 1%/6min | 100mL | 1 | 5 | 5 | 5 | 25 | 13 | 0 | 13 | N |
| S-25 | BUCCAL | - | - | 200mL | 1 | 4 | 5 | 5 | 20 | 13 | 0 | 13 | N |
| S-26 | C | 200 | - | 200mL | 1 | 5 | 5 | 5 | 25 | 13 | 0 | 13 | N |
| S-27 | BUCCAL | - | - | 200mL | 2 | 15 | 5 | 10 | 75 | 13 | 0 | 13 | N |
| S-28 | C | 200 | - | 200mL | 2 | 5 | 5 | 10 | 25 | 13 | 0 | 13 | N |
| S-29 | BUCCAL | - | - | 200mL | 2 | 10 | 5 | 10 | 50 | 13 | 0 | 13 | N |
| S-30 | C | 200 | - | 200mL | 0.125 | 3 | 8 | 1 | 24 | 13 | 0 | 13 | N |

**Appendix A, Table A1. Continued.**

| Samples | Coverage | Amount (in $mm^2$) | Destain | Final Vol. | QB results (ng/mL) | fluoro results (ng/mL) | PCR Vol (in mL) | ng for PCR - QB | ng for PCR - fluoro. | # Loci | Alleles below 150 | Total alleles above 150 | Mixture? |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S-31 | BUCCAL | - | - | 200mL | 1 | 4 | 5 | 5 | 20 | 13 | 0 | 13 | N |
| S-32 | M | 200 | 0.2%/30min | 100mL | 0.25 | 3 | 5 | 1.25 | 15 | 2 | 2 | 0 | N |
| S-33 | M | 200 | 0.2%/30min | 100mL | 0.5 | 8 | 5 | 2.5 | 40 | 6 | 1 | 5 | N |
| S-34 | M | 200 | 0.2%/30min | 100mL | 0.5 | 5 | 5 | 2.5 | 25 | 6 | 3 | 3 | N |
| S-35 | M | 200 | 0.2%/30min | 100mL | 0.5 | 5 | 5 | 2.5 | 25 | 5 | 1 | 4 | N |
| S-36 | M | 200 | 0.2%/30min | 100mL | 2 | 17 | 5 | 10 | 85 | 13 | 0 | 13 | N |
| S-37 | M | 200 | 0.2%/30min | 100mL | 2 | 13 | 5 | 10 | 65 | 10 | 2 | 8 | N |
| S-38 | M | 200 | 0.2%/30min | 100mL | 0.5 | 3 | 5 | 2.5 | 15 | 5 | 2 | 3 | N |
| S-39 | M | 200 | 0.2%/30min | 100mL | 1 | 8 | 5 | 5 | 40 | 12 | 4 | 8 | N |
| S-40 | M | 200 | 0.2%/30min | 100mL | 2 | 12 | 5 | 10 | 60 | 13 | 0 | 13 | N |
| S-41 | M | 200 | 0.2%/30min | 100mL | 2 | 14 | 5 | 10 | 70 | 13 | 1 | 12 | N |
| S-42 | M | 200 | 0.2%/30min | 100mL | 2 | 13 | 5 | 10 | 65 | 10 | 1 | 9 | N |
| S-43 | M | 200 | 0.2%/30min | 100mL | 0.5 | 6 | 5 | 2.5 | 30 | 12 | 1 | 11 | Y |
| S-44 | M | 200 | 0.2%/30min | 100mL | 2 | 40 | 5 | 10 | 200 | 13 | 1 | 12 | N |
| S-45 | M | 200 | 0.2%/30min | 100mL | 1 | 6 | 5 | 5 | 30 | 13 | 1 | 12 | Y |
| S-46 | M | 200 | 0.2%/30min | 100mL | 1 | 13 | 5 | 5 | 65 | 5 | 2 | 3 | N |
| S-47 | M | 200 | 0.2%/30min | 100mL | 1 | 16 | 5 | 5 | 80 | 11 | 3 | 8 | N |
| S-48 | M | 200 | 0.2%/30min | 100mL | 2 | 16 | 5 | 10 | 80 | 13 | 1 | 12 | N |
| S-49 | M | 200 | 0.2%/30min | 100mL | 0.5 | 6 | 5 | 2.5 | 30 | 4 | 2 | 2 | N |
| S-50 | M | 200 | 0.2%/30min | 100mL | 2 | 15 | 5 | 10 | 75 | 10 | 1 | 9 | N |
| S-51 | M | 200 | 0.2%/30min | 100mL | 2 | 8 | 5 | 10 | 40 | 13 | 2 | 11 | N |
| S-52 | - | 200 | - | 100mL |  | 11 | 5 |  | 55 | 13 | 0 | 13 | Y |
| S-53 | - | 200 | - | 100mL |  | 8 | 5 |  | 40 | 13 | 0 | 13 | Y |
| S-54 | - | 200 | - | 100mL |  | 27 | 5 |  | 135 | 11 | 2 | 9 | Y |
| S-55 | - | 200 | - | 100mL |  | 34 | 5 |  | 170 | 13 | 0 | 13 | Y |
| S-56 | - | 200 | - | 100mL |  | 7 | 5 |  | 35 | 4 | 2 | 2 | N |
| S-57 | - | 200 | - | 200mL |  | 1 | 5 |  | 5 | 0 | 0 | 0 | N |
| S-58 | - | 200 | - | 200mL |  | 1 | 5 |  | 5 | 0 | 0 | 0 | N |
| S-59 | - | 200 | - | 200mL |  | 3 | 5 |  | 15 | 13 | 0 | 13 | N |
| S-60 | - | 200 | - | 200mL |  | 4 | 5 |  | 20 | 13 | 0 | 13 | N |
| S-61 | - | 200 | - | 200mL |  | 2 | 5 |  | 10 | 13 | 0 | 13 | N |

**Appendix A, Table A2. Sample set S-1 – S-9. Samples listed with allele designation, peak height in relative fluorescent units (rfu), and size in bases. Samples denoted with (a) and (b) are results of a mixed profile (a sample that has multiple alleles, consistent with a mixture of DNA of more than one person). Blank fields indicate loci or allele locations where no signal was detected.**

| Sample | D3S1358 | | vWA | | FGA | | D8S1179 | | D21S11 | | D18S531 | | D5S818 | | D13S317 | | D7S820 | | D16S539 | | TH01 | | TPOX | | CSF1PO | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **S-1(a)** | 15 | 15 | 16 | 16 | 22 | 22 | 14 | 14 | 28 | 30 | 21 | 17 | 13 | 11 | 11 | 12 | 11 | | 9 | 10 | 8 | 9.3 | 8 | 9 | 8 | 8 |
| Height | 2248 | 2248 | 913 | 913 | 526 | 526 | 1916 | 1916 | 298 | 223 | 106 | 92 | 791 | 693 | 200 | 254 | 84 | | 247 | 216 | 835 | 606 | 356 | 301 | 318 | 318 |
| Size | 123.1 | 123.1 | 175 | 175 | 232.3 | 232.3 | 149.2 | 149.2 | 200.7 | 208.6 | 321.4 | 304 | 157.1 | 148.3 | 217.1 | 221.1 | 276.4 | | 245.4 | 249.4 | 179.3 | 186.2 | 223 | 227 | 288.1 | 288.1 |
| **S-1(b)** | 14 | 17 | | | | | | | 32 | | 15 | | 12 | 12 | | | | | 13 | | | | 11 | | | |
| Height | 131 | 144 | | | | | | | 76 | | 78 | | 154 | 154 | | | | | 95 | | | | 145 | | | |
| Size | 119.2 | 131.6 | | | | | | | 216.5 | | 295.3 | | 152.8 | 152.8 | | | | | 261.5 | | | | 235 | | | |
| **S-2** | 17 | 17 | 16 | 17 | 19 | 20 | 13 | 13 | 29 | 31.2 | 14 | 14 | 11 | 12 | 11 | 11 | | | 11 | 11 | 7 | 9.3 | 8 | 10 | 16 | |
| Height | 1462 | 1462 | 235 | 230 | 131 | 96 | 1334 | 1334 | 140 | 91 | 99 | 99 | 723 | 581 | 128 | 128 | | | 160 | 160 | 368 | 362 | 104 | 80 | 97 | |
| Size | 131.6 | 131.6 | 175 | 179 | 220.2 | 224.2 | 144.7 | 144.7 | 204.7 | 214.5 | 291.2 | 291.2 | 148.3 | 152.8 | 217.1 | 217.1 | | | 253.5 | 253.5 | 175.2 | 186.2 | 223 | 230.9 | 322.2 | |
| **S-3** | 17 | 17 | 14 | 15 | 22 | 23 | 11 | 12 | 29 | 30 | 16 | | 9 | 13 | 10 | 11 | | | 11 | 13 | 7 | 8 | 8 | 10 | 11 | |
| Height | 2250 | 2250 | 514 | 463 | 236 | 136 | 1720 | 1415 | 304 | 215 | 97 | | 881 | 584 | 274 | 187 | | | 89 | 83 | 517 | 401 | 196 | 205 | 78 | |
| Size | 131.6 | 131.6 | 167.1 | 171.1 | 232.3 | 236.3 | 135.9 | 140.1 | 204.7 | 208.6 | 299.5 | | 139.5 | 157.1 | 213.1 | 217.1 | | | 253.5 | 261.5 | 175.2 | 179.3 | 223 | 230.9 | 300.5 | |
| **S-4** | 14 | 17 | | | | | 13 | 14 | | | | | 11 | 12 | | | | | | | 9.3 | 9.3 | | | | |
| Height | 321 | 151 | | | | | 113 | 151 | | | | | 105 | 79 | | | | | | | 122 | 122 | | | | |
| Size | 119.2 | 131.6 | | | | | 144.7 | 149.2 | | | | | 148.3 | 152.8 | | | | | | | 186.2 | 186.2 | | | | |
| **S-5** | 18 | 18 | 14 | 18 | 20 | 21 | 13 | 14 | 29 | 31.2 | 14 | 16 | 11 | 13 | 8 | 11 | 8 | 9 | 13 | 13 | 8 | 8 | 8 | 11 | | |
| Height | 2132 | 2132 | 477 | 303 | 255 | 154 | 897 | 1001 | 327 | 238 | 98 | 113 | 758 | 695 | 251 | 136 | 85 | 101 | 246 | 246 | 1104 | 1104 | 315 | 237 | | |
| Size | 135.7 | 135.7 | 167.1 | 183 | 224.2 | 228.2 | 144.7 | 151.4 | 204.7 | 214.5 | 291.2 | 299.5 | 148.3 | 157.1 | 205 | 217.1 | 264.1 | 268.2 | 261.5 | 261.5 | 179.3 | 179.3 | 223 | 235 | | |
| **S-6** | 16 | 18 | 17 | 17 | | | 10 | 13 | 30 | 30 | | | 11 | 11 | | | | | | | 7 | 8 | | | | |
| Height | 307 | 288 | 176 | 176 | | | 460 | 345 | 121 | 121 | | | 475 | 475 | | | | | | | 138 | 118 | | | | |
| Size | 127.4 | 135.7 | 179 | 179 | | | 131.8 | 144.7 | 208.6 | 208.6 | | | 148.3 | 148.3 | | | | | | | 175.2 | 179.3 | | | | |
| **S-7** | 16 | 18 | 16 | 17 | 24 | 24 | 13 | 13 | 28 | 32.2 | 13 | 13 | 11 | 12 | 12 | 13 | | | 9 | | 6 | 6 | 8 | 8 | | |
| Height | 914 | 664 | 283 | 212 | 203 | 203 | 1568 | 1568 | 163 | 94 | 76 | 76 | 637 | 542 | 97 | 96 | | | 96 | | 714 | 714 | 217 | 217 | | |
| Size | 127.4 | 135.7 | 175 | 179 | 240.4 | 240.4 | 144.7 | 144.7 | 200.7 | 218.4 | 287 | 287 | 148.3 | 152.8 | 221.1 | 225 | | | 245.4 | | 171.3 | 171.3 | 223 | 223 | | |
| **S-8** | 15 | 16 | 13 | 15 | | | 10 | 14 | 28 | 30 | | | 8 | 11 | 12 | 13 | | | | | 6 | 7 | 8 | | | |
| Height | 765 | 511 | 268 | 230 | | | 762 | 615 | 118 | 116 | | | 471 | 456 | 122 | | | | | | 244 | 246 | 82 | | | |
| Size | 123.1 | 127.4 | 162.8 | 171.1 | | | 131.8 | 149.2 | 200.7 | 208.6 | | | 135.3 | 148.3 | 221.1 | | | | | | 171.3 | 175.2 | 223 | | | |
| **S-9** | 15 | 15 | 17 | 18 | 24 | 24 | 11 | 13 | 28 | 29 | | | 11 | 13 | 8 | | | | 12 | | 6 | 6 | 8 | 11 | | |
| Height | 1627 | 1627 | 209 | 160 | 160 | 160 | 874 | 748 | 135 | 162 | | | 448 | 481 | 86 | | | | 81 | | 583 | 583 | 131 | 78 | | |
| Size | 123.1 | 123.1 | 179 | 183 | 240.4 | 240.4 | 135.9 | 144.7 | 200.7 | 204.7 | | | 148.3 | 157.1 | 205 | | | | 257.5 | | 171.3 | 171.3 | 223 | 235 | | |

**Appendix A, Table A3. Sample set S-32 - S-41, treated with 0.2% HCl destain for 30 minutes. Blank fields indicate loci or allele locations where no signal was detected.**

| Sample | D3S1358 | | vWA | | FGA | | D8S1179 | | D21S11 | | D18S531 | | D5S818 | | D13S317 | | D7S820 | | D16S539 | | TH01 | | TPOX | | CSF1PO | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **S-32** | 15 | 18 | | | | | 10 | | | | | | | | | | | | | | | | | | | |
| Height | 106 | 100 | | | | | 79 | | | | | | | | | | | | | | | | | | | |
| Size | 123.2 | 135.6 | | | | | 131.8 | | | | | | | | | | | | | | | | | | | |
| **S-33** | 14 | 17 | 15 | 19 | | | 11 | 13 | | | | | 11 | 11 | | | | | | | 8 | 9.3 | 8 | 8 | | |
| Height | 845 | 571 | 238 | 153 | | | 623 | 409 | | | | | 485 | 485 | | | | | | | 261 | 217 | 104 | 104 | | |
| Size | 119.2 | 131.6 | 171.1 | 186.9 | | | 135.9 | 144.7 | | | | | 148.3 | 148.3 | | | | | | | 179.3 | 186.2 | 223 | 223 | | |
| **S-34** | 15 | 18 | 15 | 18 | 21 | 21 | 10 | 12 | | | | | 11 | 13 | | | | | | | 6 | 9.3 | | | | |
| Height | 499 | 357 | 135 | 115 | 80 | 80 | 335 | 302 | | | | | 226 | 199 | | | | | | | 177 | 99 | | | | |
| Size | 123.2 | 135.6 | 171.1 | 183 | 228.2 | 228.2 | 131.8 | 140.1 | | | | | 148.3 | 157.1 | | | | | | | 171.3 | 186.2 | | | | |
| **S-35** | 13 | 17 | 18 | 18 | | | 12 | 12 | | | | | 11 | 12 | | | | | | | 6 | 6 | | | | |
| Height | 901 | 351 | 160 | 160 | | | 555 | 555 | | | | | 273 | 225 | | | | | | | 149 | 149 | | | | |
| Size | 115.2 | 131.6 | 183 | 183 | | | 140.1 | 140.1 | | | | | 148.3 | 152.8 | | | | | | | 171.3 | 171.3 | | | | |
| **S-36** | 15 | 16 | 14 | 16 | 22 | 22 | 10 | 15 | 28 | 30 | 13 | 14 | 12 | 13 | 11 | 12 | 9 | 11 | 12 | 12 | 7 | 9.3 | 8 | 8 | 9 | 10 |
| Height | 5914 | 5368 | 2377 | 1716 | 1170 | 1170 | 4370 | 2779 | 1059 | 778 | 163 | 213 | 2703 | 2112 | 543 | 501 | 168 | 165 | 848 | 848 | 1390 | 1141 | 1514 | 1514 | 153 | 190 |
| Size | 123.2 | 127.4 | 167.1 | 175 | 232.3 | 232.3 | 131.8 | 153.6 | 200.7 | 208.6 | 287 | 291.2 | 152.8 | 157.1 | 217.1 | 221.1 | 268.2 | 276.4 | 257.5 | 257.5 | 175.2 | 186.2 | 223 | 223 | 292.1 | 296.2 |
| **S-37** | 15 | 15 | 16 | 17 | 24 | 25 | 8 | 14 | 30 | 32.2 | | | 11 | 12 | 9 | 14 | | | 11 | 11 | 9 | 9.3 | 8 | 8 | | |
| Height | 6638 | 6638 | 1039 | 794 | 115 | 110 | 4135 | 1890 | 281 | 221 | | | 1959 | 1471 | 274 | 155 | | | 148 | 148 | 314 | 268 | 222 | 222 | | |
| Size | 123.2 | 123.2 | 175 | 179 | 240.4 | 244.4 | 123.6 | 149.2 | 208.6 | 218.4 | | | 148.3 | 152.8 | 209.1 | 229 | | | 253.5 | 253.5 | 183.2 | 186.2 | 223 | 223 | | |
| **S-38** | 15 | 16 | 14 | 19 | | | 10 | 15 | | | | | 10 | 11 | | | | | | | 7 | 9.3 | | | | |
| Height | 493 | 472 | 187 | 94 | | | 335 | 219 | | | | | 313 | 219 | | | | | | | 78 | 82 | | | | |
| Size | 123.2 | 127.4 | 167.1 | 186.9 | | | 131.8 | 153.6 | | | | | 143.9 | 148.3 | | | | | | | 175.2 | 186.2 | | | | |
| **S-39** | 14 | 17 | 17 | 18 | 22 | 25 | 10 | 12 | 29 | 33.2 | | | 12 | 13 | 8 | 12 | 11 | 10 | 11 | 13 | 6 | 9 | 8 | 8 | 7 | |
| Height | 1460 | 973 | 336 | 371 | 219 | 101 | 1026 | 898 | 224 | 132 | | | 688 | 611 | 207 | 156 | 93 | | 147 | 101 | 561 | 358 | 405 | 405 | 85 | |
| Size | 119.2 | 131.5 | 179 | 183 | 232.3 | 244.4 | 131.8 | 140.1 | 204.7 | 222.4 | | | 152.8 | 157.1 | 205 | 221.1 | 276.4 | | 253.5 | 261.5 | 171.3 | 183.2 | 223 | 223 | 283.9 | |
| **S-40** | 16 | 17 | 17 | 19 | 20 | 20 | 12 | 14 | 30 | 32 | 15 | 17 | 12 | 12 | 10 | 12 | 9 | 11 | 11 | 13 | 7 | 9.3 | 8 | 8 | 10 | 14 |
| Height | 5979 | 7160 | 3890 | 3005 | 3219 | 3219 | 6727 | 5776 | 1939 | 1699 | 519 | 456 | 7272 | 7272 | 1537 | 1141 | 489 | 348 | 1193 | 1059 | 3115 | 2612 | 3489 | 3489 | 440 | 312 |
| Size | 127.4 | 131.5 | 179 | 186.9 | 224.2 | 224.2 | 140.1 | 149.2 | 208.6 | 216.5 | 295.3 | 304 | 152.8 | 152.8 | 213.1 | 221.1 | 268.2 | 276.4 | 253.5 | 261.5 | 175.2 | 186.2 | 223 | 223 | 296.2 | 313.9 |
| **S-41** | 15 | 15 | 15 | 17 | 20 | 22 | 12 | 13 | 29 | 30 | 13 | 13 | 12 | 13 | 11 | 13 | 11 | 12 | 11 | 12 | 6 | 6 | 11 | 11 | 11 | 11 |
| Height | 6974 | 6974 | 1361 | 1086 | 430 | 411 | 3340 | 2900 | 652 | 450 | 141 | 141 | 1986 | 1718 | 269 | 237 | 105 | 91 | 442 | 354 | 3116 | 3116 | 872 | 872 | 154 | 154 |
| Size | 123.2 | 123.2 | 171.1 | 179 | 224.2 | 232.3 | 140.1 | 144.7 | 204.7 | 208.6 | 287 | 287 | 152.8 | 157.1 | 217.1 | 225 | 276.4 | 280.3 | 253.5 | 257.5 | 171.3 | 171.3 | 235 | 235 | 300.5 | 300.5 |

**Appendix A, Table A3 continued. Sample set S-42 – S-51, treated with 0.2% HCl destain for 30 minutes. Blank fields indicate loci or allele locations where no signal was detected.**

| Sample | D3S1358 | | vWA | | FGA | | D8S1179 | | D21S11 | | D18S531 | | D5S818 | | D13S317 | | D7S820 | | D16S539 | | TH01 | | TPOX | | CSF1PO | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **S-42** | 14 | 17 | 17 | 20 | 19 | 22 | 12 | 13 | 28 | 30 | | | 11 | 13 | 11 | 12 | | | 12 | 12 | 9 | 9 | 6 | 9 | | |
| Height | 3013 | 2141 | 762 | 391 | 270 | 172 | 1820 | 1669 | 196 | 179 | | | 1169 | 945 | 86 | 89 | | | 135 | 135 | 575 | 575 | 143 | 106 | | |
| Size | 119.2 | 131.5 | 179 | 190.8 | 220.2 | 232.3 | 140.1 | 144.7 | 200.7 | 208.6 | | | 148.3 | 157.1 | 217.1 | 221.1 | | | 257.5 | 257.5 | 183.2 | 183.2 | 215.2 | 227 | | |
| **S-43(a)** | 15 | 17 | 16 | 17 | 25 | 25 | 13 | 13 | 29 | 29 | 13 | 13 | 11 | 12 | 10 | 10 | 8 | | 11 | 11 | 9.3 | 9.3 | 8 | 9 | | |
| Height | 1378 | 1343 | 994 | 410 | 197 | 197 | 2532 | 2532 | 308 | 308 | 224 | 224 | 1423 | 619 | 281 | 281 | 81 | | 201 | 201 | 428 | 428 | 215 | 113 | | |
| Size | 123.2 | 131.5 | 175 | 179 | 244.4 | 244.4 | 144.7 | 144.7 | 204.7 | 204.7 | 287 | 287 | 148.3 | 152.8 | 213.1 | 213.1 | 264.1 | | 253.5 | 253.5 | 186.2 | 186.2 | 223 | 227 | | |
| **S-43(b)** | 16 | 18 | | | 22 | 23 | 12 | 14 | 28 | 30 | | | | | 8 | 9 | | | 13 | | | | 11 | | | |
| Height | 124 | 227 | | | 127 | 124 | 104 | 248 | 198 | 83 | | | | | 167 | 96 | | | 120 | | | | 86 | | | |
| Size | 127.4 | 135.6 | | | 232.3 | 236.3 | 140.1 | 149.2 | 200.7 | 208.6 | | | | | 205 | 209.1 | | | 261.5 | | | | 235 | | | |
| **S-44** | 14 | 18 | 19 | 19 | 23 | 25 | 13 | 14 | 30 | 30 | 18 | 21 | 11 | 12 | 11 | 12 | 12 | 12 | 9 | 9 | 9 | 9.3 | 8 | 8 | 12 | 13 |
| Height | 7398 | 6784 | 5518 | 5518 | 771 | 629 | 6589 | 6434 | 2606 | 2606 | 175 | 142 | 5249 | 4375 | 894 | 788 | 269 | 269 | 1778 | 1778 | 2039 | 1978 | 2091 | 2091 | 157 | 122 |
| Size | 119.2 | 135.6 | 186.9 | 186.9 | 236.3 | 244.4 | 144.7 | 149.2 | 208.6 | 208.6 | 308.4 | 321.4 | 148.3 | 152.8 | 217.1 | 221.1 | 280.3 | 280.3 | 245.4 | 245.4 | 183.2 | 186.2 | 223 | 223 | 305.1 | 309.5 |
| **S-45(a)** | 15 | 16 | 17 | 17 | 20 | 21 | 13 | 15 | 30 | 33.2 | 17 | 19 | 11 | 12 | 12 | 13 | 8 | 12 | 13 | 13 | 7 | 6 | 8 | 8 | 12 | 12 |
| Height | 2287 | 1292 | 1011 | 1011 | 387 | 214 | 1966 | 765 | 319 | 296 | 175 | 176 | 617 | 1730 | 256 | 181 | 145 | 121 | 552 | 552 | 776 | 424 | 943 | 943 | 472 | 472 |
| Size | 123.2 | 127.4 | 179 | 179 | 224.2 | 228.2 | 144.7 | 153.6 | 208.6 | 222.4 | 304 | 312.8 | 148.3 | 152.8 | 221.1 | 225 | 264.1 | 280.3 | 261.5 | 261.5 | 175.2 | 171.3 | 223 | 223 | 305.1 | 305.1 |
| **S-45(b)** | 17 | | 20 | | | | | | 29 | | | | | | 9 | 11 | | | | | 9 | | 9 | 11 | | |
| Height | 626 | | 436 | | | | | | 293 | | | | | | 125 | 81 | | | | | 353 | | 118 | 85 | | |
| Size | 131.6 | | 190.8 | | | | | | 204.7 | | | | | | 209.1 | 217.1 | | | | | 183.2 | | 227 | 235 | | |
| **S-46** | 15 | 16 | 17 | 20 | | | 12 | 13 | | | | | 10 | 11 | | | | | | | 9 | 9.3 | | | | |
| Height | 1140 | 943 | 158 | 113 | | | 626 | 445 | | | | | 620 | 496 | | | | | | | 106 | 94 | | | | |
| Size | 123.2 | 127.4 | 179 | 190.8 | | | 140.1 | 144.7 | | | | | 143.9 | 148.3 | | | | | | | 183.2 | 186.2 | | | | |
| **S-47** | 14 | 15 | 14 | 17 | 21 | 23 | 8 | 13 | 30 | 31.2 | 13 | | 11 | 12 | 11 | 12 | | | 9 | 12 | 6 | 9.3 | 8 | 12 | | |
| Height | 3071 | 2461 | 886 | 561 | 275 | 133 | 2875 | 1657 | 234 | 309 | 84 | | 1368 | 1189 | 224 | 140 | | | 149 | 111 | 723 | 409 | 173 | 171 | | |
| Size | 119.2 | 123.2 | 167.1 | 179 | 228.2 | 236.3 | 123.6 | 144.7 | 208.6 | 214.5 | 287 | | 148.3 | 152.8 | 217.1 | 221.1 | | | 245.4 | 257.5 | 171.3 | 186.2 | 223 | 239 | | |
| **S-48** | 15 | 16 | 15 | 16 | 22 | 24 | 13 | 14 | 29 | 30 | 12 | 17 | 12 | 13 | 11 | 13 | 9 | 9 | 12 | 13 | 7 | 9.3 | 9 | 11 | 11 | 11 |
| Height | 6145 | 5340 | 2621 | 2100 | 670 | 421 | 4351 | 3433 | 793 | 702 | 164 | 169 | 2900 | 2711 | 640 | 408 | 245 | 245 | 390 | 346 | 1774 | 1411 | 417 | 506 | 117 | 117 |
| Size | 123.2 | 127.4 | 171.1 | 175 | 232.3 | 240.4 | 144.7 | 149.2 | 204.7 | 208.6 | 282.9 | 304 | 152.8 | 157.1 | 217.1 | 225 | 268.2 | 268.2 | 257.5 | 261.5 | 175.2 | 186.2 | 227 | 235 | 300.5 | 300.5 |
| **S-49** | 16 | 18 | 17 | 17 | | | 14 | 15 | | | | | 12 | 13 | | | | | | | | | | | | |
| Height | 351 | 213 | 104 | 104 | | | 199 | 157 | | | | | 122 | 115 | | | | | | | | | | | | |
| Size | 127.4 | 135.6 | 179 | 179 | | | 149.2 | 153.6 | | | | | 152.8 | 157.1 | | | | | | | | | | | | |
| **S-50** | 14 | 15 | 15 | 19 | 19 | 19 | 15 | 15 | 27 | 30 | | | 11 | 11 | 12 | 12 | | | 9 | | 6 | 7 | 8 | 8 | | |
| Height | 2822 | 2466 | 722 | 428 | 383 | 383 | 2261 | 2261 | 302 | 218 | | | 2475 | 2475 | 268 | 268 | | | 101 | | 674 | 696 | 357 | 357 | | |
| Size | 119.2 | 123.2 | 171.1 | 186.9 | 220.2 | 220.2 | 153.6 | 153.6 | 197 | 208.6 | | | 148.3 | 148.3 | 221.1 | 221.1 | | | 245.4 | | 171.3 | 175.2 | 223 | 223 | | |
| **S-51** | 15 | 18 | 17 | 17 | 21 | 26 | 14 | 14 | 29 | 32.2 | 17 | 17 | 11 | 12 | 8 | 12 | 9 | 10 | 12 | 14 | 6 | 7 | 8 | 11 | 11 | 11 |
| Height | 2974 | 2284 | 2214 | 2214 | 404 | 216 | 3407 | 3407 | 420 | 306 | 236 | 236 | 1463 | 1173 | 342 | 168 | 114 | | 163 | 158 | 943 | 731 | 195 | 205 | 78 | 78 |
| Size | 123.2 | 135.6 | 179 | 179 | 228.2 | 248.5 | 149.2 | 149.2 | 204.7 | 218.4 | 304 | 304 | 148.3 | 152.8 | 205 | 221.1 | 268.2 | | 257.5 | 265.7 | 171.3 | 175.2 | 223 | 235 | 300.5 | 300.5 |

**Appendix A, Table A4. Sample set S-52 - S-56: samples with no destain treatment. All but S-56 were mixed samples presenting more than one profile. Blank fields indicate loci or allele locations where no signal was detected.**

| Sample | D3S1358 | | vWA | | FGA | | D8S1179 | | D21S11 | | D18S531 | | D5S818 | | D13S317 | | D7S820 | | D16S539 | | TH01 | | TPOX | | CSF1PO | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **S-52(a)** | 15 | 16 | 15 | 17 | 18 | 20 | 14 | 14 | 29 | 32.2 | 12 | 17 | 10 | 12 | 11 | 11 | 10 | 10 | 11 | 13 | 6 | 6 | 8 | 8 | 12 | 13 |
| Height | 6018 | 5458 | 2438 | 2139 | 1418 | 1311 | 4911 | 4911 | 1721 | 1454 | 687 | 526 | 4435 | 3495 | 2057 | 2057 | 836 | 836 | 827 | 585 | 5478 | 5478 | 2424 | 2424 | 362 | 307 |
| Size | 123.2 | 127.4 | 171.1 | 179 | 216.1 | 224.2 | 149.2 | 149.2 | 204.7 | 218.4 | 282.9 | 304 | 143.9 | 152.8 | 217.1 | 217.1 | 272.3 | 272.3 | 253.5 | 261.5 | 171.3 | 171.3 | 223 | 223 | 305.1 | 309.5 |
| **S-52(b)** | 14 | 17 | 14 | 16 | | | 13 | | | | | | 9 | 11 | | | | | | | | | 12 | | | |
| Height | 406 | 77 | 134 | 139 | | | 673 | | | | | | 166 | 241 | | | | | | | | | 109 | | | |
| Size | 119.2 | 131.6 | 167.1 | 175 | | | 144.7 | | | | | | 139.4 | 148.3 | | | | | | | | | 239 | | | |
| **S-53(a)** | 17 | 17 | 16 | 18 | 19 | 22 | 12 | 12 | 31.2 | 31.2 | 12 | 14 | 12 | 12 | 12 | 13 | 8 | 8 | 11 | 13 | 6 | 9 | 8 | 9 | 8 | 11 |
| Height | 1345 | 1345 | 459 | 679 | 274 | 306 | 1735 | 1735 | 518 | 518 | 234 | 271 | 903 | 903 | 262 | 183 | 268 | 268 | 366 | 278 | 564 | 634 | 521 | 646 | 414 | 270 |
| Size | 131.6 | 131.6 | 175 | 183 | 220.2 | 232.3 | 140.1 | 140.1 | 214.5 | 214.5 | 282.9 | 291.2 | 152.8 | 152.8 | 221.1 | 225 | 264.1 | 264.1 | 253.5 | 261.5 | 171.3 | 183.2 | 223 | 227 | 288.1 | 300.5 |
| **S-53(b)** | 15 | 16 | 19 | | | | 10 | 15 | | | | | 10 | 11 | | | | | | | 7 | 9.3 | | | | |
| Height | 853 | 814 | 157 | | | | 865 | 624 | | | | | 499 | 404 | | | | | | | 338 | 150 | | | | |
| Size | 123.2 | 127.4 | 186.9 | | | | 131.8 | | | | | | 143.9 | 148.3 | | | | | | | 175.2 | 186.2 | | | | |
| **S-54(a)** | 17 | 18 | 17 | 18 | 21 | 22 | 13 | 14 | 28 | 30 | | | 11 | 11 | 8 | 10 | | | 11 | 13 | 7 | 7 | 8 | 8 | 13 | 13 |
| Height | 2809 | 2281 | 671 | 550 | 341 | 313 | 2743 | 2476 | 575 | 539 | | | 3146 | 3146 | 410 | 261 | | | 101 | 163 | 2303 | 2303 | 783 | 783 | 75 | 75 |
| Size | 131.6 | 135.6 | 179 | 183 | 228.2 | 232.3 | 144.7 | 149.2 | 200.7 | 208.6 | | | 148.3 | 148.3 | 205 | 213.1 | | | 253.5 | 261.5 | 175.2 | 175.2 | 223 | 223 | 308.9 | 308.9 |
| **S-54(b)** | 11 | 16 | | | | | 12 | | | | | | | | | | | | | | | | | | | |
| Height | 120 | 244 | | | | | 174 | | | | | | | | | | | | | | | | | | | |
| Size | 107.2 | 127.4 | | | | | 140.1 | | | | | | | | | | | | | | | | | | | |
| **S-55(a)** | 15 | 17 | 20 | 20 | 22 | 22 | 13 | 14 | 30 | 30 | 15 | 19 | 11 | 11 | 8 | 11 | 11 | 11 | 10 | 11 | 6 | 9.3 | 8 | 8 | 10 | 12 |
| Height | 4082 | 2763 | 854 | 854 | 272 | 272 | 2951 | 3078 | 500 | 500 | 160 | 106 | 3302 | 3302 | 294 | 323 | 153 | 153 | 293 | 210 | 943 | 764 | 477 | 477 | 150 | 150 |
| Size | 123.2 | 131.6 | 190.8 | 190.8 | 232.3 | 232.3 | 144.7 | 149.2 | 208.6 | 208.6 | 295.3 | 312.8 | 148.3 | 148.3 | 205 | 217.1 | 276.4 | 276.4 | 249.4 | 253.5 | 171.3 | 186.2 | 223 | 223 | 295.8 | 304.4 |
| **S-55(b)** | 14 | 16 | 15 | 18 | 21 | 25 | | | 31 | 32.2 | 12 | | 12 | 12 | 12 | | | | 12 | | 8 | | 6 | 11 | | |
| Height | 234 | 218 | 189 | 193 | 159 | 143 | | | 184 | 278 | 93 | | 502 | 502 | 112 | | | | 93 | | 228 | | 268 | 153 | | |
| Size | 119.2 | 127.4 | 171.1 | 183 | 228.2 | 244.4 | | | 212.6 | 218.4 | 282.9 | | 152.8 | 152.8 | 221.1 | | | | 257.5 | | 179.3 | | 215.2 | 235 | | |
| **S-56** | 14 | 17 | 15 | 15 | | | 13 | 13 | | | | | 11 | 12 | | | | | | | | | | | | |
| Height | 244 | 180 | 112 | 112 | | | 441 | 441 | | | | | 86 | 103 | | | | | | | | | | | | |
| Size | 119.2 | 131.6 | 171.1 | 171.1 | | | 144.7 | 144.7 | | | | | 148.3 | 152.8 | | | | | | | | | | | | |

**Appendix A, Table A5. Sample set S-10 - S-19, treated with 1%HCl destain for 30 minutes. 14 µL of sample was added to make a 50 µL PCR reaction in an attempt to increase the number of loci that could be typed. Blank fields indicate loci or allele locations where no signal was detected.**

| Sample | D3S1358 | | vWA | | FGA | | D8S1179 | | D21S11 | | D18S531 | | D5S818 | | D13S317 | | D7S820 | | D16S539 | | TH01 | | TPOX | | CSF1PO | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **S-10** | 14 | 15 | 16 | 18 | 18 | 22 | 8 | 12 | 29 | 29 | | | 12 | 14 | 12 | 13 | | | 9 | 11 | 6 | 8 | 9 | 9 | | |
| Height | 2361 | 2176 | 484 | 463 | 177 | 130 | 2009 | 1439 | 441 | 441 | | | 852 | 678 | 172 | 100 | | | 108 | 86 | 559 | 430 | 297 | 297 | | |
| Size | 119.2 | 123.1 | 175 | 183 | 216.1 | 232.3 | 123.6 | 140.1 | 204.7 | 204.7 | | | 152.8 | 161.4 | 221.1 | 225 | | | 245.4 | 253.5 | 171.3 | 179.3 | 227 | 227 | | |
| **S-11** | 16 | 18 | 17 | 17 | 19 | 20 | 12 | 12 | 29 | 31 | | | 12 | 12 | 8 | 8 | | | 11 | 11 | 6 | 8 | | | | |
| Height | 1596 | 1290 | 847 | 847 | 141 | 95 | 2334 | 2334 | 115 | 89 | | | 1441 | 1441 | 123 | 123 | | | 103 | 103 | 433 | 442 | | | | |
| Size | 127.4 | 135.7 | 179 | 179 | 220.2 | 224.2 | 140.1 | 140.1 | 204.7 | 212.6 | | | 152.8 | 152.8 | 205 | 205 | | | 253.5 | 253.5 | 171.3 | 179.3 | | | | |
| **S-12** | 16 | 17 | 16 | 18 | 21 | 24 | 13 | 15 | 28 | 29 | 14 | 15 | 11 | 13 | 11 | 12 | 9 | 11 | 10 | 13 | 6 | 9 | 8 | 9 | | |
| Height | 4412 | 4198 | 1369 | 999 | 270 | 241 | 3051 | 2472 | 597 | 541 | 95 | | 2547 | 1959 | 407 | 363 | 78 | | 207 | 190 | 1325 | 800 | 340 | 306 | | |
| Size | 127.4 | 131.6 | 175 | 183 | 228.2 | 240.4 | 144.7 | 153.6 | 200.7 | 204.7 | 291.2 | | 148.3 | 157.1 | 217.1 | 221.1 | 268.2 | | 249.4 | 261.5 | 171.3 | 183.2 | 223 | 227 | | |
| **S-13** | 16 | 17 | 15 | 18 | | | 13 | 13 | | | | | 10 | 11 | | | | | | | 8 | 9.3 | | | | |
| Height | 824 | 609 | 190 | 129 | | | 851 | 851 | | | | | 414 | 367 | | | | | | | 97 | 116 | | | | |
| Size | 127.4 | 131.6 | 171.1 | 183 | | | 144.7 | 144.7 | | | | | 143.9 | 148.3 | | | | | | | 179.3 | 186.2 | | | | |
| **S-14** | 15 | 17 | 14 | 16 | 21 | 24 | 11 | 13 | 30 | 32.2 | | | 9 | 12 | 7 | 11 | | | 11 | | 7 | 9.3 | | | | |
| Height | 2162 | 1538 | 662 | 436 | 107 | 77 | 1450 | 1094 | 156 | 88 | | | 1282 | 880 | 166 | 129 | | | 96 | | 378 | 217 | | | | |
| Size | 123.1 | 131.6 | 167.1 | 175 | 228.2 | 240.4 | 135.9 | 144.7 | 208.6 | 218.4 | | | 139.5 | 152.8 | 201 | 217.1 | | | 253.5 | | 175.2 | 186.2 | | | | |
| **S-15** | 15 | 15 | 15 | 18 | 20 | 23 | 13 | 14 | 27 | 28 | 13.2 | 14 | 11 | 11 | 9 | 10 | 9 | | 11 | 13 | 6 | 9.3 | 8 | 8 | 12 | 12 |
| Height | 7055 | 7055 | 1548 | 1211 | 514 | 385 | 2784 | 2364 | 661 | 524 | 82 | 129 | 4587 | 4587 | 326 | 338 | 111 | | 247 | 236 | 1551 | 1188 | 810 | 810 | 221 | 221 |
| Size | 123.1 | 123.1 | 171.1 | 183 | 224.2 | 236.3 | 144.7 | 149.2 | 197 | 200.7 | 289.1 | 291.2 | 148.3 | 148.3 | 209.1 | 213.1 | 268.2 | | 253.5 | 261.5 | 171.3 | 186.2 | 223 | 223 | 305.1 | 305.1 |
| **S-16(a)** | 16 | 17 | 18 | 18 | 19 | 24 | 14 | 15 | 28 | 33.2 | 13 | 13 | 11 | 11 | | | | | 9 | 11 | 9 | 9.3 | 9 | 9 | 10 | 10 |
| Height | 3118 | 2835 | 1297 | 1297 | 147 | 143 | 2067 | 1748 | 231 | 140 | 2692 | 2692 | 363 | 363 | | | | | 249 | 271 | 673 | 526 | 292 | 292 | 119 | 119 |
| Size | 127.4 | 131.6 | 183 | 183 | 220.2 | 240.4 | 149.2 | 153.6 | 200.7 | 222.4 | 287 | 287 | 148.3 | 148.3 | | | | | 245.4 | 253.5 | 183.2 | 186.2 | 227 | 227 | 296.2 | 296.2 |
| **S-16(b)** | 12 | | 15 | | 22 | 23 | 12 | | | | | | | | | | | | | | 7 | 8 | 8 | 11 | | |
| Height | 373 | | 332 | | 86 | | 172 | | | | | | | | | | | | | | 75 | 101 | 89 | 92 | | |
| Size | 111.1 | | 171.1 | | 232.3 | | 140.1 | | | | | | | | | | | | | | 175.2 | 179.3 | 223 | 235 | | |
| **S-17** | 14 | 15 | 16 | 16 | | | 11 | 15 | 29 | 30 | | | 12 | 12 | | | | | | | 5 | 7 | 8 | 11 | | |
| Height | 1151 | 941 | 1851 | 1851 | | | 3821 | 1939 | 103 | | | | 1910 | 1910 | | | | | | | 780 | 672 | 203 | 138 | | |
| Size | 119.2 | 123.1 | 175 | 175 | | | 135.9 | 153.6 | 204.7 | | | | 152.8 | 152.8 | | | | | | | 167.1 | 175.2 | 223 | 235 | | |
| **S-18** | 15 | 16 | 14 | 15 | | | 10 | 14 | | | | | 11 | 12 | | | | | | | 7 | 7 | | | | |
| Height | 695 | 654 | 235 | 189 | | | 753 | 407 | | | | | 379 | 332 | | | | | | | 378 | 378 | | | | |
| Size | 123.1 | 127.4 | 167.1 | 171.1 | | | 131.8 | 149.2 | | | | | 148.3 | 152.8 | | | | | | | 175.2 | 175.2 | | | | |
| **S-19** | 14 | 14 | | | | | 12 | 12 | | | | | 12 | 12 | | | | | | | | | | | | |
| Height | 412 | 412 | | | | | 214 | 214 | | | | | 150 | 150 | | | | | | | | | | | | |
| Size | 119.2 | 119.2 | | | | | 140.1 | 140.1 | | | | | 152.8 | 152.8 | | | | | | | | | | | | |

**Appendix A, Table A6. Sample set S-20 - S-31. S-20 and S-21 were destained with 0.2% HCl for 30 minutes, while S-22 and S-23 were destained with 1% HCl solution. S-24 through S-31 are control samples. Blank fields indicate loci or allele locations where no signal was detected.**

| Sample | D3S1358 | | vWA | | FGA | | D8S1179 | | D21S11 | | D18S531 | | D5S818 | | D13S317 | | D7S820 | | D16S539 | | TH01 | | TPOX | | CSF1PO | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **S-20(a)** | 16 | 17 | 17 | 18 | 22 | | 12 | 12 | 30 | 31.2 | | | 11 | 12 | 9 | | | | 11 | 11 | 8 | 9.3 | 8 | 8 | | |
| Height | 531 | 372 | 186 | 141 | 81 | | 897 | 897 | 103 | 82 | | | 439 | 243 | 91 | | | | 129 | 129 | 133 | 183 | 199 | 199 | | |
| Size | 127.4 | 131.6 | 179 | 183 | 232.3 | | 140.1 | 140.1 | 208.6 | 214.5 | | | 148.3 | 152.8 | 209.1 | | | | 253.5 | 253.5 | 179.3 | 186.2 | 223 | 223 | | |
| **S-20(b)** | 15 | | | | | | | | | | | | | | | | | | | | | | | | | |
| Height | 111 | | | | | | | | | | | | | | | | | | | | | | | | | |
| Size | 123.1 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **S-21** | 16 | 18 | 15 | 18 | 22 | 22 | 10 | 11 | 31.2 | 32.2 | 12 | 13 | 11 | 12 | 11 | 11 | 10 | 10 | 11 | 11 | 6 | 7 | 8 | 9 | 10 | 11 |
| Height | 3492 | 2832 | 1441 | 972 | 683 | 683 | 3913 | 3507 | 607 | 549 | 220 | 156 | 2329 | 2029 | 989 | 989 | 231 | 231 | 744 | 744 | 1691 | 1558 | 732 | 646 | 166 | 144 |
| Size | 127.4 | 135.6 | 171.1 | 183 | 232.3 | 232.3 | 131.8 | 135.9 | 214.5 | 218.4 | 282.9 | 287 | 148.3 | 152.8 | 217.1 | 217.1 | 272.3 | 272.3 | 253.5 | 253.5 | 171.3 | 175.2 | 223 | 227 | 296.2 | 300.5 |
| **S-22** | 15 | 17 | 15.2 | 18 | | | 12 | 14 | | | | | 12 | 13 | | | | | | | 7 | 7 | 8 | | | |
| Height | 485 | 290 | 349 | 85 | | | 295 | 260 | | | | | 227 | 138 | | | | | | | 225 | 225 | 75 | | | |
| Size | 123.1 | 131.6 | | 183 | | | 140.1 | 149.2 | | | | | 152.8 | 157.1 | | | | | | | 175.2 | 175.2 | 223 | | | |
| **S-23** | 16 | 17 | 17 | 18 | | | 13 | 14 | | | | | 9 | 11 | | | | | 9 | | 6 | 9.3 | | | | |
| Height | 414 | 366 | 124 | 132 | | | 560 | 364 | | | | | 356 | 332 | | | | | 106 | | 123 | 156 | | | | |
| Size | 127.4 | 131.6 | 179 | 183 | | | 144.7 | 149.2 | | | | | 139.5 | 148.3 | | | | | 245.4 | | 171.3 | 186.2 | | | | |
| **S-24(2)** | 16 | 17 | 18 | 20 | 20 | 21 | 12 | 13 | 28 | 31.2 | 16 | 16 | 11 | 12 | 9 | 11 | 7 | 12 | 9 | 10 | 9.3 | 9.3 | 8 | 10 | 11 | 11 |
| Height | 4564 | 4256 | 3303 | 2903 | 3565 | 3334 | 5159 | 5007 | 5036 | 4621 | 5689 | 5689 | 5025 | 4174 | 3260 | 3415 | 2145 | 2084 | 3899 | 4141 | 5029 | 5029 | 5108 | 5164 | 5883 | 5883 |
| Size | 127.4 | 131.6 | 183 | 190.8 | 224.2 | 228.2 | 140.1 | 144.7 | 200.7 | 214.5 | 299.6 | 299.6 | 148.3 | 152.8 | 209.1 | 217.1 | 260.1 | 280.3 | 245.4 | 249.4 | 186.2 | 186.2 | 223 | 230.9 | 300.5 | 300.5 |
| **S-25(2)** | 16 | 17 | 18 | 20 | 20 | 21 | 12 | 13 | 28 | 31.2 | 16 | 16 | 11 | 12 | 9 | 11 | 7 | 12 | 9 | 10 | 9.3 | 9.3 | 8 | 10 | 11 | 11 |
| Height | 5645 | 5091 | 3406 | 3251 | 3876 | 3472 | 5180 | 5342 | 5240 | 5052 | 5856 | 5856 | 4908 | 4839 | 3902 | 3573 | 2261 | 2089 | 3888 | 3921 | 5745 | 5745 | 4984 | 4840 | 5351 | 5351 |
| Size | 127.4 | 131.6 | 183 | 190.8 | 224.2 | 228.2 | 140.1 | 144.7 | 200.7 | 214.5 | 299.6 | 299.6 | 148.3 | 152.8 | 209.1 | 217.1 | 260.1 | 280.3 | 245.4 | 249.4 | 186.2 | 186.2 | 223 | 230.9 | 300.5 | 300.5 |
| **S-26(2)** | 13 | 15 | 14 | 17 | 20 | 23 | 14 | 15 | 29 | 32 | 12 | 16 | 10 | 11 | 11 | 13 | 9 | 10 | 12 | 12 | 8 | 9.3 | 8 | 11 | 12 | 12 |
| Height | 3845 | 3443 | 2636 | 2293 | 2072 | 1649 | 3722 | 3499 | 2793 | 3003 | 1877 | 1545 | 3229 | 3072 | 1919 | 1493 | 1404 | 1117 | 2885 | 2885 | 2570 | 2326 | 2147 | 1799 | 2792 | 2792 |
| Size | 115.2 | 123.1 | 167.1 | 179 | 224.2 | 236.3 | 149.2 | 153.6 | 204.7 | 216.5 | 282.9 | 299.6 | 143.9 | 148.3 | 217.1 | 225 | 268.2 | 272.3 | 257.5 | 257.5 | 179.3 | 186.2 | 223 | 235 | 305.1 | 305.1 |
| **S-27(2)** | 13 | 15 | 14 | 17 | 20 | 23 | 14 | 15 | 29 | 32 | 12 | 16 | 10 | 11 | 11 | 13 | 9 | 10 | 12 | 12 | 8 | 9.3 | 8 | 11 | 12 | 12 |
| Height | 5997 | 6096 | 5789 | 5690 | 3863 | 3452 | 3882 | 4615 | 4956 | 6128 | 2721 | 2193 | 4269 | 3135 | 4080 | 3805 | 2062 | 1933 | 6517 | 6517 | 4532 | 5880 | 6108 | 6041 | 5773 | 5773 |
| Size | 115.2 | 123.1 | 167.1 | 179 | 224.2 | 236.3 | 149.2 | 153.6 | 204.7 | 216.5 | 282.9 | 299.6 | 143.9 | 148.3 | 217.1 | 225 | 268.2 | 272.3 | 257.5 | 257.5 | 179.3 | 186.2 | 223 | 235 | 305.1 | 305.1 |
| **S-28(2)** | 16 | 17 | 16 | 18 | 22 | 23 | 10 | 14 | 28 | 30 | 11 | 13 | 11 | 12 | 10 | 12 | 10 | 10 | 11 | 12 | 8 | 9.3 | 8 | 9 | 9 | 10 |
| Height | 4887 | 2751 | 4178 | 4102 | 4110 | 3416 | 4160 | 5230 | 5861 | 5561 | 3867 | 3812 | 5226 | 5307 | 3829 | 4011 | 4685 | 4685 | 4172 | 3677 | 4941 | 4480 | 4906 | 4819 | 4331 | 4187 |
| Size | 127.4 | 131.6 | 175 | 183 | 232.3 | 236.3 | 131.8 | 149.2 | 200.7 | 208.6 | 278.8 | 287 | 148.3 | 152.8 | 213.1 | 221.1 | 272.3 | 272.3 | 253.5 | 257.5 | 179.3 | 186.2 | 223 | 227 | 292.1 | 296.2 |
| **S-29(2)** | 16 | 17 | 16 | 18 | 22 | 23 | 10 | 14 | 28 | 30 | 11 | 13 | 11 | 12 | 10 | 12 | 10 | 10 | 11 | 12 | 8 | 9.3 | 8 | 9 | 9 | 10 |
| Height | 5961 | 2297 | 5564 | 4998 | 4266 | 3756 | 3168 | 5274 | 4760 | 5784 | 3459 | 3410 | 4938 | 5160 | 4875 | 4544 | 4671 | 4671 | 6599 | 6433 | 4555 | 5446 | 6097 | 6216 | 5955 | 5995 |
| Size | 127.4 | 131.6 | 175 | 183 | 232.3 | 236.3 | 131.8 | 149.2 | 200.7 | 208.6 | 278.8 | 287 | 148.3 | 152.8 | 213.1 | 221.1 | 272.3 | 272.3 | 253.5 | 257.5 | 179.3 | 186.2 | 223 | 227 | 292.1 | 296.2 |
| **S-30(2)** | 15 | 15 | 18 | 19 | 21 | 22 | 10 | 14 | 30 | 31 | 13 | 13 | 12 | 12 | 11 | 12 | 9 | 12 | 12 | 13 | 9.3 | 9.3 | 8 | 11 | 10 | 12 |
| Height | 1441 | 1441 | 485 | 484 | 598 | 454 | 716 | 605 | 559 | 665 | 1037 | 1037 | 1137 | 1137 | 467 | 470 | 427 | 291 | 297 | 271 | 671 | 671 | 449 | 409 | 287 | 385 |
| Size | 123.2 | 123.2 | 183 | 186.9 | 228.2 | 232.3 | 131.8 | 149.2 | 208.6 | 212.6 | 287 | 287 | 152.8 | 152.8 | 217.1 | 221.1 | 268.2 | 280.3 | 257.5 | 261.5 | 186.2 | 186.2 | 223 | 235 | 296.2 | 305.1 |
| **S-31(2)** | 15 | 15 | 18 | 19 | 21 | 22 | 10 | 14 | 30 | 31 | 13 | 13 | 12 | 12 | 11 | 12 | 9 | 12 | 12 | 13 | 9.3 | 9.3 | 8 | 11 | 10 | 12 |
| Height | 6582 | 6582 | 3917 | 3063 | 3126 | 2866 | 4859 | 4825 | 4409 | 4331 | 6135 | 6135 | 5420 | 5420 | 3280 | 2821 | 1833 | 1835 | 297 | 271 | 671 | 671 | 449 | 409 | 287 | 385 |
| Size | 123.2 | 123.2 | 183 | 186.9 | 228.2 | 232.3 | 131.8 | 149.2 | 208.6 | 212.6 | 287 | 287 | 152.8 | 152.8 | 217.1 | 221.1 | 268.2 | 280.3 | 257.5 | 261.5 | 186.2 | 186.2 | 223 | 235 | 296.2 | 305.1 |

**Appendix A, Table A7. Thin-Prep samples, unstained. Two samples gave no profile, while three gave complete profiles. Blank fields indicate loci or allele locations where no signal was detected.**

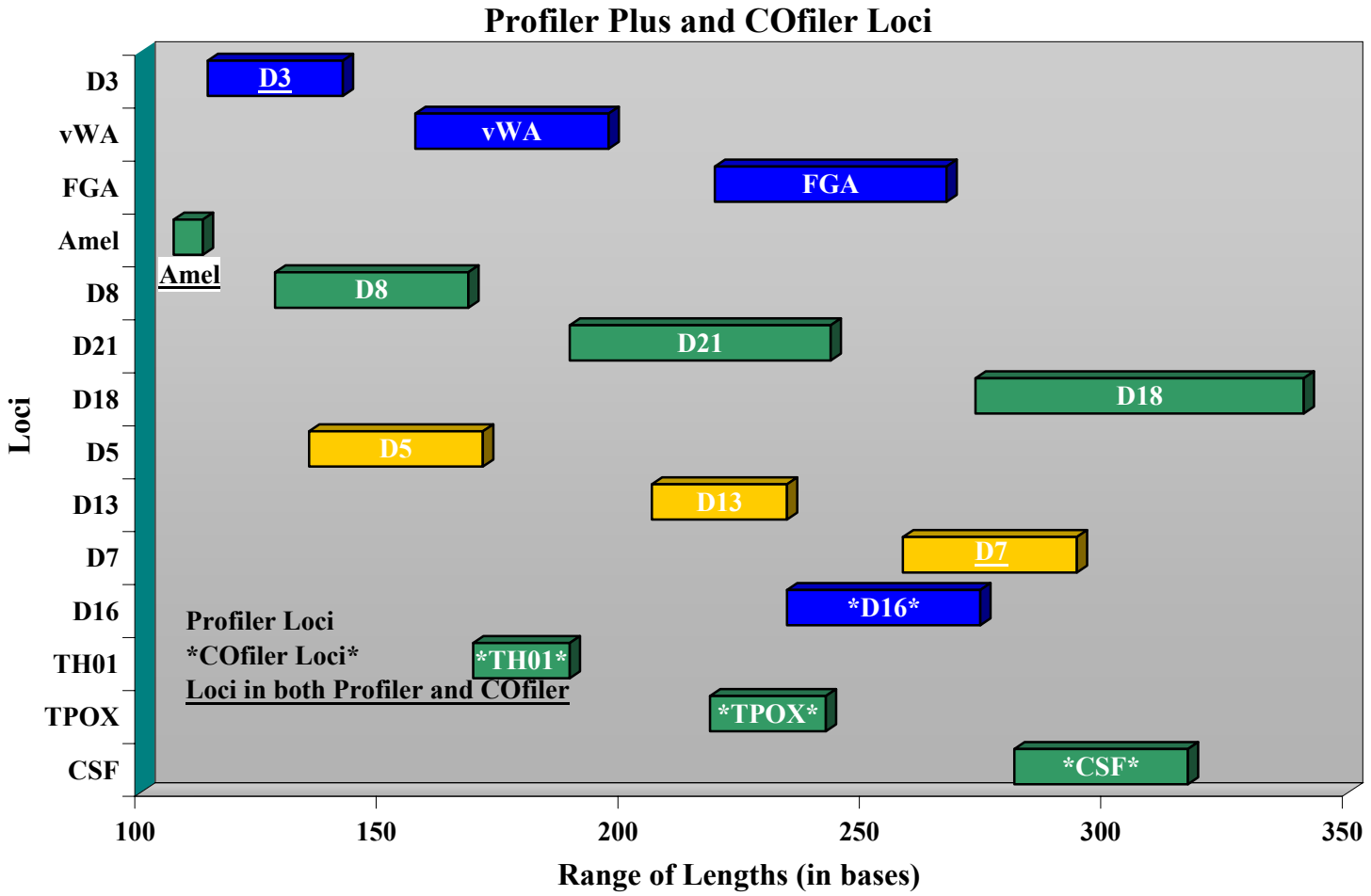
| Sample | D3S1358 | | vWA | | FGA | | D8S1179 | | D21S11 | | D18S531 | | D5S818 | | D13S317 | | D7S820 | | D16S539 | | TH01 | | TPOX | | CSF1PO | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **S-57** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Height | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Size | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **S-58** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Height | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Size | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **S-59** | 16 | 18 | 14 | 18 | 23 | 25 | 11 | 14 | 28 | 30 | 18 | 19 | 11 | 13 | 11 | 13 | 9 | 12 | 10 | 13 | 9 | 9.3 | 8 | 9 | 10 | 12 |
| Height | 775 | 770 | 587 | 445 | 595 | 550 | 743 | 791 | 923 | 872 | 763 | 688 | 783 | 757 | 496 | 607 | 475 | 418 | 736 | 605 | 588 | 586 | 915 | 1004 | 712 | 846 |
| Size | 127.4 | 135.6 | 167.1 | 183 | 236.3 | 244.4 | 135.9 | 149.2 | 200.7 | 208.6 | 308.4 | 312.8 | 148.3 | 157.1 | 217.1 | 225 | 268.2 | 280.3 | 249.4 | 261.5 | 183.2 | 186.2 | 223 | 227 | 4187 | 305.1 |
| **S-60** | 16 | 18 | 16 | 16 | 20 | 22 | 9 | 11 | 28 | 28 | 18 | 20 | 11 | 12 | 10 | 11 | 8 | 11 | 12 | 13 | 7 | 9.3 | 8 | 9 | 12 | 14 |
| Height | 1131 | 967 | 1551 | 1551 | 749 | 732 | 1376 | 1082 | 2236 | 2236 | 809 | 1060 | 936 | 932 | 842 | 727 | 586 | 716 | 781 | 794 | 860 | 957 | 947 | 1169 | 712 | 680 |
| Size | 127.4 | 135.6 | 175 | 175 | 224.2 | 232.3 | 127.6 | 135.9 | 200.7 | 200.7 | 308.4 | 317.5 | 148.3 | 152.8 | 213.1 | 217.1 | 264.1 | 276.4 | 257.5 | 261.5 | 175.2 | 186.2 | 223 | 227 | 305.1 | 312.8 |
| **S-61** | 14 | 15 | 17 | 20 | 24 | 26 | 14 | 14 | 28 | 32.2 | 12 | 15 | 7 | 12 | 11 | 12 | 8 | 10 | 10 | 11 | 7 | 9 | 8 | 11 | 10 | 10 |
| Height | 1107 | 925 | 538 | 553 | 861 | 780 | 1712 | 1712 | 1176 | 1118 | 784 | 720 | 960 | 833 | 784 | 773 | 703 | 553 | 794 | 924 | 951 | 772 | 1245 | 1127 | 1633 | 1633 |
| Size | 119.2 | 123.2 | 179 | 190.8 | 240.4 | 248.5 | 149.2 | 149.2 | 200.7 | 218.4 | 282.9 | 295.3 | 131.1 | 152.8 | 217.1 | 221.1 | 264.1 | 272.3 | 249.4 | 253.5 | 175.2 | 183.2 | 223 | 235 | 4187 | 4187 |

**Appendix A, Table 8. Population statistics. Results for samples S-1 – S-30 for the four major populations of Eastern Tennessee. * Denotes samples that were mixed profiles and calculated with a mixture equation to obtain statistical analsysis.**

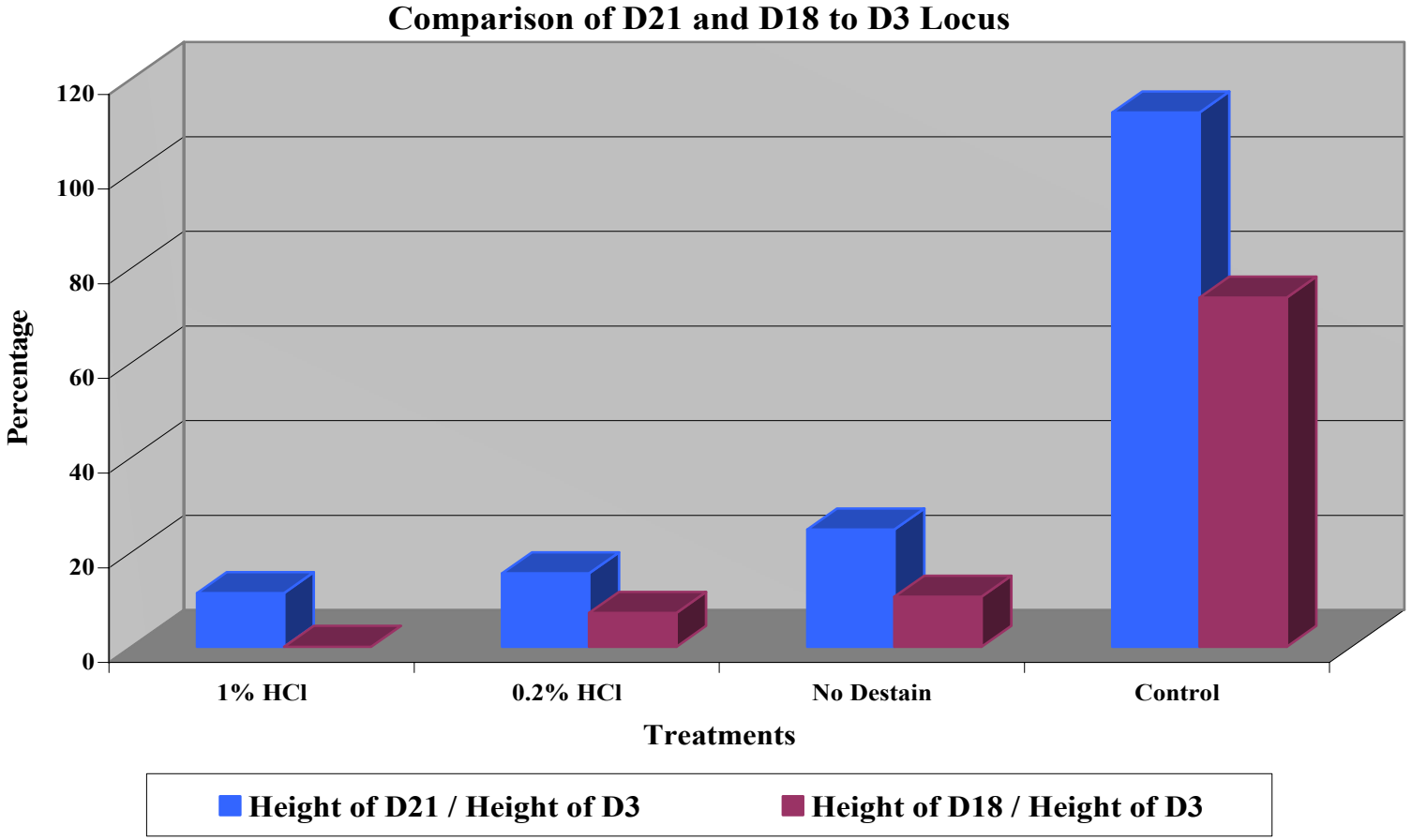
| | Caucasian | African-American | Southeastern Hispanic | Southwestern Hispanic |
|---|---|---|---|---|
| S-1* | 7.283E+10 | 1.533E+09 | 2.088E+11 | 2.160E+11 |
| S-2 | 9.579E+12 | 7.663E+14 | 8.058E+12 | 4.415E+13 |
| S-3 | 2.098E+17 | 8.889E+16 | 1.086E+17 | 1.048E+18 |
| S-4 | 4.439E+03 | 6.281E+04 | 1.707E+04 | 2.115E+04 |
| S-5 | 7.252E+14 | 6.627E+16 | 4.290E+15 | 8.977E+17 |
| S-6 | 6.566E+06 | 1.987E+08 | 4.630E+06 | 6.623E+06 |
| S-7 | 2.415E+13 | 9.050E+14 | 2.092E+13 | 7.704E+13 |
| S-8 | 4.040E+10 | 8.117E+09 | 9.107E+10 | 4.566E+10 |
| S-9 | 1.154E+12 | 3.610E+14 | 1.919E+12 | 1.566E+13 |
| S-10 | 1.103E+09 | 1.531E+08 | 2.667E+09 | 1.735E+10 |
| S-11 | 3.320E+12 | 3.226E+14 | 3.300E+12 | 5.342E+13 |
| S-12 | 1.416E+14 | 5.565E+14 | 5.562E+14 | 4.054E+16 |
| S-13 | 5.692E+05 | 1.908E+06 | 1.462E+06 | 2.103E+06 |
| S-14 | 1.647E+12 | 2.389E+13 | 1.484E+12 | 6.887E+11 |
| S-15 | 6.180E+16 | 9.872E+18 | 1.760E+17 | 1.035E+18 |
| S-16* | 4.136E+06 | 1.399E+06 | 5.271E+06 | 4.446E+07 |
| S-17 | 5.195E+09 | 1.464E+09 | 1.999E+09 | 8.606E+08 |
| S-18 | 1.030E+06 | 2.927E+05 | 6.231E+05 | 3.587E+05 |
| S-19 | 1.674E+04 | 3.871E+04 | 1.005E+05 | 1.061E+05 |
| S-20* | 2.675E+09 | 9.025E+11 | 4.776E+09 | 4.444E+08 |
| S-21 | 2.074E+15 | 4.866E+17 | 1.369E+16 | 2.997E+16 |
| S-22 | 2.990E+07 | 6.680E+06 | 2.970E+07 | 1.617E+07 |
| S-23 | 1.556E+07 | 1.611E+08 | 8.203E+06 | 2.990E+07 |
| S-24 | 1.211E+19 | 9.001E+20 | 3.499E+19 | 1.178E+20 |
| S-25 | 1.211E+19 | 9.001E+20 | 3.499E+19 | 1.178E+20 |
| S-26 | 1.553E+17 | 4.486E+18 | 3.136E+17 | 1.780E+18 |
| S-27 | 1.553E+17 | 4.486E+18 | 3.136E+17 | 1.780E+18 |
| S-28 | 1.281E+16 | 9.960E+16 | 1.265E+17 | 1.473E+17 |
| S-29 | 1.281E+16 | 9.960E+16 | 1.265E+17 | 1.473E+17 |
| S-30 | 5.942E+14 | 3.164E+17 | 2.876E+15 | 2.230E+15 |

**Appendix A, Table A8 – Population statistics continued. Statistical results for samples S-31 – S-61.**

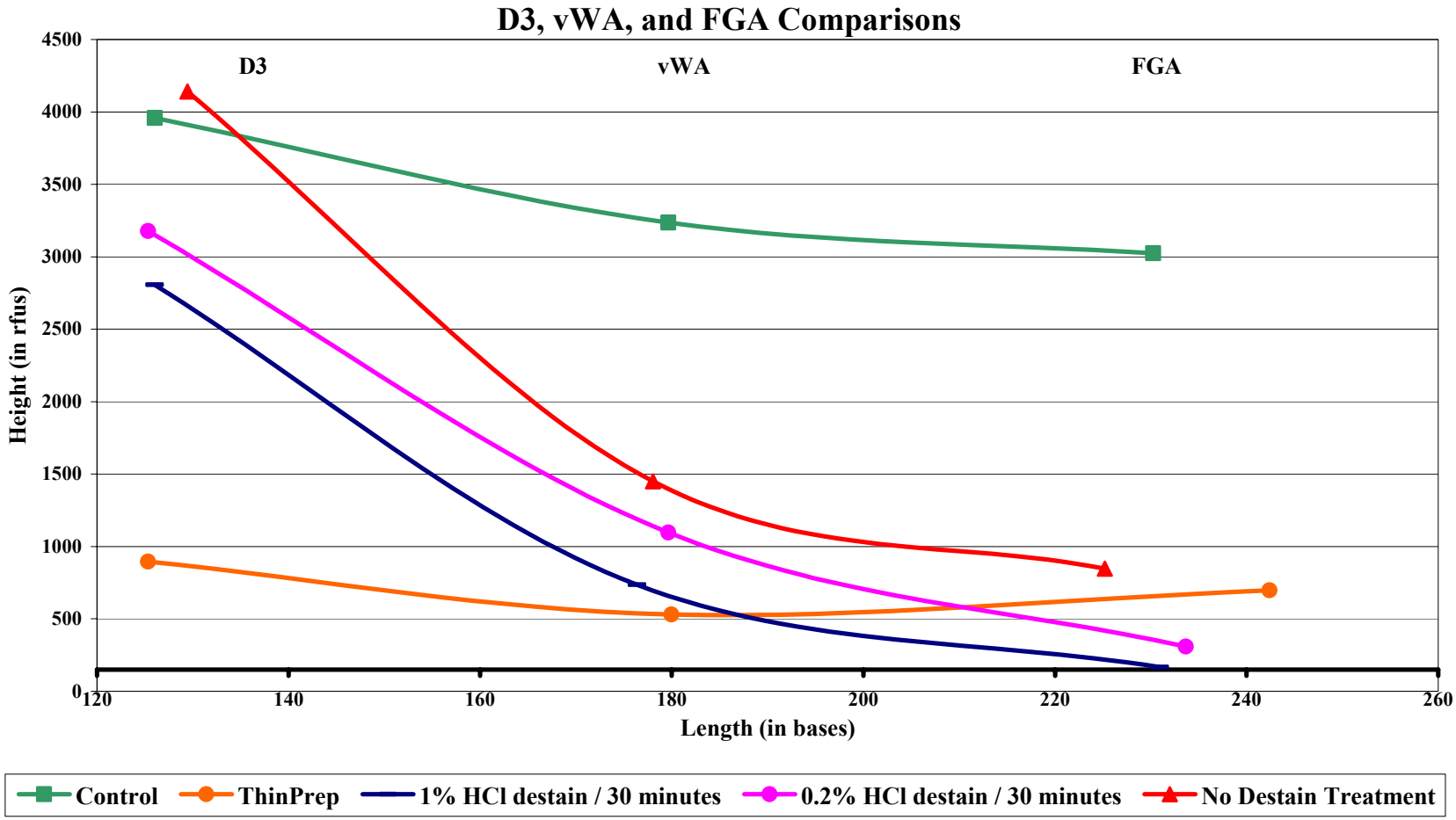
| | Caucasian | African-American | Southeastern Hispanic | Southwestern Hispanic |
|---|---|---|---|---|
| S-31 | 5.942E+14 | 3.164E+17 | 2.876E+15 | 2.230E+15 |
| S-32 | 1.103E+03 | 3.615E+04 | 9.940E+02 | 1.460E+03 |
| S-33 | 5.708E+06 | 1.000E+08 | 4.929E+07 | 5.208E+07 |
| S-34 | 1.614E+07 | 1.843E+09 | 5.464E+07 | 1.266E+08 |
| S-35 | 1.091E+07 | 3.489E+07 | 4.401E+07 | 3.912E+07 |
| S-36 | 1.748E+15 | 2.170E+16 | 1.516E+16 | 1.516E+16 |
| S-37 | 5.821E+12 | 9.709E+13 | 7.605E+11 | 1.910E+11 |
| S-38 | 5.420E+06 | 1.766E+07 | 8.361E+06 | 2.539E+06 |
| S-39 | 3.144E+16 | 2.106E+17 | 7.722E+16 | 2.695E+17 |
| S-40 | 5.426E+16 | 5.214E+17 | 3.188E+17 | 7.435E+17 |
| S-41 | 1.502E+15 | 2.551E+17 | 1.078E+15 | 1.033E+15 |
| S-42 | 6.472E+13 | 3.089E+13 | 4.486E+14 | 1.664E+16 |
| S-43* | 5.328E+08 | 1.258E+10 | 2.874E+08 | 1.096E+08 |
| S-44 | 3.639E+17 | 1.306E+19 | 1.055E+18 | 2.079E+18 |
| S-45* | 3.953E+09 | 2.240E+10 | 3.589E+09 | 1.481E+10 |
| S-46 | 3.280E+06 | 1.125E+07 | 2.352E+06 | 3.709E+06 |
| S-47 | 2.274E+13 | 2.414E+16 | 8.780E+13 | 9.524E+13 |
| S-48 | 7.241E+14 | 5.005E+14 | 1.100E+15 | 2.618E+16 |
| S-49 | 4.090E+04 | 3.415E+04 | 3.104E+04 | 1.388E+05 |
| S-50 | 7.032E+14 | 2.818E+13 | 1.308E+15 | 1.801E+15 |
| S-51 | 3.602E+16 | 2.212E+18 | 2.455E+16 | 1.969E+17 |
| S-52* | 1.581E+10 | 1.949E+12 | 2.694E+10 | 6.974E+10 |
| S-53* | 6.215E+10 | 4.871E+11 | 3.565E+11 | 3.300E+11 |
| S-54* | 1.339E+10 | 1.372E+11 | 9.718E+09 | 6.266E+09 |
| S-55* | 2.752E+07 | 1.210E+08 | 3.651E+07 | 1.950E+07 |
| S-56 | 3.631E+04 | 3.771E+04 | 1.048E+05 | 2.889E+05 |
| S-57 | 0.000E+00 | 0.000E+00 | 0.000E+00 | 0.000E+00 |
| S-58 | 0.000E+00 | 0.000E+00 | 0.000E+00 | 0.000E+00 |
| S-59 | 6.653E+16 | 3.329E+17 | 1.563E+17 | 4.027E+18 |
| S-60 | 3.019E+18 | 9.346E+18 | 1.644E+19 | 2.420E+20 |
| S-61 | 1.334E+18 | 5.903E+16 | 1.184E+17 | 1.329E+17 |



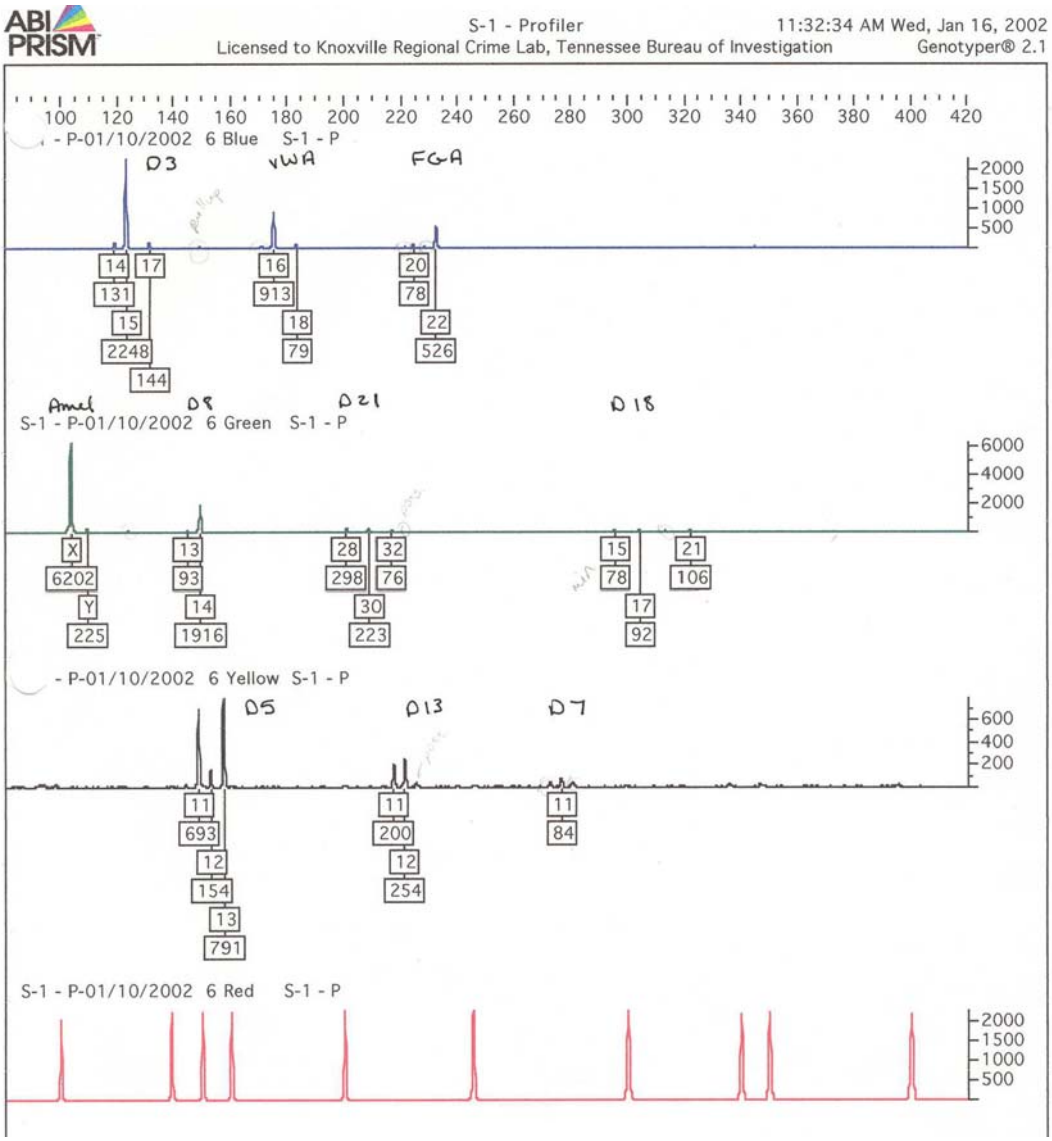

**Appendix A, Figure A1. Profiler Plus and COfiler Loci. The thirteen core CODIS loci and their range of possible allele lengths in bases (for Applied Biosystems). The color denotes the fluorescent dye attached to the specific primers as they are analyzed during capillary electrophoresis.**



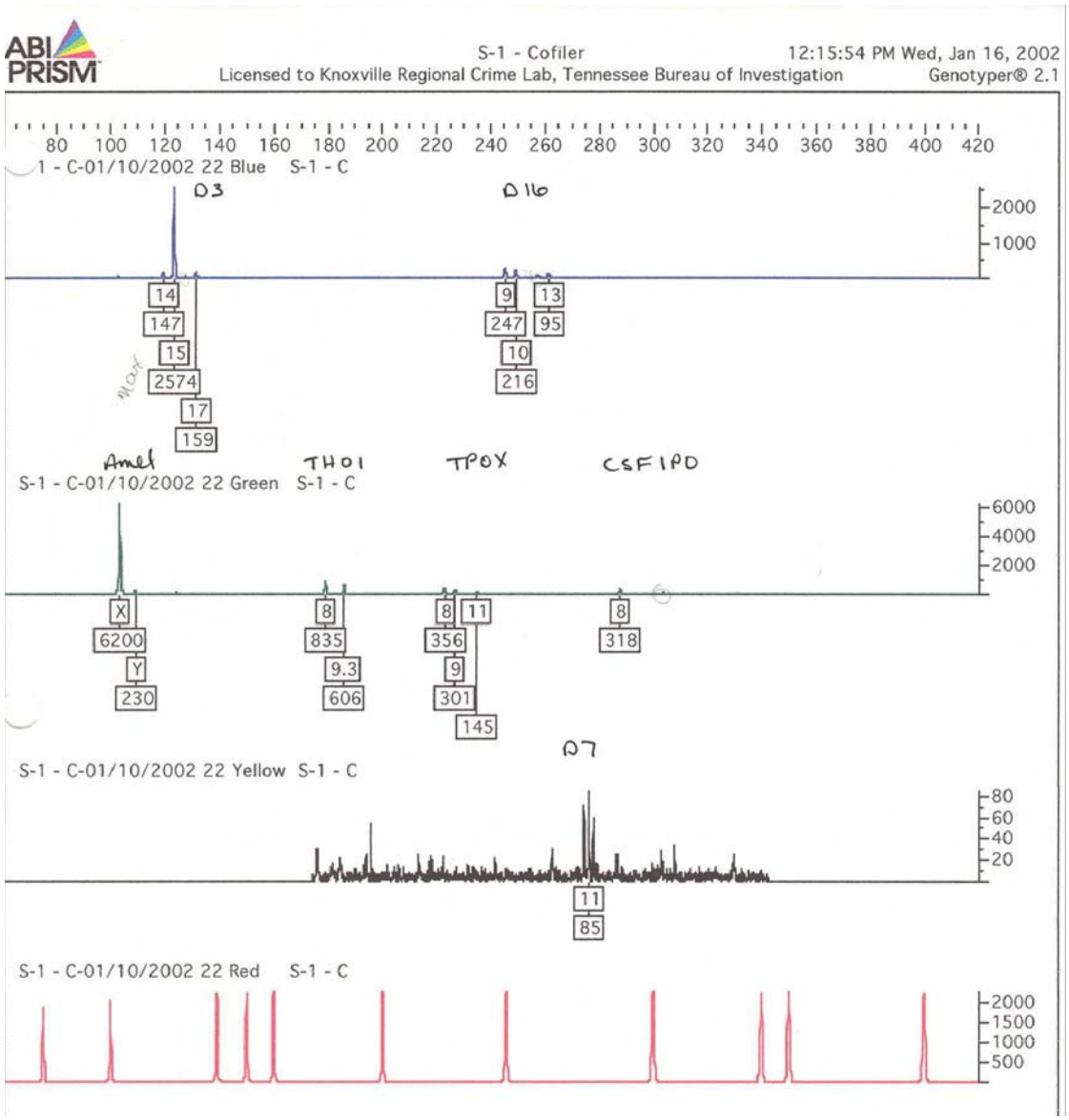

**Appendix A, Figure A2. Comparison of D21 and D18 to D3 Locus. The peak heights of alleles at D18 and D21 were divided by the peak height of D3, a smaller locus, to show degradation. Note that as [HCl] decreases, the percentage height of D18 and D21 increase.**



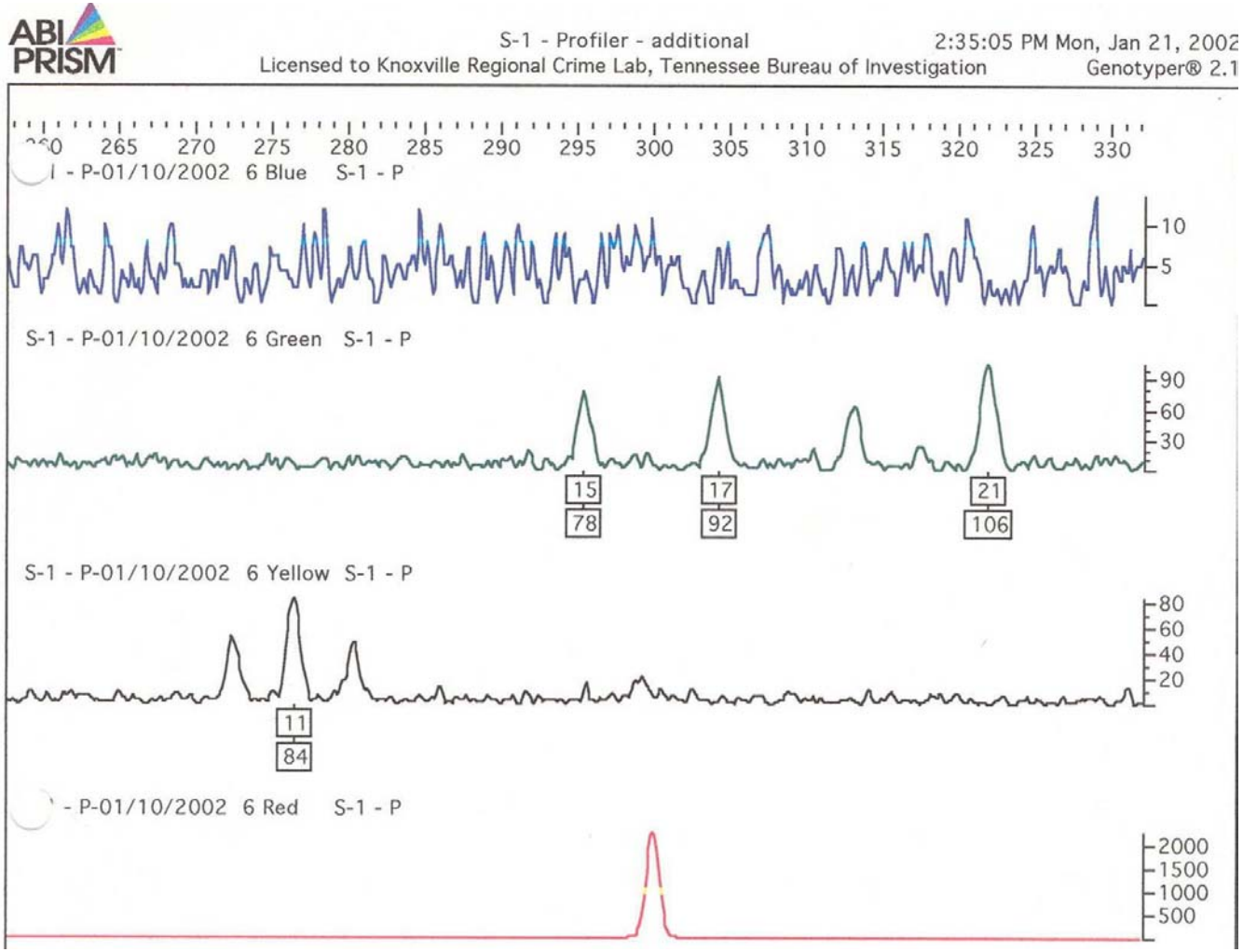

**Appendix A, Figure A3. D3, vWA and FGA Comparisons. Alleles were averaged for different destain groups at the D3, vWA, and FGA loci. Note that while the Thin Prep and Control samples show little change as the length of alleles increases, the peak heights of archived samples decrease dramaticallly**



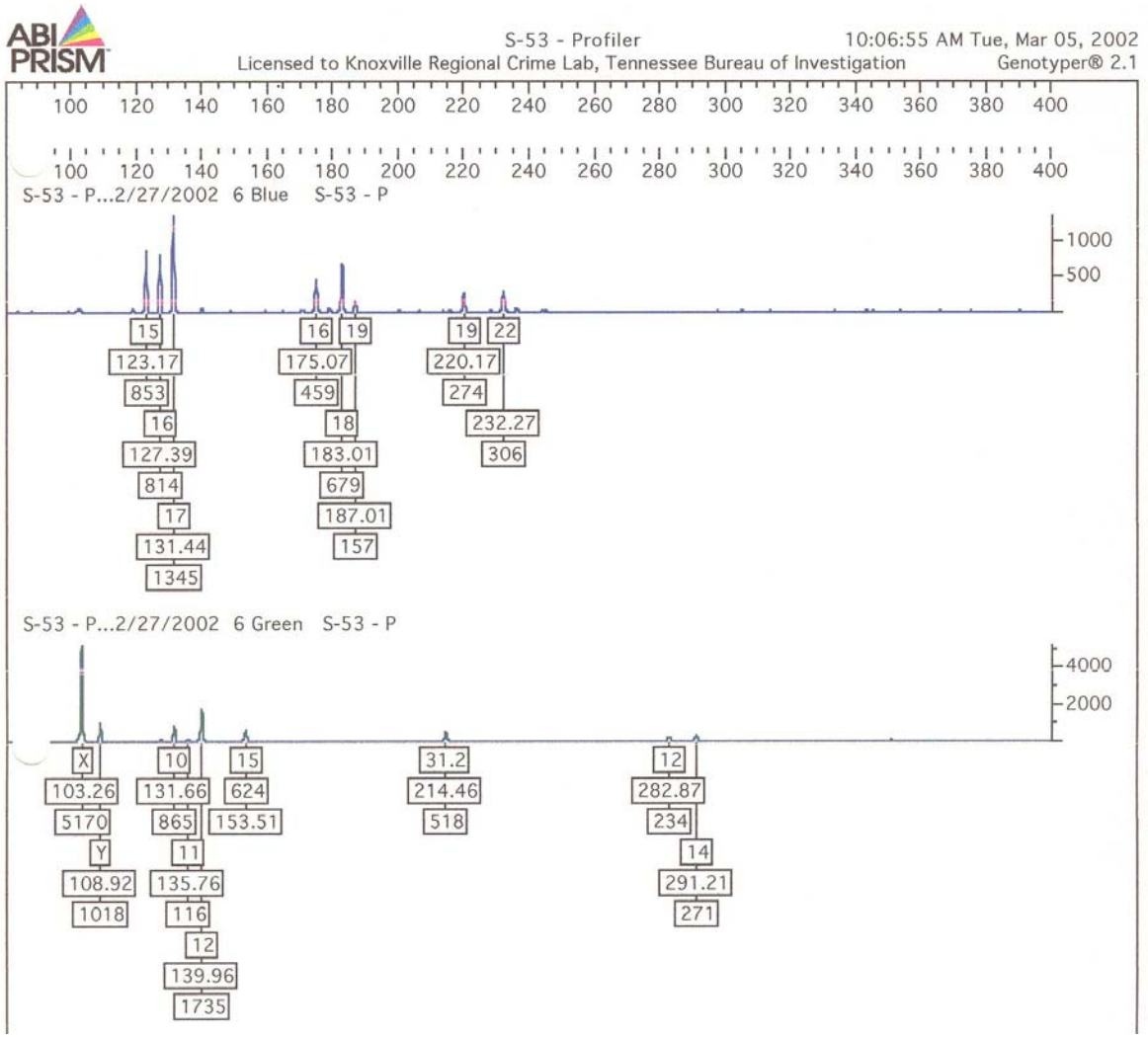

**Appendix A, Figure A4a. Electropherograms. Example of a mixed profile where more than one person's DNA is present. This is a Profiler electropherogram: the allele is the first box beneath the peak and the the peak height in rfus is below it. The loci designation is written above each set of alleles.**



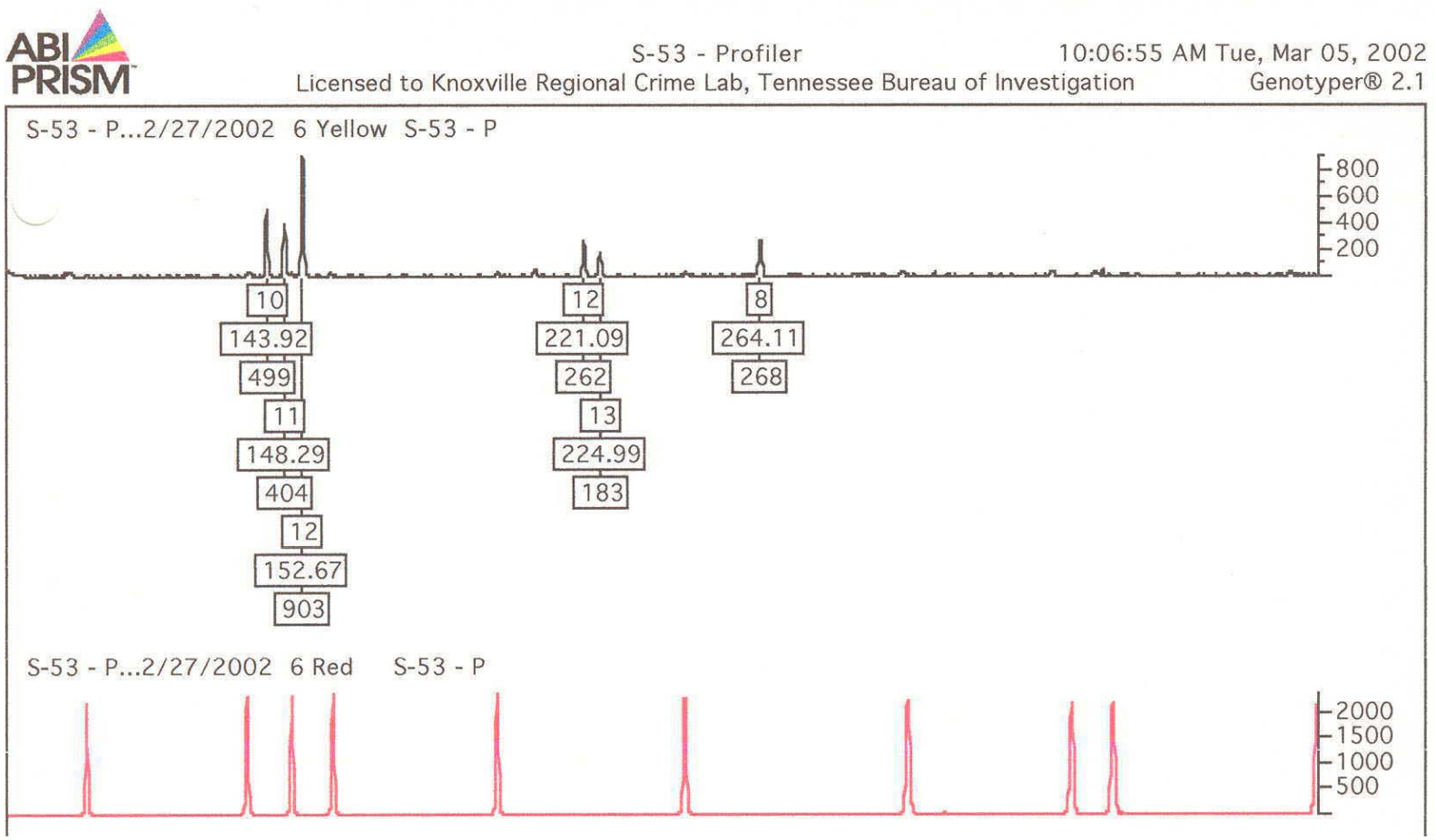

**Appendix A, Figure A4b. Electropherograms. Sample S-1, an example of a mixed profile – the COfiler electropherogram of Sample 1.**



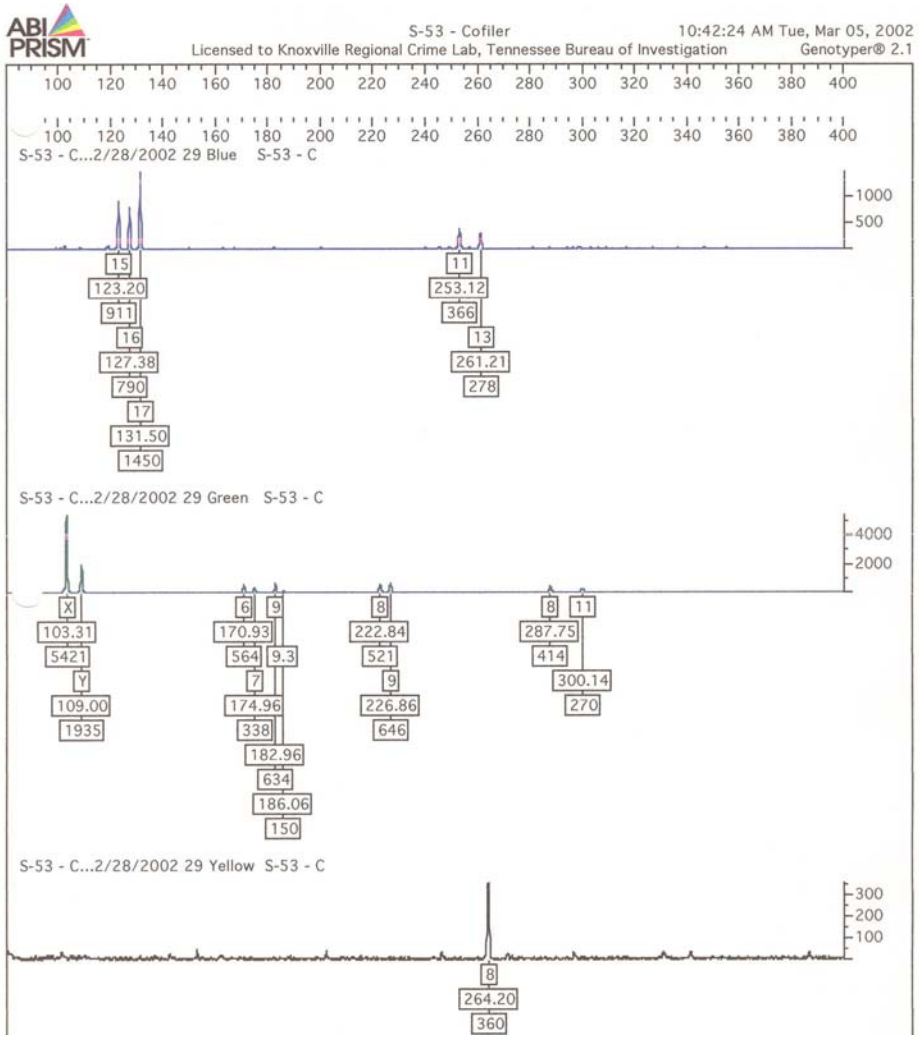

**Appendix A, Figure A4c. Electropherograms. From Sample 1, an example of loci where some alleles were called above 75 rfu, but other alleles were not called that appeared below 75 rfu.**



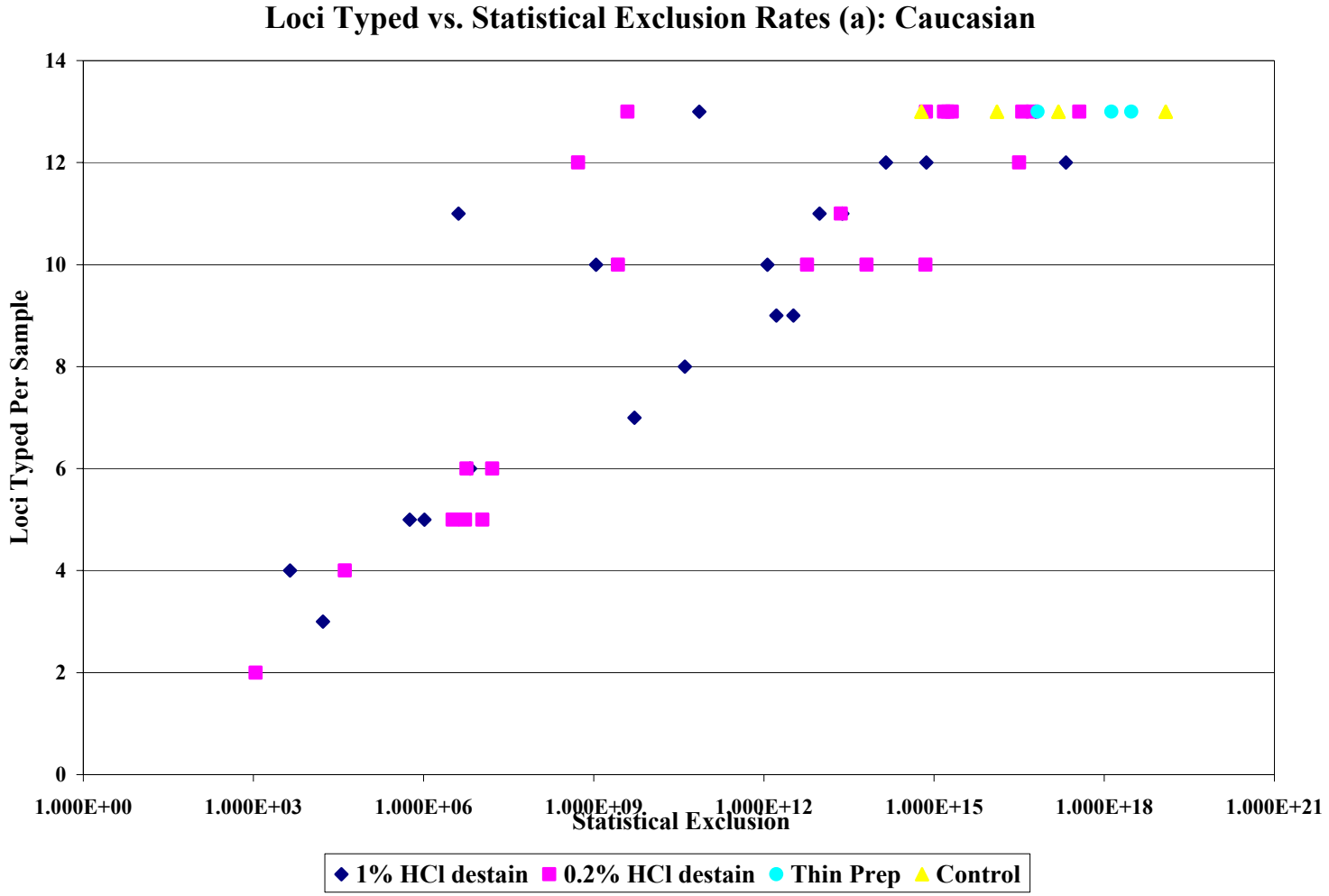

**Appendix A, Figure A5. Mixed Sample. S-53 is an example of a mixed sample, where a second DNA sample is present in the profile. This is evident first by the detection of a Y allele at the Amelogenin locus, but also by the presence of multiple alleles at the different loci.**



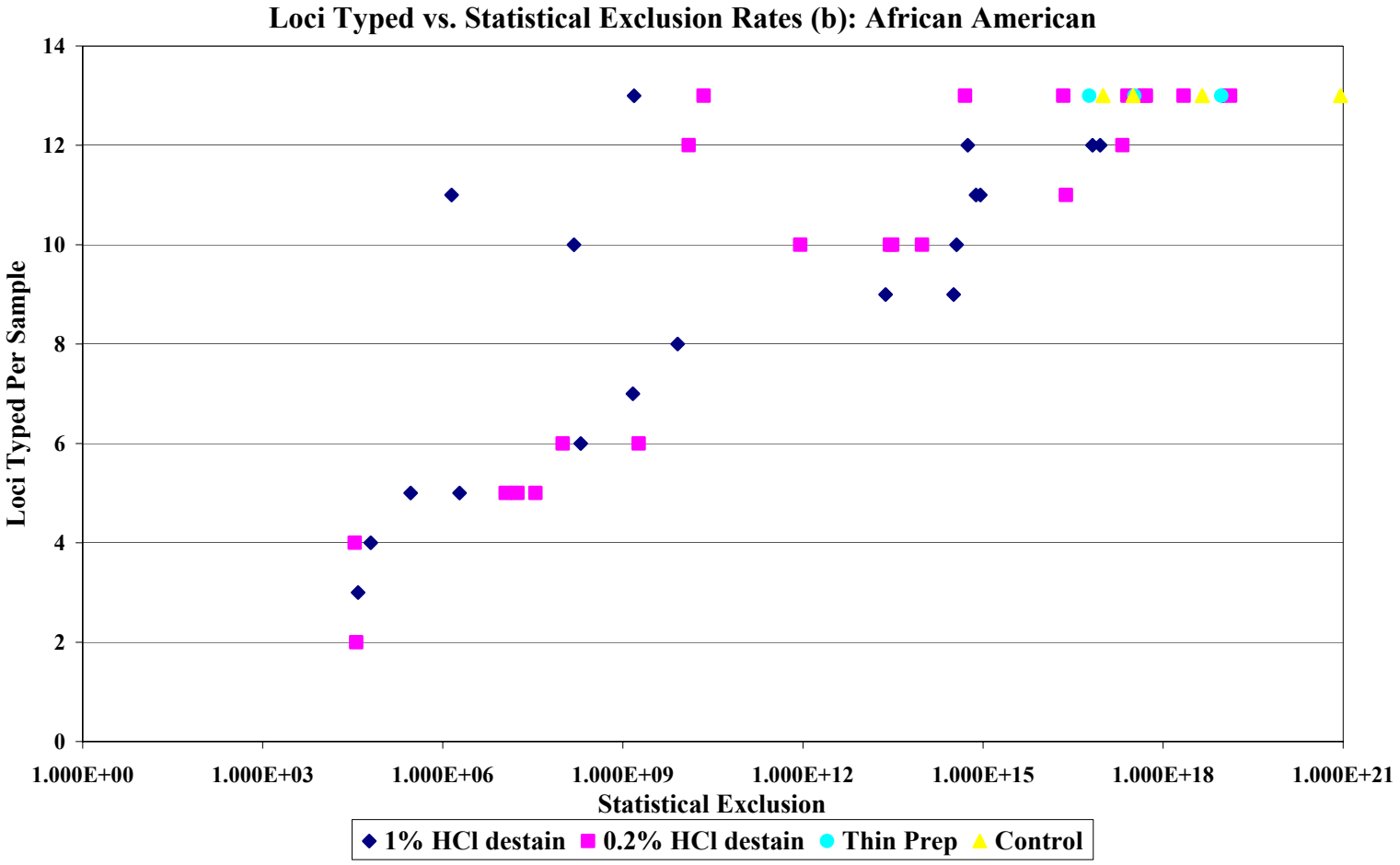

**Appendix A, Figure A5 continued. Mixed Sample. S-53 is an example of a mixed sample, where contaminating DNA is present in the profile. This is evident by the presence of multiple alleles at the different loci. This is the second part of the Profiler electropherogram for S-53; the red line denotes the ROX standard for this sample.**



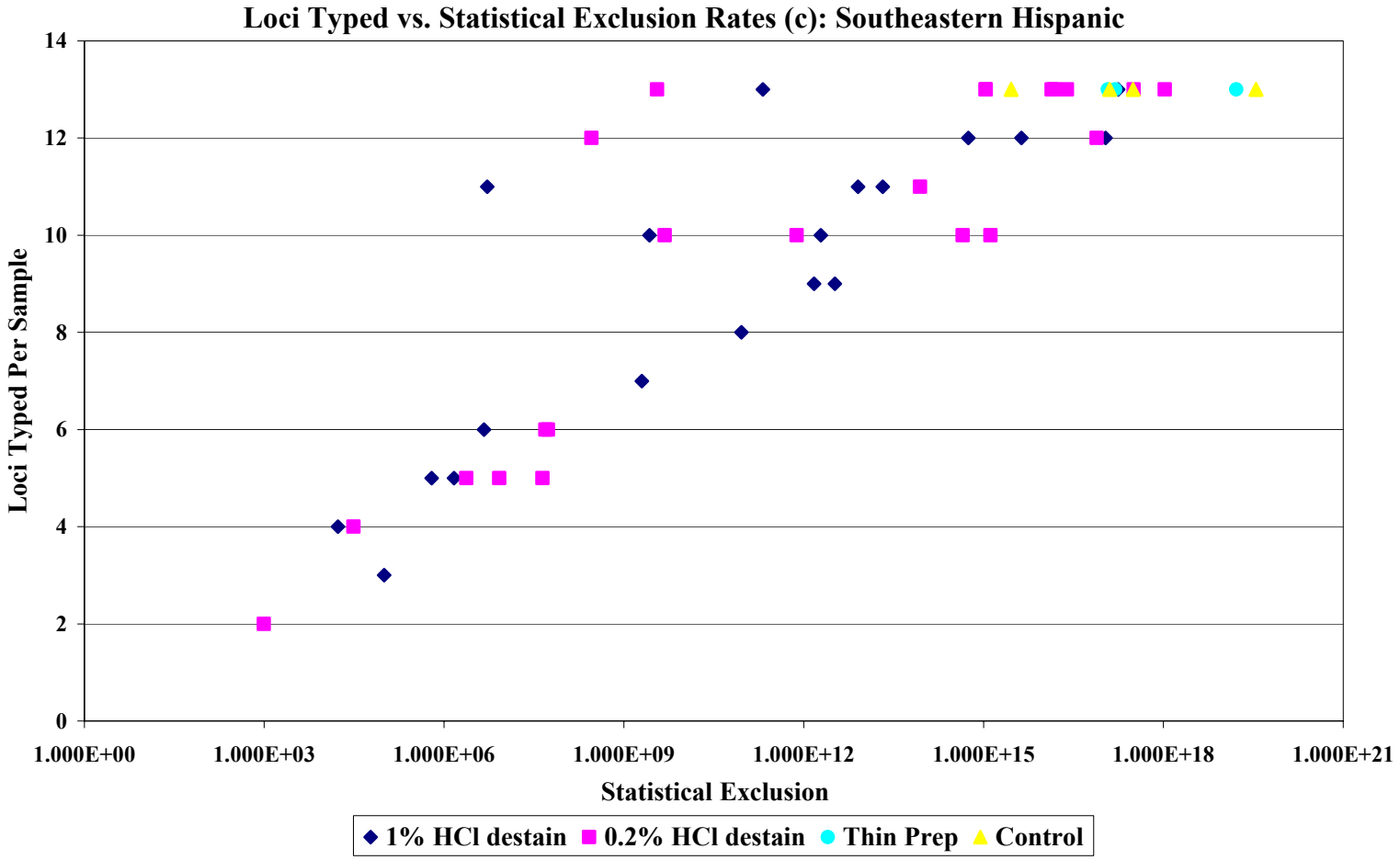

**Appendix A, Figure A5, continued. Mixed Sample. S-53 is a mixed sample with two profiles present. This is the COfiler electropherogram for this sample.**



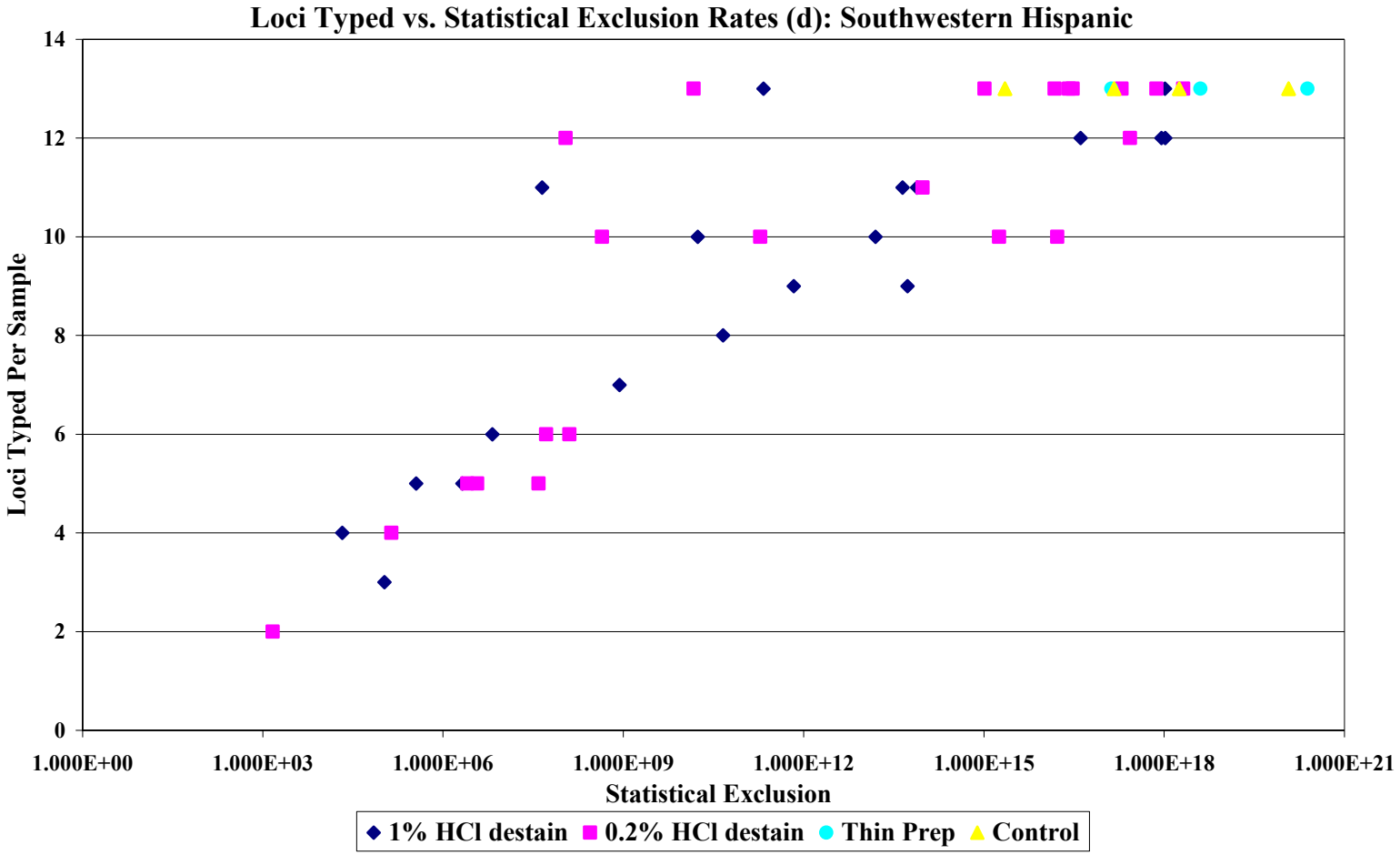

**Appendix A, Figure A6a. Loci Typed vs. Statistical Exclusion Rates (a): Caucasian. This shows the number of loci each sample typed within destain groups versus the exclusion rate that was calculated for the sample. Note that as the number of loci typed increased, the exclusion also increased.**




**Appendix A, Figure A6b. Loci Typed vs. Statistical Exclusion Rates (b): African American.**




**Appendix A, Figure A6c. Loci Typed vs. Statistical Exclusion Rates (c): Southeastern Hispanic.**




**Appendix A, Figure A6d. Loci Typed vs. Statistical Exclusion Rates (d): Southwestern Hispanic.**

# Appendix B

# Appendix B – Consent Form

## The Use of Pap Slides as a Source of DNA to Assist in the Identification of Missing Persons

### Introduction

This letter is to inform you about an opportunity to participate in a research study. The study will try to determine whether or not it is possible to identify an unknown individual using DNA from a prepared pathological slide. If the study is successful, the information gathered here will help in the identification of missing persons.

### Information about your involvement in the study

If you choose to be involved in this study, the following will take place. After you have read and fully understand this form you will need to sign it and return it to the medical staff. During the course of your regular exam, the practitioner will make two Pap slides - the one that is normally sent for evaluation and the second to be used in our research. In addition, the practitioner will take a soft brush (swab) and collect some cells from the inside of your mouth on the cheek. These cells will be used to extract DNA as well and the usefulness of the DNA from the two sources will be compared.

There is no additional procedure for you to go through and no additional cost to you - the practitioner will simply make a duplicate slide and the mouth cell swab for the research project. Before the research begins all information that may link the slide to you personally will be removed from the slide and swab and this informed consent, thus your anonymity will be maintained. *In other words, at no time during the research will your slide or the swab be linked back to you, nor will the researchers have any knowledge of your participation in the project.* Our objective is simply to see if DNA can be retrieved from the slide, not to identify you or any other individual.

### Risks

We anticipate no measurable risk to you. The slide that will be obtained will be taken the same time that your normal Pap smear is completed, so no additional procedure is required. The collection of cheek cells on the swab is a routine non-invasive procedure as well. Maintaining confidentiality and anonymity are of the highest importance to us, and we will take steps to ensure both.

### Benefits

This research we hope will make it possible for law enforcement investigators and other medical professionals to identify human remains using prepared medical slides as an information source.

Participant's initials _____

There is no direct benefit to you except our appreciation for your voluntary participation in meeting this goal.

**Confidentiality**

The information in the study will be kept confidential. Data will be stored securely and will be made available only to persons conducting the study. All personal identifiers will be removed from the slides and swabs before they are given to the researchers. No reference will be made in oral or written reports which could link participants to the study. In addition, any DNA information obtained will not be added to any forensic database.

**Compensation**

There is no compensation available for voluntary participants of this study.

**Emergency Medical Treatment**

The University of Tennessee does not "automatically" reimburse participants for medical claims. If physical injury is suffered in the course of this research, please notify the investigator in charge: Dr Karla J Matteson, 865-544-9449.

**Contact information**

If you have questions at any time about the study or the procedures (or you have experienced adverse effects as a result of participating in this study), you may contact the researcher: Dr Karla J Matteson at 865-544-9449. If you have questions about your rights as a participant, contact the Institutional Review Board office of the Graduate School of Medicine at 865-544-9781.

**Participation**

Your participation in this study is voluntary; you may decline to participate without penalty. If you decide to participate, you may withdraw from the study at any time without penalty.

**Consent**

I have read the above information. I have received a copy of this form. I agree to participate in this study.

Participant's Signature: _____ Date: _____

Witness's Signature: _____ Date: _____

# Vita

Jennifer Lee Millsaps was born in Waynesville, North Carolina on November 6, 1976. She moved to Maryville, TN in 1986 where she attended grade school and high school in Alcoa, TN. She graduated from Alcoa High School in 1995. She graduated Magna Cum Laude from Maryville College in 2000 with a B.A. in Biochemistry and a minor in Psychology. From there, she went to the University of Tennessee, Knoxville and received a M.S. in Life Sciences with a concentration in Genome Science and Technology in 2002.

Jennifer is currently employed with the Tennessee Bureau of Investigation and is pursuing a career in forensic science.