Feller, Elizabeth A., Evaluation of the AmpFℓSTR® MiniFiler™ Typing Kit: Mixture Studies and Non-Probative Sample Studies. Master of Science (Forensic Genetics), May, 2009, 42 pp., 2 illustrations, references, 11 titles.

This project was aimed to assemble and assess data from internal validations of AmpFℓSTR® MiniFiler™ PCR Amplification Kit by forensic laboratories across the United States. After compilation, data was evaluated for quality of testing, results, and concordance within and between participating laboratories. It was concluded that MiniFiler™ can successfully amplify DNA from multiple sources in mixtures of neat and degraded samples, as well as enhance DNA profiles obtained for several types of samples with suspected PCR inhibition or degradation. The data was collected into a final report with discussions and conclusions to the findings for submission to the National DNA Indexing System (NDIS) Approval Board for authorization to use forensic DNA genotypes generated using MiniFiler™ in national DNA databases.

EVALUATION OF THE AMPFℓSTR® MINIFILER™ TYPING KIT: MIXTURE STUDIES AND NON-PROBATIVE SAMPLE STUDIES

INTERNSHIP PRACTICUM REPORT

Presented to the Graduate Council of the

Graduate School of Biomedical Sciences

University of North Texas

Health Science Center at Fort Worth

In Partial Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

Elizabeth A. Feller, B.S.

Fort Worth, Texas

May 2009

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I would like to thank my major professor and graduate advisor, Dr. John Planz, for this practicum opportunity and for guidance during my internship. In addition I would like to thank my other professors, classmates and coworkers at UNTHSC, all of whom have made an indelible impact on me, my learning, my research, and my future. I could not go on without also thanking my husband, who always gives me a new perspective and a reason to ask *why*.

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CHAPTER 1

INTRODUCTION

Once a genetic profile has been obtained from a sample using STR fragment analysis, it can be compared to other known genetic profiles to see if a match can be made in which some or all of the alleles detected are observed in both profiles. In the instances of missing persons cases or convicted felons repeatedly committing crimes, it is helpful to develop a database of DNA profiles accessible by forensics personnel across the United States to ease communication between laboratories and make more connections between seemingly unrelated crimes or individuals. Such databases include the Combined DNA Indexing System (CODIS) and the National DNA Indexing System (NDIS), which are regulated by government boards and committees and provide forensics laboratories the ability to submit, store, and search for DNA profiles created from the analysis of human samples.

In order for a DNA profile to be entered into one of these databases, it must contain a minimum number of designated NDIS/CODIS loci (the CODIS "core STR loci") and the profile must have been obtained through approved methods. These include thoroughly validated methods and techniques. Each method or technique, including commercially-made "kits", must undergo developmental validation performed by the sponsoring company or manufacturer, in addition to internal validation performed by each lab interested in using the method or technique prior to its incorporation in the laboratory's general procedures. These validation studies include specific components such as:

1. Testing the method's robustness with a variety of procedural conditions (including limit of detection, mixture analysis, reproducibility and precision studies)

2. Assessing the method's production or control of artifacts

3. Testing the method for concordance with previously validated methods and with the identical method used in another laboratory or with alternative instruments

4. Evaluation the method's performance on mock casework sample or non-probative samples for its effectiveness in actual forensic testing

Each validation must also meet quality assurance requirements and follow standard quality control measures in place for each participating laboratory. In most cases these are consistent with the guidelines established by the Scientific Working Group on DNA Analysis Methods (SWGDAM) and the American Society of Crime Laboratory Directors Laboratory Accreditation Board (ASCLD-LAB), governmental bodies that allow for standardization of techniques and analysis between forensic laboratories.

Upon completion of all necessary validation studies the method may be submitted to the NDIS Approval Board for consideration. The Board will consider several aspects of the validation studies in making their decision, including:

1. Concordance studies

2. Mixed sample analysis

3. Non-probative sample analysis

4. Population studies

5. Precision studies

6. Proficiency and/or Qualifying sample studies

7. Reproducibility

8. Sensitivity studies (used to determine limits of detection and optimal template concentration)

9. Any relevant literature submitted for publication that pertain to the internal validation studies

10. Any other necessary information for the Board to determine the method's suitability and compatibility for use at NDIS

If the NDIS Approval Board deems the method acceptable for forensic use and for inclusion in the list of approved methods, it will notify each laboratory of the update so they can begin their internal validations or, if they have been completed, so that the laboratory can begin using the method in routine forensic work where it is applicable.

The AmpF*ℓ*STR® MiniFiler™ PCR Amplification Kit was developed by Applied Biosystems® as a way to amplify loci that are more prone to degradation or inhibition in compromised samples. One marked difference that distinguishes MiniFiler™ from other kits is that the primers are located as close as possible to the start of the target region, which results in shorter PCR amplicons (all less than 260bp). The loci selected for inclusion in MiniFiler™ was determined by surveying several laboratories who currently perform STR fragment analysis, as well as analyzing those loci that would produce the smaller amplicon sizes and loci that are frequently subject to degradation or inhibition. The MiniFiler™ kit uses the following loci in its multiplex reaction: D2S1338, D18S51, D21S11, FGA, D16S539, CSF1PO, D7S820, D13S317, and amelogenin. These loci all produce amplicons greater than 200bp in length in currently used

kits and are all informative sites with high allelic variability. These nine loci are labeled using the 5-dye chemistry system also used in other Applied Biosystems® products. In addition, the primers have non-nucleotide linkers attached to them, which allows for better DNA fragment migration and more loci to be labeled with the same dye without risking allelic overlap between loci.

Another difference between MiniFiler™ and other currently available kits is its protocols; namely, the *Taq* DNA polymerase is included in the Master Mix and the PCR parameters have been adapted for the optimal amplification of the shorter amplicons and to maximize primer binding specificity. These changes were also put into place to allow for the PCR reaction to overcome any inhibitors present and to ensure that degraded samples have the best opportunity for accurate amplification. The MiniFiler™ kit should be strongly considered as an enhancement tool for DNA analysis in which currently used kits cannot produce distinct genetic profiles due potentially to inhibition or degradation of the DNA or the amplification process.

In order to MiniFiler™ to be considered by the NDIS Approval Board as a kit suitable for generating profiles that can be uploaded into NDIS, several laboratories across the United States were recruited to performed independent internal validations using MiniFiler™. The internal validations done by each participating laboratory were performed in conjunction with the developmental validation study conducted by Julio Mulero and the Research/Development team of Applied Biosystems®. Once the internal validations were completed, their results and conclusions were submitted to the National DNA Indexing System (NDIS) Approval Board to assess the performance of MiniFiler™ and to approve the uploading of MiniFiler™-generated STR profiles into NDIS. The NDIS Approval Board requested more information regarding MiniFiler™ use with mixtures and non-probative samples before making a final decision on the

approval of MiniFiler™ for routine forensic laboratory use in casework analysis. Therefore, all of the data from each lab's internal validation that concerned mixture studies and non-probative sample analysis has been compiled and evaluated, then presented as a final report addendum to the original report.

This internship practicum has the following goals:

1. To compile data from the internal validations performed at each participating laboratory.

2. To evaluate data collected for concordance and to identify any differences in data that are not readily explained.

3. To present the data and the conclusions made from the data as a final report suitable for inclusion in the revised report being submitted to the NDIS Approval Board.

CHAPTER II

MATERIALS AND METHODS

The laboratories that participated in the validation of the MiniFiler™ kit include 5 laboratories across the country, all of whom are currently accredited (to maintain confidentiality they are Labs A through E). Each laboratory used a variety of samples for their validation studies. In particular, 2-person mixture samples were prepared in ratios that ranged from 20:1 to 1:1; in some instances, 3-person and 4-person mixture samples were also prepared with similar ratios. For non-probative samples, each laboratory used a variety of known and unknown samples for rigorous analysis. These included bone samples, whole blood, stains, and tissue preparations, each extracted with several methods including organic PCIA extraction, Chelex extraction, and extraction using robotics platforms such as the Promega® Maxwell 16® instrument.

To accurately assess MiniFiler™'s abilities and limitations, each sample was run according to the manufacturer's provided protocol (previously approved through developmental validation performed by Applied Biosystems®). Each laboratory used at least one ABI® genetic analyzer for DNA fragment separation and analysis. In some instances for non-probative samples, the injection time was increased from the protocol's recommended 5 seconds to 10 and 20 seconds, depending on the sample type and results from the standard injection and related results from testing the sample using the AmpFℓSTR® Identifiler® Human PCR Amplification Kit. In several instances samples were run in replicate.

Each laboratory submitted their data from their mixture studies and analyses of non-probative samples to Dr. John Planz, who then saved it to CD for analysis. The data was sent in

various forms including Excel spreadsheets, .PDF files, raw data and electropherograms. The data was then sorted into each category (mixture and non-probative sample studies) and the results were compared for each laboratory. Then the data was compiled into universal formats with visual aids and evaluated for concordance or lack thereof between the results of each laboratory. Discussion sections and conclusions were made for each participating laboratory's data, as well as overall conclusions for the MiniFiler™ kit. The final report was submitted to the SWGDAM committee for review for eventual submission in addition to the original report submitted to the NDIS Approval Board regarding MiniFiler™.

CHAPTER III

RESULTS

Mixture Studies

Each participating laboratories conducted mixture studies at various major:minor contributor ratios and with varying criteria to define the identification of a mixture. Each study reported the detection of multiple alleles using the MiniFiler™ kit for at least one locus for all mixture ratios (see Figure 1), and each also reported full profiles of both contributors for the 1:1 mixture samples. Total input DNA for each mixture varied between studies, ranging from 0.4ng/μL to 1.0ng/μL. A variety of mixtures were tested between all five laboratories, including:

- 2-person mixtures of males and females in ratios ranging from 20:1 to 1:20
- 2-person mixtures of 2 males in ratios ranging from 20:1 to 1:20
- 4-person mixtures of males and females in ratios ranging from 17:1:1:1 to 4:3:2:1
- Mixtures using neat samples in several ratios
- Mixtures using degraded samples in several ratios

Criteria for potential mixtures were defined as observing:

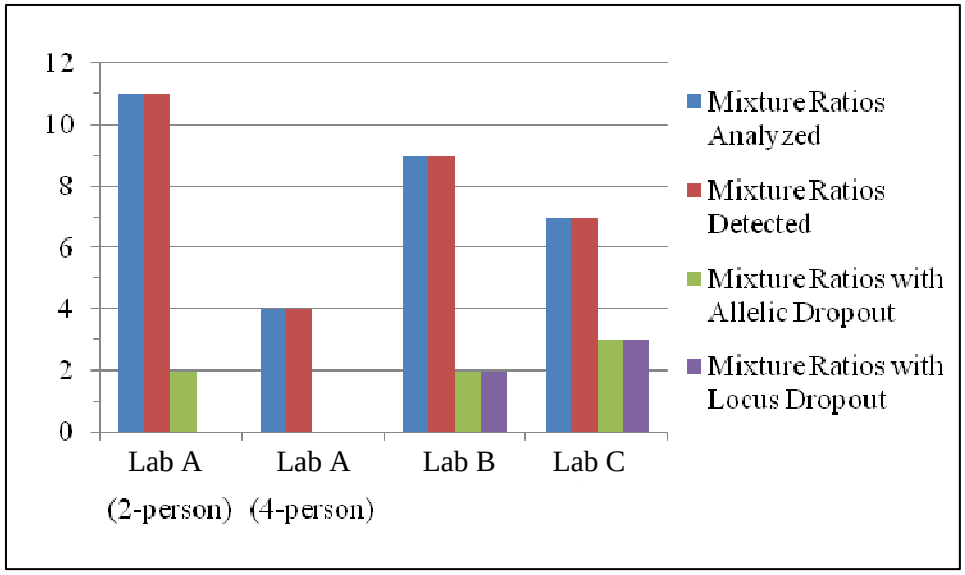
-Seeing more than 2 alleles at any locus

-Peaks in stutter positions greater than the expected amount of stutter for that position

-Heterozygous peak-height imbalances greater than 30%

One mixture study demonstrated that an estimated 87% of minor alleles were detectable in 2-person mixtures of 19:1 and 1:19. Of the other ratios for 2-person mixtures, some minor alleles could not be distinguished from the alleles of the major contributor at a 2:1 ratio, but almost 100% of the minor alleles were distinguishable once the ratio was changed to 4:1. All alleles were detected and accounted for in 4-person mixtures tested for all ratios (see Figure 1).

Figure 1. Mixture Ratios Analyzed by Participating Validating Laboratories using MiniFiler™.



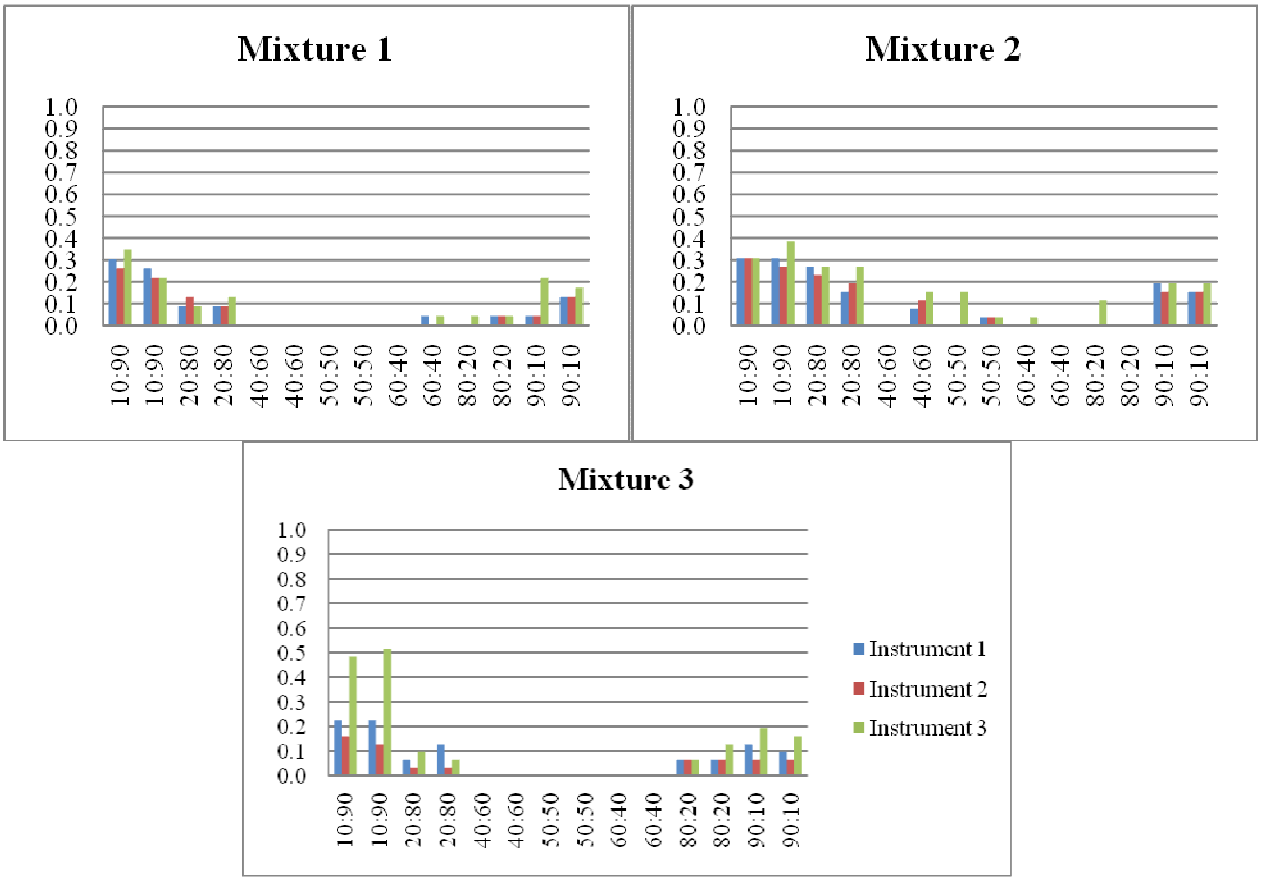
Some laboratories conducted additional tests, such as the ability to use peak-height imbalances to predict the major:minor contributor ratio. In most instances, each study was performed in duplicate or triplicate and on at least one genetic analyzer to ensure concordance within the laboratory.

Mixtures were detected in samples for nearly all mixture ratios and were most apparent in the 1:1 ratios and ratios that were close to 1:1. Once the mixture ratio exceeded 4:1 allelic dropout was observed for the minor contributor, either from decreased amplification or from the minor allele being filtered out as stutter from a major contributor allele. In studies that also

included degraded DNA as samples, it was observed that there was greater variability in the detection of minor alleles. Two studies reported more severe peak-height imbalances for the D7S820, FGA, and D16S539 loci, which in some cases resulted in allelic dropout of the minor alleles.

Overall these results demonstrate the potential use for MiniFiler™ in enhancing DNA profiles in mixtures where the major and minor contributors' DNA is in near-equal concentrations, but may be less reliable when the mixture ratio exceeds 4:1. As shown, the detection of minor alleles can be influenced by the genotype of the major contributor, as the minor alleles may fall in a stutter position and thus be filtered by an expert system.

Figure 2. Allelic Dropout Observed for Three Mixtures at Varying Mixture Ratios.



Non-Probative Sample Studies

Each laboratory conducted several studies using MiniFiler™ for the analysis of non-probative casework samples that produced full, partial and no profiles during previous analyses. Samples included a variety of tissue extracts (using organic extraction or Chelex extraction), bone extracts (using organic extraction), and Chelex-extracted reference bloodstains. All samples were amplified in replicate for testing. Each sample was previously analyzed using one or more currently NDIS-approved kit, including:

- Identifiler®
- PowerPlex 16®
- Profiler Plus® and COfiler®

Reference samples all had full profiles generated from one of these kits, while mock case samples and non-probative samples had either partial profiles or no profiles. For some samples, the injection time was varied to determine if any additional alleles could be accurately detected using both the kits mentioned above and MiniFiler™ (some samples underwent 20-second injections in addition to the standard injection time for the genetic analyzer being used). Once the samples were run, the alleles were analyzed using various expert systems software platforms. Profiles were considered "full" if all alleles were above 100RFU or 200RFU for heterozygous loci and homozygous loci, respectively.

In all studies the results from MiniFiler™ were concordant with alleles identified using one of the aforementioned kits. Samples that had allelic dropout using one of these kits had various successes with MiniFiler™ ranging from no improvement in the sample's profile to complete restoration of undetected alleles, and MiniFiler™ overall increased the resolution of all

alleles in the genotypes. Peak-height ratios for heterozygous loci were well above the minimum peak-height ratio specified by Applied Biosystems® and several participating laboratories. Some laboratories also focused their studies on artifacts produced by the MiniFiler™ chemistry. For bloodstain samples it was observed that stutter peaks fell below the standard detection thresholds for expert systems software.

In some samples from one study minor alleles were detected and the samples could be identified as potential mixtures; possible sources of contamination were eliminated as contributors (analysts, other samples, and controls) so these two samples may be true mixtures. With this exclusion of possible sources for minor alleles, the findings suggest that additional DNA was introduced during sample collection or processing prior to the samples arriving at the DNA laboratory.

One laboratory reported discordance between replicate testing using MiniFiler™ at the D2S1338 locus; because this locus is not included in the kits used to test the samples previously (Profiler Plus® and COfiler®) it was not determined at the time if it was a singular incident or if the primers used in the MiniFiler™ kit cover a region in the DNA with a primer binding mutation site.

MiniFiler™ was able to enhance the profiles of several samples in terms of allele detection, genotype resolution and signal strength, including those that exhibited PCR inhibition and/or degradation. However, its success was dependent partially on the DNA template concentration (when the concentration fell below 40pg/μL, more partial profiles were obtained from analysis).

CHAPTER IV

DISCUSSION

It can be concluded that the studies from these five laboratories demonstrate MiniFiler™'s variability in mixture detection. Because peak-height ratios for heterozygous loci are inconsistent, the assignment of major and minor components is strongly advised against. The success of MiniFiler™ in distinguishing between mixtures and single-source samples may be hindered by the sample's initial condition and any presence of inhibition, despite numerous examples of MiniFiler™'s ability to overcome these obstacles. In addition, the increased occurrence of artifacts would complicate any alterations in call thresholds and the distinction of true alleles from stutter peaks and stochastic amplification events. MiniFiler™'s best use for mixture interpretation is to enhance profiles from degraded or otherwise compromised samples, and the kit should not be relied upon to separate alleles into major and minor components for any sample type.

MiniFiler™ also performs well in enhancing incomplete profiles obtained from samples that are compromised by degradation or PCR inhibition. MiniFiler™ is useful in instances of low copy number DNA, in which preservation of the sample itself remains of utmost importance for the possibility of future testing. However, the use of MiniFiler™ must come with a caveat that analysts using the kit must be well-versed in its sensitivity to the amount of input DNA used, as well as possible obstacles in data interpretation. These include the observances of stutter, amplified artifacts and variable peak imbalances. Peak-height ratios at heterozygous loci had a wide range both within each laboratory and between the five labs, indicating that data interpretation may be difficult at times during routine casework. Call thresholds may have to be altered to account for low signal strength from loci with extreme peak-height ratio imbalances,

possible primer binding mutations or that undergo unpredictable amplification events. Together, these findings indicate that MiniFiler™ is best used on single-source samples or mixtures of equal ratios to enhance incomplete profiles due to degradation, low copy number or inhibition.

CHAPTER VI

CONCLUSIONS

It was concluded that the MiniFiler™'s limit of detection for mixtures is partially influenced by the major:minor contributor ratio as well as the genotype combinations at each locus. These factors imply that the background information provided for a case as well as the stutter definition and peak-height ratios should be taken into consideration when interpreting potential mixtures using MiniFiler™. Overall these results demonstrate the potential use for MiniFiler™ in enhancing DNA profiles in mixtures where the major and minor contributors' DNA is in near-equal concentrations, but may be less reliable when the mixture reaches a more extreme major:minor contributor ratio.

MiniFiler™ shows great potential in recovering alleles lost in current STR typing due to degradation or PCR inhibition. All studies were able to provide evidence that the MiniFiler™ kit can be used to enhance DNA profiles from a variety of sample types that were extracted using a wide variety of methods. These findings indicate that integration of MiniFiler™ into current laboratory practices would have minimal interruption of normal activity, because no procedures need to be specifically used for the correct use of the MiniFiler™ kit.

Future studies that could be performed to assess MiniFiler™'s genotyping abilities should include specific known PCR inhibitors in varying ratios and concentrations, stains and samples of varying age or stages of degradation/decomposition, and samples allowed to comingle as would be observed with buried remains from several individuals. In addition, it may prove useful to determine if any explosive substrates could inhibit amplification using MiniFiler™ for the use of this kit in mass disaster scenarios in which an explosion occurred. The

variation observed in peak-height ratios should be rigorously investigated prior to use of MiniFiler™ for known mixture samples if an attempt to distinguish between major and minor contributors will be made, or if the mixture ratio is determined to be greater than 4:1.

Along with the developmental validation study performed by Applied Biosystems®, these internal validation studies provide concrete conclusions that the MiniFiler™ kit can be a useful tool in forensic DNA analysis and could aide in current investigations and the re-opening of cold cases to examine aged or otherwise degraded biological evidence. For enhancing DNA profiles that may be subject to inhibition or degradation in single-source and mixture samples, the NDIS Approval Board should heavily consider MiniFiler™ as an addition to the list of approved human DNA PCR amplification kits used to generate DNA profiles for uploading into national DNA databases.

APPENDIX 1

EVALUATION OF THE AMPFℓSTR® MINIFILER™ TYPING KIT: MIXTURES

Evaluation of AmpFℓSTR® MiniFiler™ Typing Kit: Mixture Analysis

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Because mixture analysis and interpretation can play a major role in casework processing, the MiniFiler™ kit was robustly tested for its mixture resolving power, stutter at each locus, the highest and lowest ratios for which mixtures can be detected, and the ability for laboratories to correctly interpret and report mixture results from MiniFiler™.

A total of five accredited laboratories performed mixture study components for their internal validation of MiniFiler™ (to maintain confidentiality they are Labs A-E). Lab A conducted mixture studies involving 2-person mixtures at various ratios from 1:19 to 19:1 (both male:male and male:female mixtures) and a 4-person mixture (1 female:3 males) at the following mixture ratios: 17:1:1:1, 14:3:2:1, 4:3:2:1, and 1:1:1:1. Each mixture had a total input DNA of 1ng for amplification and used DNA extracted from whole blood samples extracted using Chelex. Injections were made for 5 and 10 seconds on the ABI® 3130-A Genetic Analyzer; each run also included single runs of each contributor's DNA sample as single-source controls.

Criteria for potential mixtures were defined as:

-Seeing more than 2 alleles at any locus

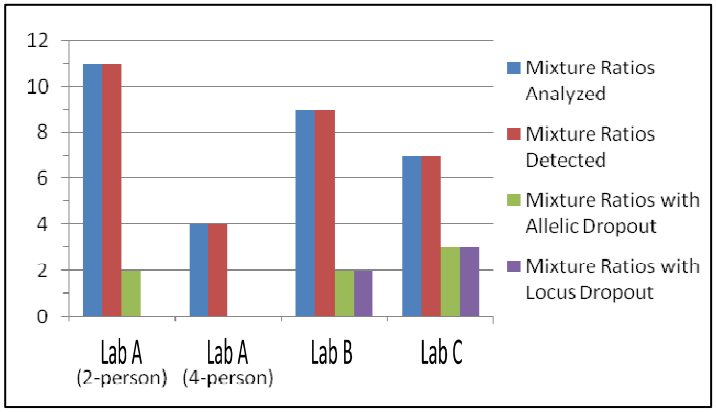
-Peaks in stutter positions greater than the expected amount of stutter for that position

-Heterozygous peak-height imbalances greater than 30%

The Lab A mixture study demonstrated that an estimated 87% of minor alleles were detectable in the 2-person mixtures of 19:1 and 1:19. Of the other ratios for the 2-person mixtures, some minor alleles could not be distinguished from the alleles of the major contributor at a 2:1 ratio, but almost 100% of the minor alleles were distinguishable once the ratio was changed to 4:1. All alleles were detected and accounted for in the 4-person mixtures for all ratios (see Figure 1).

It was concluded that the MiniFiler™'s limit of detection for mixtures is partially influenced by the major:minor contributor ratio and by the genotype combinations at each locus. These factors imply that the background information provided for a case as well as the stutter definition and peak-height ratios should be taken into consideration when interpreting potential mixtures using MiniFiler™.

Figure 1. Mixture Ratios Analyzed by Participating Validating Laboratories using MiniFiler™.



Lab B conducted 2-person mixture studies for degraded and neat samples, using initial DNA concentrations for each sample of 0.5ng/μL at the following ratios: 20:1, 10:1, 5:1, 3:1, 1:1, 1:3, 1:5, 1:10 and 1:20. Initial samples were chosen based on allelic diversity at each locus. Once the samples were combined, 1μL of each mixture was amplified with MiniFiler™ and run on the ABI® 3100 using 10-second injections.

The evaluation of each mixture included:

-Ability to detect a mixture

-Noticeable peak-height imbalances in heterozygous loci

-Ability of use the peak-height ratios to predict the mixture ratio of one contributor to another

Mixtures were apparent at the 20:1 ratio for neat and degraded samples; in general it was more difficult to detect a mixture using degraded DNA samples due to the variability in the detection of minor alleles. For neat DNA mixtures, peak-height imbalances were observed for heterozygous loci in all ratios (see Lab B Table 5), with the most significant imbalance seen at D7S820 at the 3:1 ratio. Predictions of mixture ratios were also made for the neat DNA mixtures to help determine how reproducible each mixture ratio is using MiniFiler™ (see Lab B Table 6). Overall, the 1:1, 3:1 and 5:1 ratios provided the most precision and consistency across the entire profile for neat DNA mixtures.

Lab B Table 5. Assessment of Peak Height Imbalance in Heterozygous Loci.

Ratio of A:B (KLH:SY)	Most Imbalanced Locus	Peak Height Ratio of Imbalance
1:1	D7S820	62%
1:3	D21S11	56%
1:5	D21S11	60%
1:10	D16S539	56%
1:20	D2S1338	77%
20:1	D21S11	50%
10:1	D2S1338	45%
5:1	D2S1338	54%
3:1	D7S820	33%

Lab B Table 6. Calculated Mixture Ratios Based on Peak Height.

Proposed Ratio of A:B A = KLH, B = SY	Actual Ratio of A:B (range)
1:1	1:1
1:3	1:3
1:5	1:3 to 1:6
1:10	1:4 to 1:15
1:20	1:20
20:1	1:5.5 to 1:21
10:1	1:5 to 1:15
5:1	1:2.5 to 1:6
3:1	1:2 to 1:3

The Lab C validation study included analyzing several 2-person mixture studies in triplicate at the following ratios: 20:1, 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:10 and 1:20. Each DNA mixture had a total input DNA of 0.4ng for amplification using MiniFiler™. The mixtures were analyzed by using peak-height imbalances at heterozygous loci to exclude "major component" alleles and alleles shared by the major and minor contributors. Along with amelogenin, 8 loci were analyzed for each mixture (see Lab C Table 1). It was observed that three separate loci did not have any detectable minor alleles (D7S820 and Amelogenin for 1:20 mixture and D16S539 for 1:10 mixture). In addition, the 1:10 mixture had one allele undetected at FGA for one of its injections, but the allele was detected in the two subsequent injections. In the 20:1 mixture, D21S11 and D18S51 detected both minor alleles but for each locus one allele was filtered out as stutter, as it fell in the stutter position for one of the major alleles at the locus. For all other mixture ratios

and all loci, all minor alleles that did not overlap with major alleles were detected and allele calls were consistent for all injections and ratios.

Lab C Table 1. Minor Component Alleles (Excluding alleles shared with Major Component).

Description	D13S317	D7S820	Amel	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
20:1 Mix	8	13	N/A	18,23	29*	N/A	21*	9	22,23
10:1 Mix	8	13	N/A	18,23	29,30	N/A	15,21	9	22,23
5:1 Mix	8	13	N/A	18,23	29,30	N/A	15,21	9	22,23
2:1 Mix	8	13	N/A	18,23	29,30	N/A	15,21	9	22,23
1:2 Mix	11,12	11	Y	16,19	30.2,31	9	16,20	10	20,25
1:5 Mix	11,12	11	Y	16,19	30.2,31	9	16,20	10	20,25
1:10 Mix	11,12	11	Y	16,19	30.2,31	-	16,20	10	20**
1:20 Mix	11,12	-	-	16,19	30.2,31	9	16,20	10	20,25

* = Both minor alleles detected at this locus, but one fell in the stutter position of a major allele and filtered out as stutter.
** = Minor 25 allele was not detected in the first injection of this sample, but was detected in the second and third injections.

- = No minor alleles detected

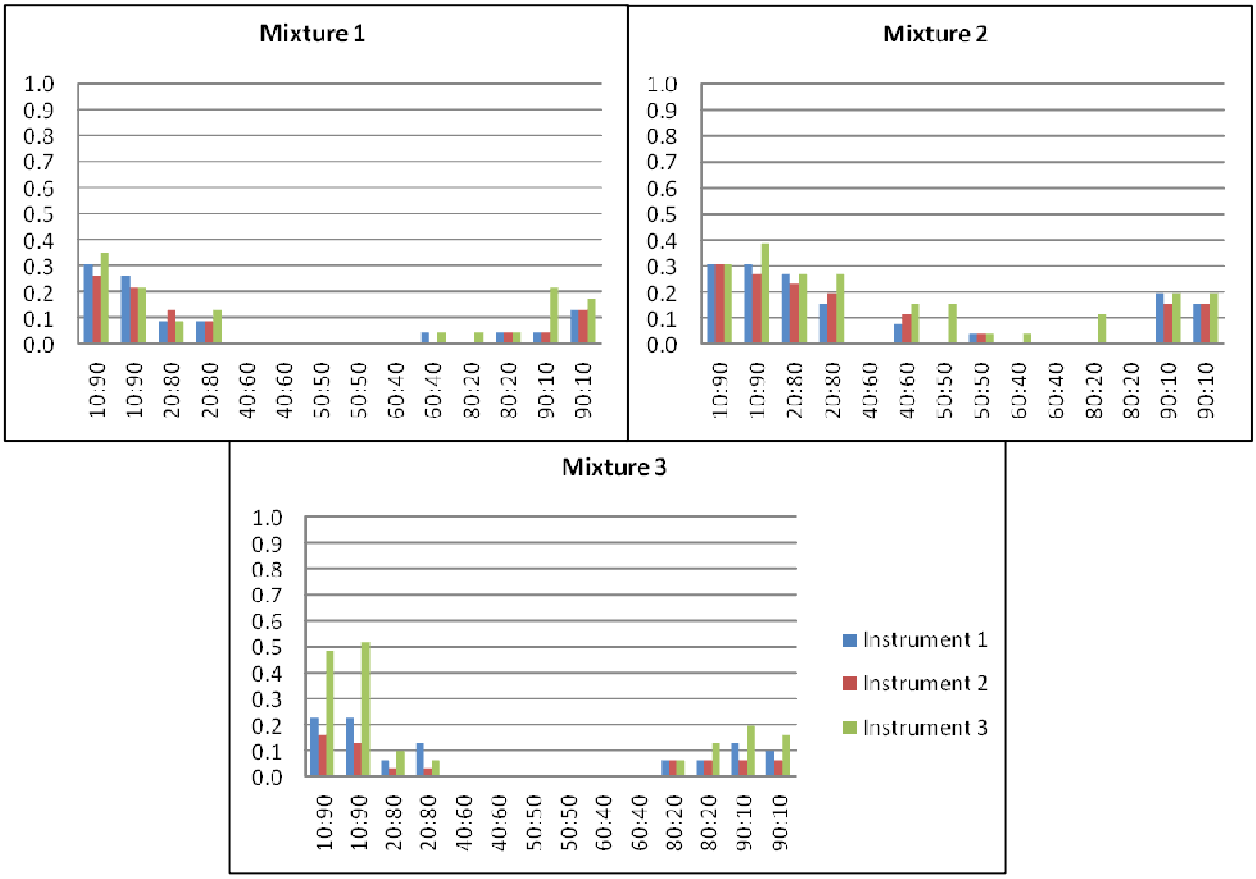
N/A = Not applicable, no non-overlapping alleles at this locus.

Lab D conducted three 2-person mixture studies in duplicate on three different instruments at several ratios (10:90, 20:80, 40:60, 50:50, 60:40, 80:20 and 90:10). For the more extreme mixture ratios (10:90 and 90:10) there was more noticeable allele dropout, as well as a higher number of loci exhibiting this dropout. (see Lab D Table 1 and Figure 1). In two mixture studies full profiles were obtained with all alleles present for nearly all of the 40:60, 50:50 and 60:40 mixture ratios (with the exception of 2 samples at the 60:40 ratio). The third mixture study experienced allele dropout in at least one of the mentioned ratios on all three instruments. Overall these results demonstrate the potential use for MiniFiler™ in enhancing DNA profiles in mixtures where the major and minor contributors' DNA is in near-equal concentrations, but may be less reliable when the mixture ratio exceeds 60:40.

Lab D Table 1. Summary of Results from Three 2-person Mixture Studies (Mixture 1 has 23 total alleles present; Mixture 2 has 26 total alleles present; Mixture 3 has 31 total alleles present).

	Mixture 1			Mixture 2			Mixture 3		
	Instrument 1	Instrument 2	Instrument 3	Instrument 1	Instrument 2	Instrument 3	Instrument 1	Instrument 2	Instrument 3
Ratio	**Average Allele Dropout/Average Loci Exhibiting Dropout**								
10:90	6.5 / 5	5.5 / 4	6.5 / 5	8 / 6	7.5 / 6	9 / 6.5	7 / 5	4.5 / 4	15.5 / 9
20:80	2 / 2	2.5 / 2.5	2.5 / 2	5.5 / 4.5	5.5 / 4	7 / 5	3 / 2.5	1 / 1	2.5 / 2.5
40:60	-	-	-	1/ 1	2 / 1.5	2 / 1.5	-	-	-
50:50	-	-	-	0.5 / 0.5	0.5 / 0.5	2.5 / 2	-	-	-
60:40	0.5 / 0.5	-	0.5 / 0.5	-	-	0.5 / 0.5	-	-	-
80:20	0.5 / 0.5	0.5 / 0.5	1 / 1	-	-	1.5 / 1.5	2 / 2	2 / 2	3 / 3
90:10	2 / 1.5	2 / 1.5	4.5 / 3.5	4.5 / 3.5	4 / 3	5 / 3.5	3.5 / 2.5	2 / 1.5	5.5 / 3.5

Lab D Figure 1. Relative Allelic Dropout Observed for Three Mixtures at Varying Mixture Ratios.



The fifth laboratory, Lab E, conducted two sets of 2-person mixture studies; the mixtures were selected to best cover the allelic range of the loci between each pair of individuals. Samples of each mixture were made in the following dilutions: 20:1, 15:1, 10:1, 5:1, 3:1, 1:1, 1:3, 1:5, 1:10, 1:15 and 1:20. The mixtures were all prepared to obtain similar peak heights between individuals for the 1:1 dilution. All samples were then amplified using MiniFiler™ and run on an ABI® 3130 Genetic Analyzer.

Mixtures were characterized by the following criteria:

- Two or more alleles present at a locus for several loci

- Severe peak-height imbalances at a heterozygous locus at one or more loci

The results of the capillary electrophoresis indicated that multiple alleles were detected in all dilutions to the extent that each sample could be distinguished as a mixture. All alleles were detected in the 1:1 mixture, but the other mixtures generated incomplete profiles. Allelic dropout was more pronounced as the mixture ratio became more extreme, though minor contributor alleles were still detected for at least one loci in both the 1:20 and 20:1 mixtures and no locus had complete allelic dropout across all dilutions.

Overall, it can be concluded that the studies from these five laboratories demonstrate MiniFiler™'s variability in mixture detection. Because peak-height ratios for heterozygous loci can be inconsistent, the assignment of major and minor components is strongly advised against. The success of MiniFiler™ in distinguishing between mixtures and single-source samples may be hindered by the sample's initial condition and any presence of inhibition, despite numerous examples of MiniFiler™'s ability to overcome these obstacles. In addition, the increased occurrence of artifacts would complicate any alterations in call thresholds and the distinction of true alleles from stutter peaks and stochastic amplification events. MiniFiler™'s best use in terms of mixture analysis and interpretation is to enhance profiles from degraded or otherwise compromised samples, and the kit should not be relied upon to separate alleles into major and minor components. Compared to other currently NDIS-approved PCR amplification kits, MiniFiler™ performs at the same level in terms of mixture identification and allele detection. In some instances MiniFiler™ was more sensitive in its detection of minor alleles than most of the currently used kits would be, indicating that MiniFiler™ could be of great use in mixtures with lower concentrations of DNA for one or more contributors.

APPENDIX 2

EVALUATION OF THE AMPFℓSTR® MINIFILER™ TYPING KIT: NONPROBATIVES

Evaluation of AmpFℓSTR® MiniFiler™ Typing Kit: Non-Probative Studies

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A critical component of the validation of MiniFiler™ for routine forensic use is studies that scrutinize reproducibility and the ability to use MiniFiler™ to generate reliable DNA profiles from common casework samples. The use of non-probative casework samples and mock case samples allows for the evaluation of MiniFiler™'s success and potential shortcomings in analyzing routine samples whose condition would warrant the use of MiniFiler™ to enhance incomplete profiles or obtain profiles for samples that previously generated no results due to low copy number, inhibition or degradation.

Lab A conducted several studies using non-probative casework samples that produced full, partial and no profiles during previous analyses. Samples included a variety of tissue extracts (using organic extraction or Chelex extraction), bone extracts (using organic extraction), and Chelex-extracted reference bloodstains. All samples were amplified by four different analysts using MiniFiler™ (some samples in duplicate); in previous work, each sample was genotyped using Promega® PowerPlex 16® (a currently validated kit) so the genotypes could be referenced for testing the concordance of MiniFiler™ with a kit already in use in routine casework. Each sample was diluted to approximately 0.5ng/μL input DNA then requantified before amplification. If the sample could not reach the target concentration, 10uL of sample extract was used for amplifying with MiniFiler™. The samples were then analyzed on the ABI® 3130XL Genetic Analyzer using 5- and 10-second injections (in some cases, 20-second injections were also performed).

Once the samples were run, the alleles were analyzed using GeneMapper ID® with 40RFU as the minimum intensity for determining the quality of the peak heights. Profiles were considered "full" if all alleles were above 100RFU or 200RFU for heterozygous loci and homozygous loci (see Lab A Table 1). It was observed that in most instances MiniFiler™ was capable of genotype enhancement, peak balance, and overcoming PCR inhibition and/or DNA degradation.

Lab A Table 1. Non-Probative Sample Results by Sample Type.

Sample Type	PP16® Results	Identifiler® Results (5sec)	Identifiler® Results (10sec)	Identifiler® Results (20sec)	MiniFiler™ Results (5sec)	MiniFiler™ Results (10sec)	MiniFiler™ Results (20sec)
Reference Bloodstain (Chelex)	D21 artifact	Full profile, RFUs 600-1000, possible D21 artifact	Full profile, RFUs ~2000, possible D21 artifact	N/A	Full profile, RFUs ~3000, no D21 artifact	Full profile, RFUs >6000, no D21 artifact	N/A
	Severe D5 imbalance	Full profile, RFUs ~1000-2000, no D5 imbalance	Full Profile, RFUs ~2000-4000, slight FGA imbalance, no D5 imbalance	N/A	Full profile, RFUs ~200- >4000	Full profile, RFUs ~4000- >8000	N/A
	Severe FGA imbalance	Full profile, RFUs ~2000-3000, FGA imbalance	Full profile, RFUs ~4000-6000, FGA imbalance	N/A	Full profile, RFUs 300 -6000, FGA imbalance	Full profile, RFUs >7000, FGA imbalance	N/A
Bode Swab	Full Profile	Partial profile (FGA below threshold)	Full profile	Full profile	Full profile	Full profile	N/A
	Partial Profile	Partial profile†‡	Partial profile‡	Partial profile ‡	Full profile	Full profile	N/A
	Partial Profile‡	Partial profile‡	Full profile	Full profile	Full profile	Full profile	N/A

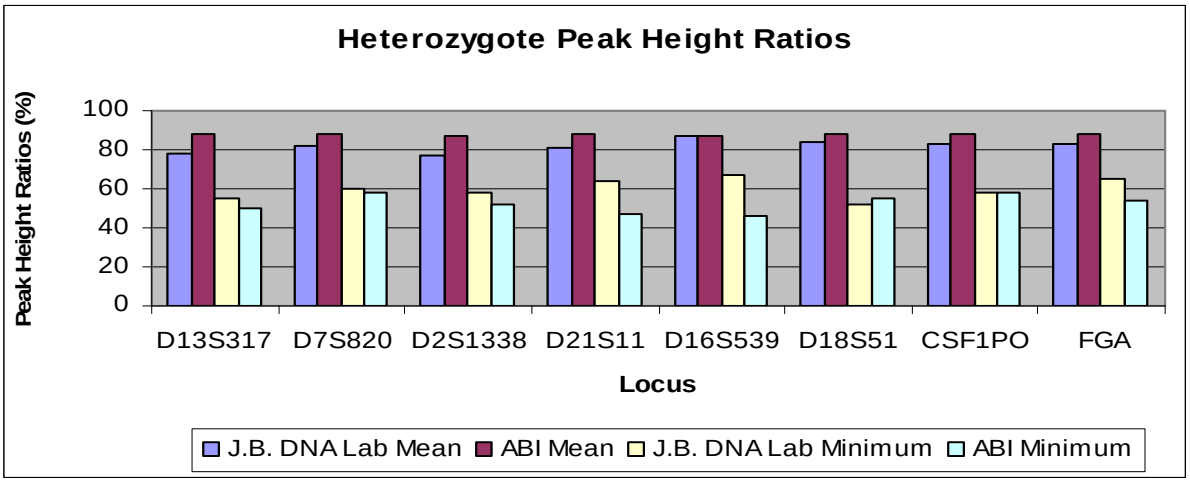
Sample Type	PP16® Results	Identifiler® Results (5sec)	Identifiler® Results (10sec)	Identifiler® Results (20sec)	MiniFiler™ Results (5sec)	MiniFiler™ Results (10sec)	MiniFiler™ Results (20sec)
Bone (Organic)	Complete	59% full profile, RFUs 300-1500, minor peak imbalance (D13, D2); 41% partial profile*†‡	87.5% full profile, RFUs 500-3000, minor peak imbalance (D13, D2, CSF); 12.5% partial profile*†‡	Full profile when performed (4 times)	Full profile, RFUs 1000-2000	Full profile, RFUs 2000-5000	N/A
	Partial	50% partial profile (11/16 loci) ‡, TH01/D13 imbalanced; 50% no profile	50% partial profile (11/16 loci) ‡, TH01/D13 imbalanced; 50% no profile	50% Partial profile (16/16 loci) ‡; 50% no profile	50% Full profile, RFUs 1000-3000, D13 imbalance; 50% partial profile (up to 1/9 loci)	50% full profile, RFUs 2000-4000, D13 imbalance; 50% partial profile (3/9 loci)	Partial profile when performed (up to 3/9 loci)
	No result	No profile	15% partial profile (up to 7/16 loci); 85% no profile	8% partial profile (up to 6/16 loci); 92% no profile	No profile	No profile	66% partial profile (up to 2/9 loci); 34% no profile
Bone (DNA IQ)	No results	No profile	No profile	No profile	Partial profile (1 or 2 alleles present)	Partial profile (up to 2/9 loci) ‡	Partial profile (up to 7/9 loci) ‡, several artifacts
Hair		25% partial profile; 75% no profile	25% partial profile; 75% no profile	50% partial profile; 50% no profile	25% full profile; 75% partial profile (up to 3/9 loci)	25% full profile; 75% partial profile (up to 7/9 loci)	Partial profile (1/9 to 9/9 loci‡)
Spinal Cord (Chelex)	Degraded or inhibited	No profile	No profile	No profile	50% partial profile (1/9 alleles present); 50% no profile	Partial profile (2/9 loci)	Partial profile when performed (3/9 loci)
	Low partial profiles	67% partial profile (up to 16/16 loci) ‡; 33% no profile	67% partial profile (up to 16/16 loci) ‡; 33% no profile	33% full profile; 67% no profile	33% full profile; 67% no profile	33% full profile; 67% no profile	No profile when performed
Tissue (Chelex)	No results	50% partial profile (up to 13/16 loci); 50% no profile	50% partial profile (up to 16/16 loci); 50% no profile	75% partial profile (up to 16/16 loci); 25% no profile	50% full profile; 25% partial profile (up to 2/9 loci); 25% no profile	50% full profile; 25% partial profile (up to 6/9 loci); 25% no profile	Partial profile when performed (up to 6/9 loci)
	Amelogenin imbalance	Full profile, RFUs <5000 then drop off, amelogenin imbalance	Full profile, RFUs <7000 then drop off, amelogenin imbalance	N/A	Full profile, RFUs <5000, minor amelogenin imbalance	Full profile, RFUs >7000, no amelogenin imbalance	N/A
	Inhibition	86% partial profile (up to 15/16 loci) †; 14% no profile	86% partial profile (up to 16/16 loci) †; 14% no profile	86% partial profile (up to 15/16 loci) †; 14% no profile	43% full profile‡; 43% partial profile (up to 3/9 loci); 14% no profile	43% full profile, RFUs 600-3000; 43% partial profile (up to 5/9 loci); 14% no profile	Partial profile when performed (5/9 loci)
	Severe D5/FGA imbalance	Full profile, RFUs 1000-3000; no D5 imbalance	Full profile, RFUs 2000-4000; no D5 imbalance; some FGA imbalance	Full profile when performed (RFUs ~4000, no D5 imbalance)	Full profile, RFUs >4000, some FGA imbalance	Full profile, RFUs >6000, some FGA imbalance	N/A
	Penta D Split Peaks	Full profile, RFUs 1500-3000, some drop off	Full profile, RFUs 3000-6000, some drop off	N/A	D16 dropout, RFUs 3000-6000	D16 dropout, RFUs >6000	N/A
Tissue (Organic)	Inhibition	80% partial profile (up to 10/16 loci) †; 20% no profile	80% partial profile (up to 13/16 loci) †; 20% no profile	80% partial profile (up to 13/16 loci, ILS pull-up) †; 20% no profile	Full profile‡, RFUs 400-800	Full profile‡, RFUs >800	Partial profile when performed (up to 9/9 loci)

** Some samples experienced injection problems or failures* *† Dropout observed* *‡Peaks below threshold*

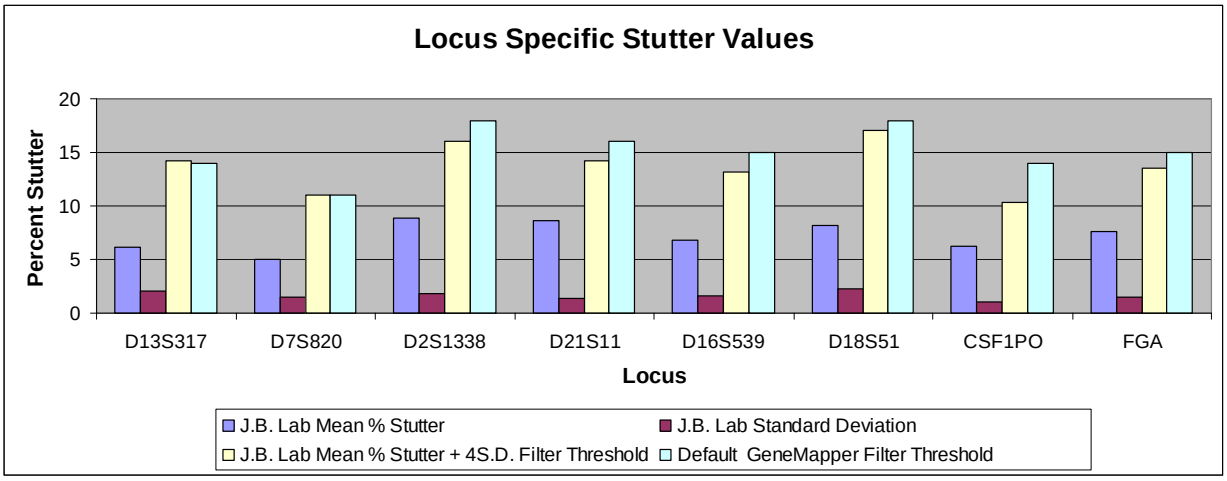
Lab B is in the process of analyzing their non-probative samples using MiniFiler™. Their experimental design intends to use reference samples and epithelial fractions of differential extractions from degraded samples. The goal of their study is to obtain complete DNA profiles from those samples that previously provided complete DNA profiles when analyzed using Identifiler®, thus providing examples of concordance between the two kits.

Lab C performed casework studies on 18 known samples (bloodstains) and 11 evidence samples. The bloodstain samples provided results concordant with previous testing using Identifiler®, and heterozygote peak-height ratios were consistent with those reported in the MiniFiler™ Users Guide (see Lab C Figure 1). Stutter percentages fell below the standard thresholds for GeneMapper® software (see Lab C Figure 2).

Lab C Figure 1. Heterozygote peak-height ratios for known bloodstain reference samples.



Lab C Figure 2. Locus-specific stutter values for known bloodstain reference samples.



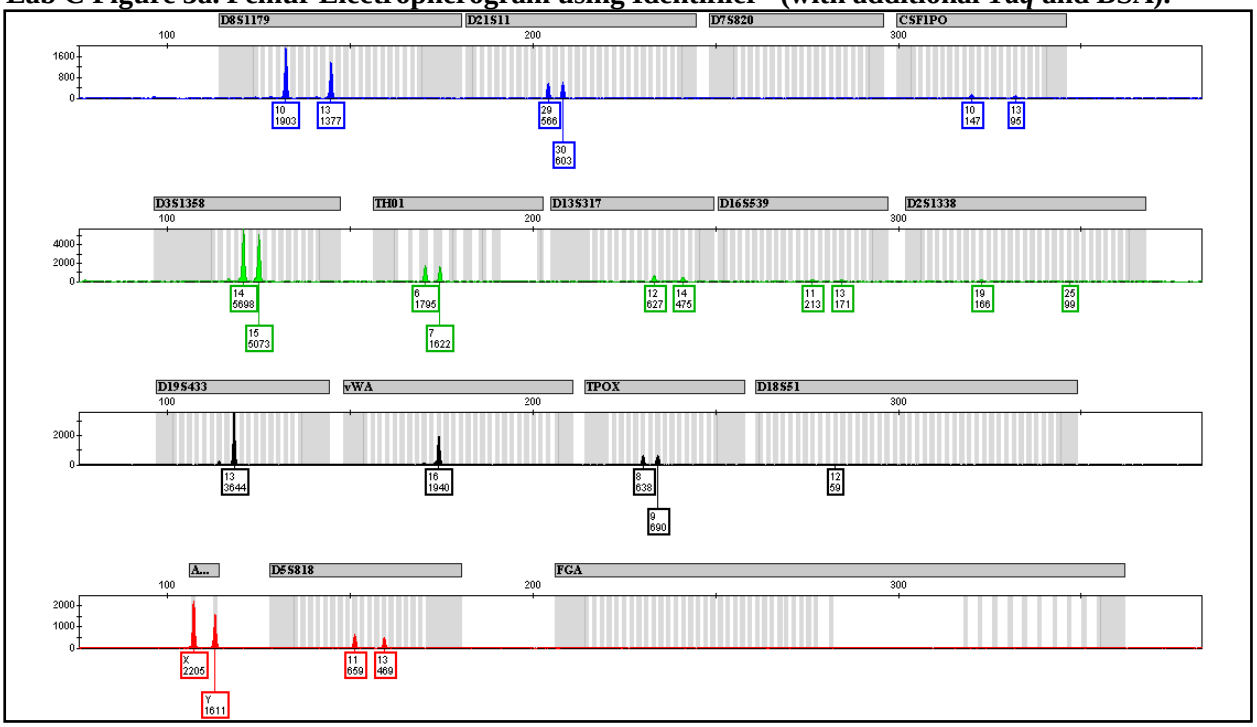
The 11 non-probative samples were chosen because they provided only partial profiles using other validated methods (see Lab C Table 1). Sample types included bone, formalin-fixed paraffin embedded tissue and expectorated sunflower seeds. Not only were the results obtained using MiniFiler™ in agreement with previous allele calls, but the use of MiniFiler™ allowed for better genotype resolution and additional allele calls in some cases. This was most apparent in the tissue samples which were highly degraded and had several PCR inhibitors present. In samples 6 and 10 minor alleles were detected; possible sources of contamination were eliminated as contributors (analysts, other samples, and controls) so these two samples may actually be mixtures. A similar situation is present for samples 3, 4, and 5, which all came from the same individual. These samples appear to be mixtures, and with the same exclusion of possible sources for minor alleles, findings suggest that additional DNA was introduced during sample collection or processing prior to the samples arriving at the DNA laboratory.

Lab C Table 1. Non-probative sample descriptions.

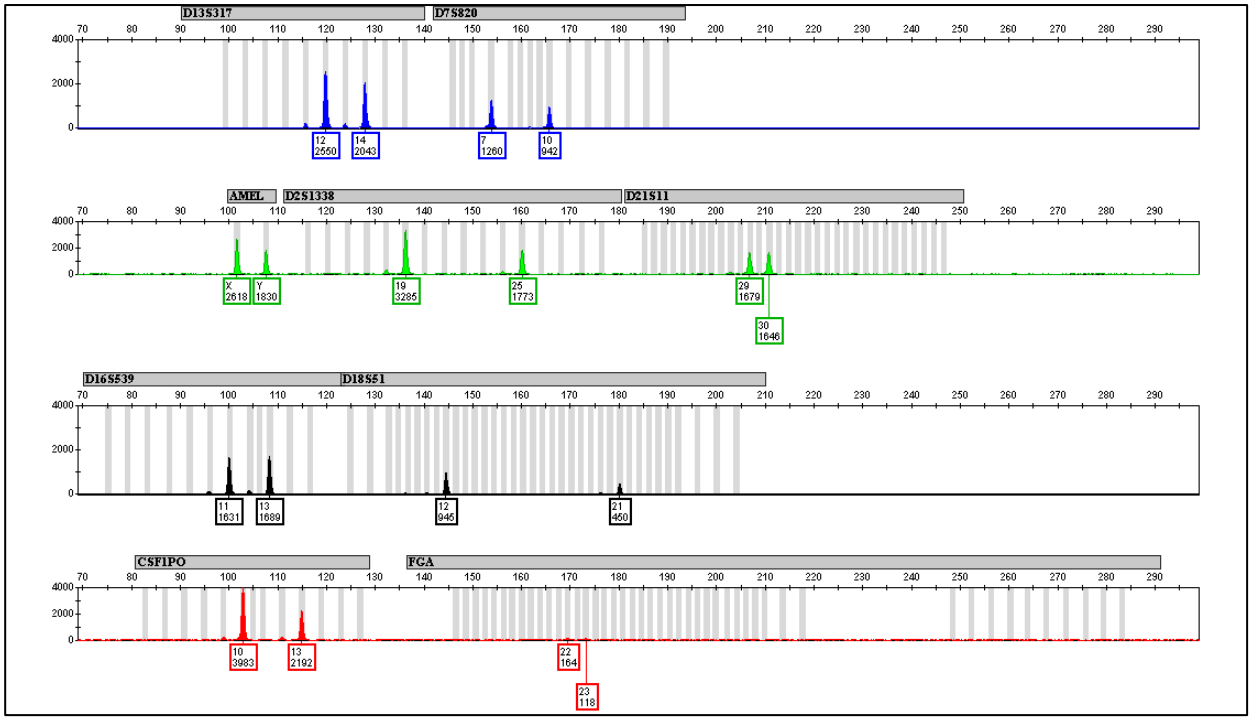
MiniFiler™ Sample #	Sample Type	Sample Type	Dilution	~µL Remaining
1	Extract from Sunflower Seed	Training Sample	Neat	>20
2	Femur	Training Sample	Neat	20
3	Paraffin Embedded Tissue	Case Sample	Neat	14
4	Paraffin Embedded Tissue	Case Sample	Neat	15
5	Paraffin Embedded Tissue	Case Sample	Neat	13
6	Hip Bone	Case Sample	Neat	>20
7	Femur	Case Sample	Neat	15
8	Femur	Case Sample	Neat	14
9	Femur	Case Sample	Neat	14
10	Tibia	Case Sample	Neat	14
11	Femur	Case Sample	1:10	14

The following are electropherograms for various non-probative samples comparing the effectiveness of Identifiler® and MiniFiler™ in generating DNA profiles from “challenging” samples (see Lab C Figures 3a-7). These demonstrate the usefulness of MiniFiler™ in enhancing DNA profiles for cases of highly degraded samples or those with significant amounts of PCR inhibitors that traditionally used kits cannot overcome. Not only are the genotypes enhanced from using the MiniFiler™ kit, but in most cases peak heights imbalances were corrected and the prevalence of artifacts and stochastic effects was decreased. Baseline noise was markedly depressed using MiniFiler™, indicating improved PCR amplification and increased specificity of the miniSTR primers. The increased PCR cycle numbers also contribute to the success of MiniFiler™, allowing for more complete amplification of the longer amplicons targeted in the kit. In all, the applications of MiniFiler™ are vast as seen with the variety of sample types and variation of inhibition and/or degradation demonstrated here.

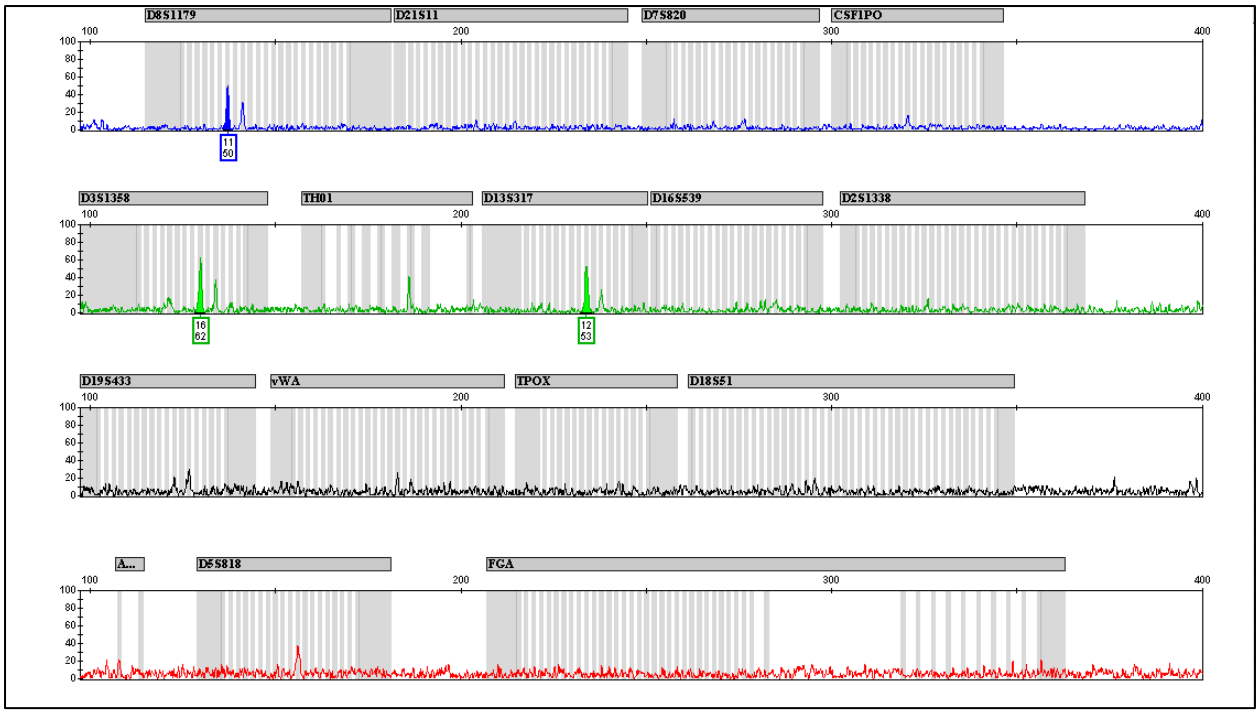
Lab C Figure 3a. Femur Electropherogram using Identifiler® (with additional *Taq* and BSA).



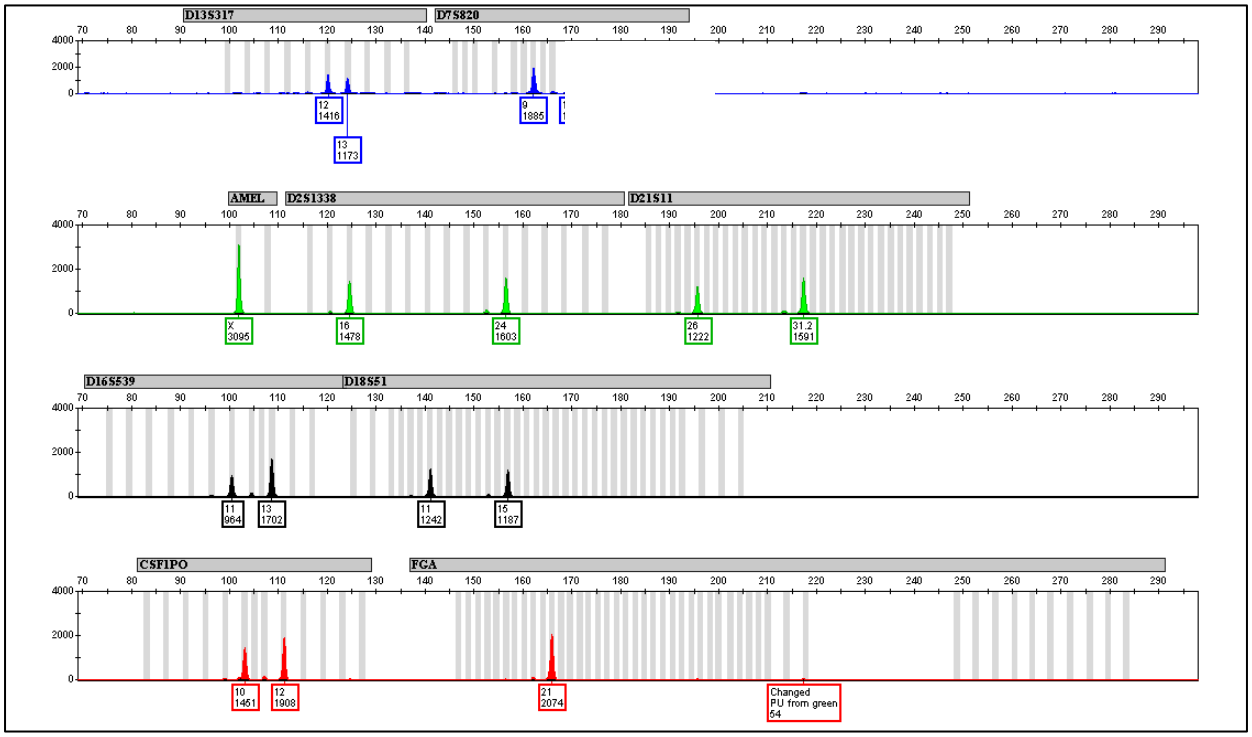
Lab C Figure 3b. Femur electropherogram using MiniFiler™.



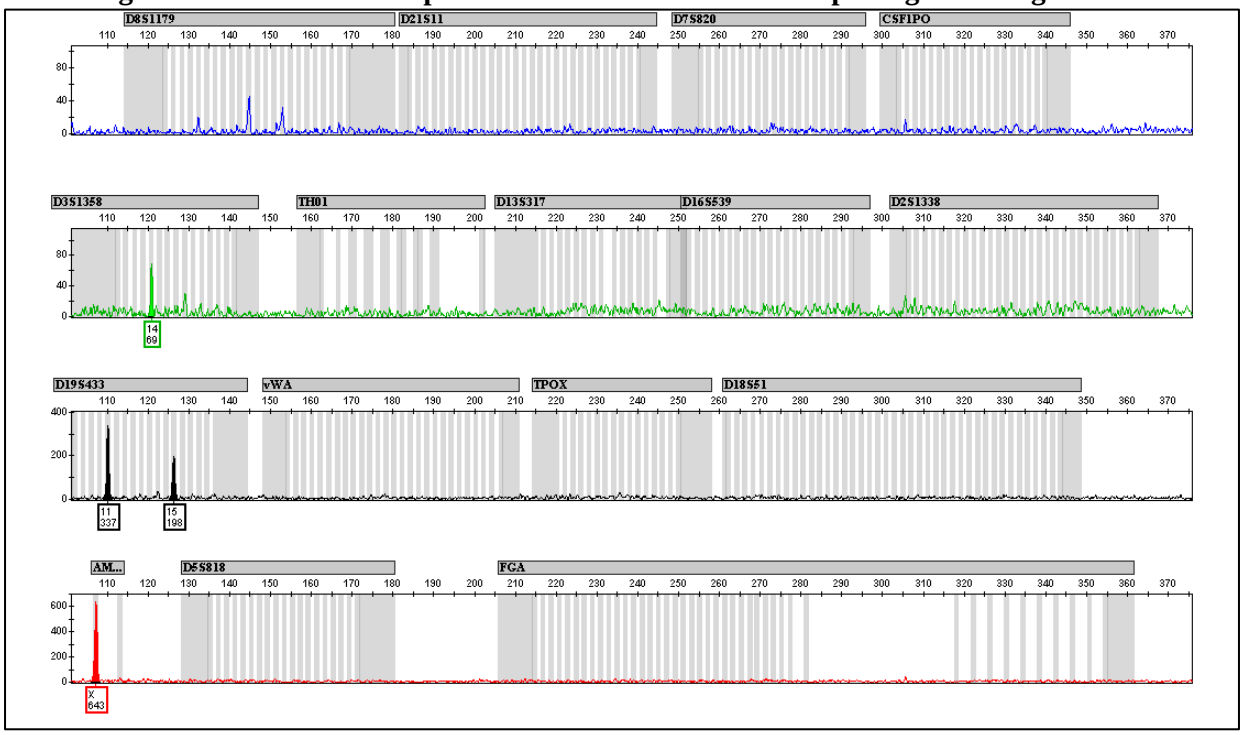
Lab C Figure 4a. Expectorated sunflower seed electropherogram using Identifiler® and various amounts of input DNA template (0.25ng DNA shown).



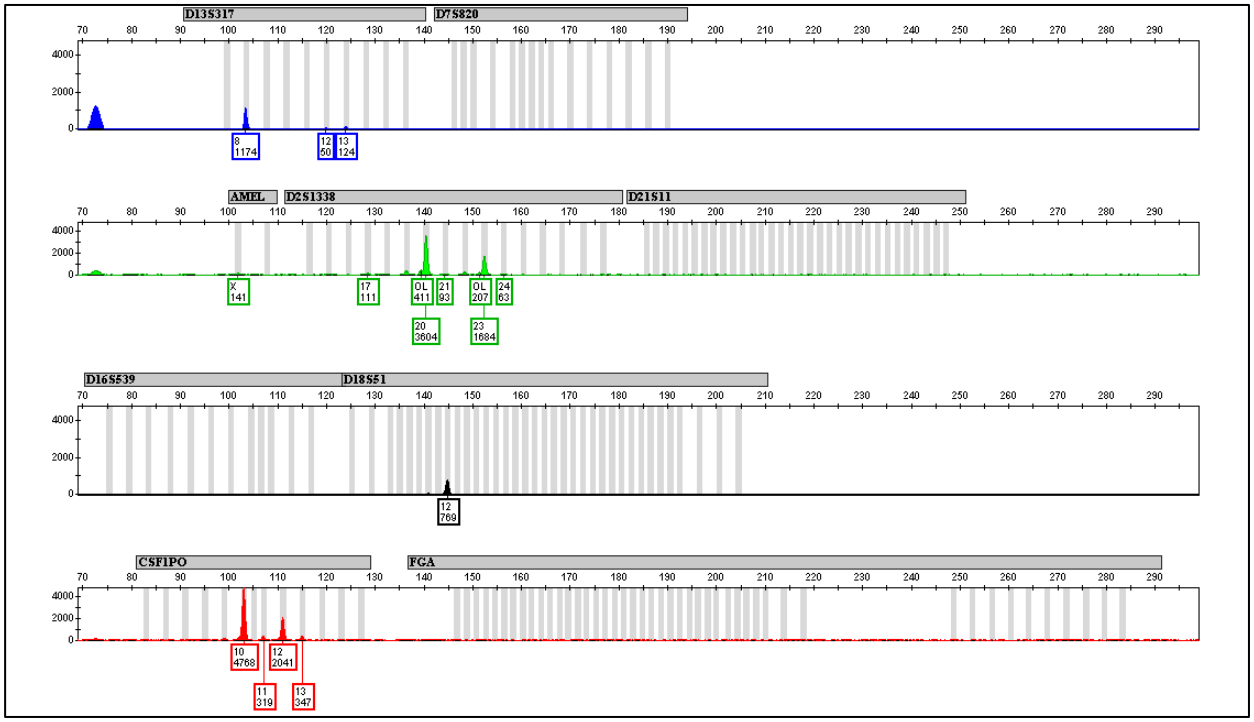
Lab C Figure 4b. Expectorated sunflower seed electropherogram using MiniFiler™.



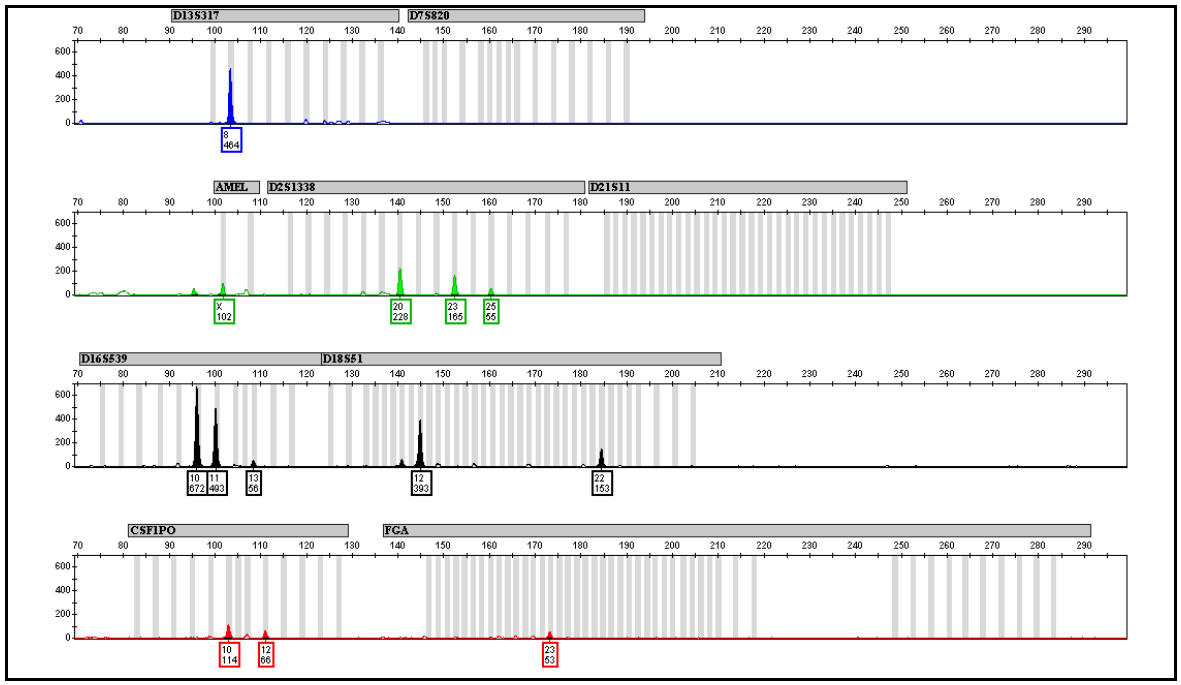
Lab C Figure 5a. Formalin-fixed paraffin embedded tissue electropherogram using Identifiler®.



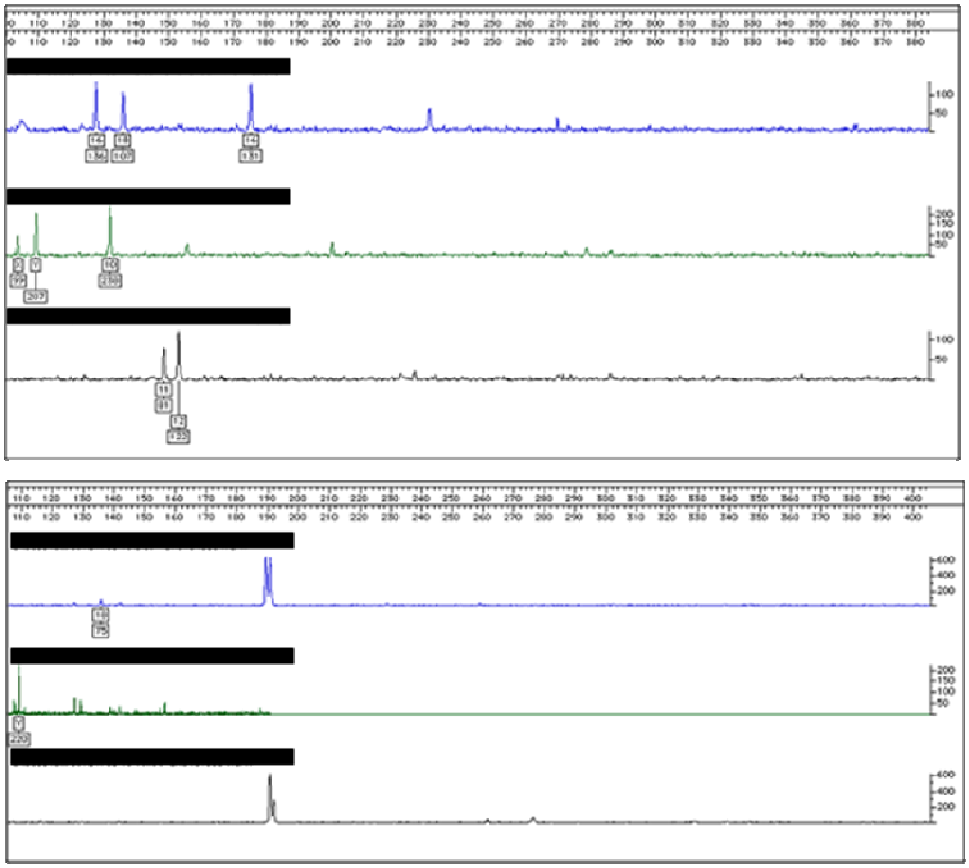
Lab C Figure 5b. Formalin-fixed paraffin embedded tissue electropherogram using MiniFiler™.



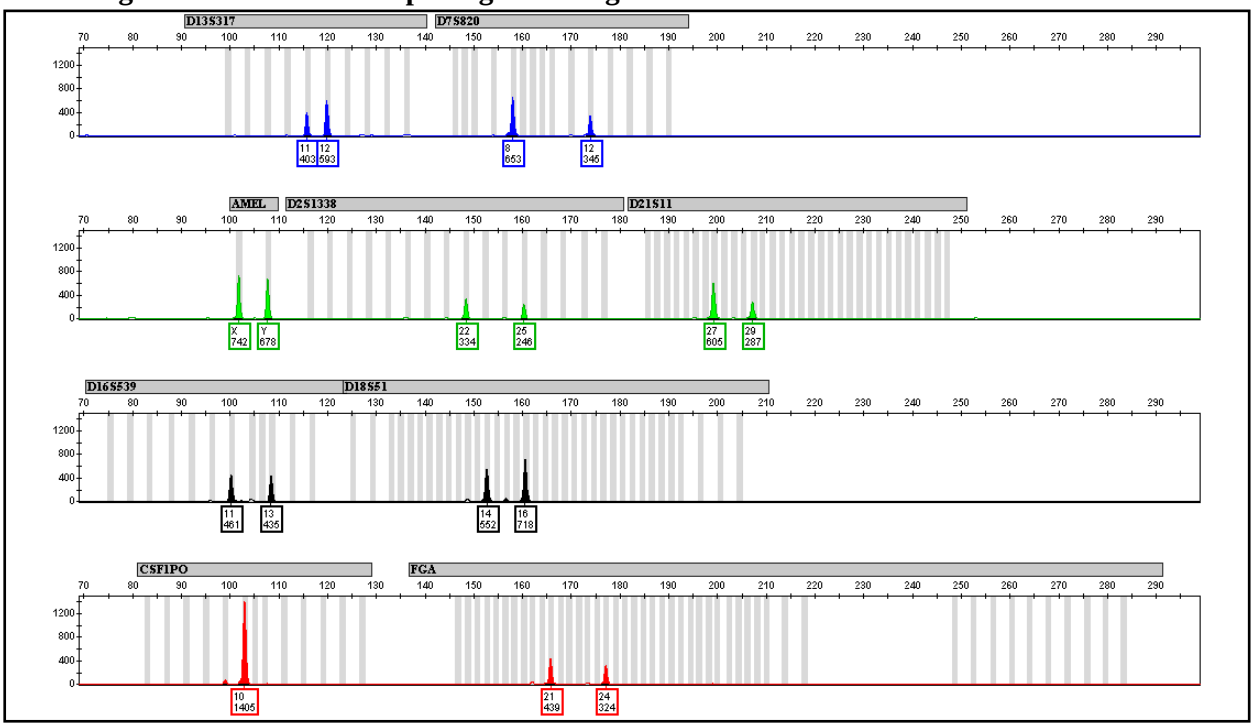
Lab C Figure 6. Electropherogram from amplification of 0.8µL of a 1:5 dilution using MiniFiler™.



Lab C Figure 7a. Femur electropherogram using Profiler Plus®/COfiler®.



Lab C Figure 7b. Femur electropherogram using MiniFiler™.



Lab D performed several concordance studies on unidentified human remains (UHR) using Profiler Plus® ID, COfiler®, and MiniFiler™, to establish the new kit as reliable and capable of enhancing incomplete profiles previously attained. Fortyeight samples from skeletal remains were chosen to exhibit a range of degradation based on the profile results obtained from using Profiler Plus® ID and COfiler® (varying from no profile to 12 STR loci detected). For the study, amplification parameters were altered to allow for 30 PCR cycles. In addition, capillary electrophoresis was performed with 1µL of amplified product injected for 10 seconds at 3kV. In some cases additional 20-second injections and 2µL reload with 20-second injections were used to improve the signal strength of alleles at heterozygous loci to surpass the detection thresholds and peak-height ratio limits (call threshold 100RFU, reporting threshold 400RFU for homozygotic loci and minimum peak-height ratio of 35% for heterozygotic loci).

The study performed by Lab D resulted in concordant results between all loci that overlapped between the kits used. In one sample, MiniFiler™ detected only one allele of two at D16S539 (this locus was determined by COfiler® to be heterozygous). Futher investigation showed that the second allele fell below the call threshold, which in turn pulled the peak-height ratio below the minimum 35%. One other sample presented problems with interpretation at the D2S1338 locus; initial analysis showed one allele well above the call thresholds and one allele below, but another sample taken from the same UHR did not detect the lesser allele. Because D2S1338 is not a locus included in Profiler Plus® ID and COfiler®, it is not clear if the discordance between the two samples is from a primer binding mutation at the locus or if it is a solitary incident of allelic dropout.

MiniFiler™ was able to enhance the profiles of twenty three samples, including some which experienced probable PCR inhibition and/or degradation. Samples were grouped according to their

estimated DNA concentration according to Quantifiler® (see Lab D Table 1) (it is noted that Quantifiler® often overestimates the actual amount of DNA template in instances of low copy and degradation). Where Profiler Plus® ID and COfiler® often resulted in only smaller amplicons being successfully detected, MiniFiler™ was able to recover missing loci and increase the signal strength of true alleles not detected based on set thresholds. However, when DNA template concentration fell below 40pg, neither Profiler Plus® ID/COfiler® nor MiniFiler™ was very successful in generating profiles.

Lab D Table 1. UHR Concordance Study: Data Recovery per Quantifiler® Concentrations.

		PROCO Total		MiniFiler™ Total		Net MiniFiler™		CODIS 13 + D2 Total	
Concentration (ng/µl)	**Number of Samples**	**STR Loci Recovered**	**Percent Recovery**	**STR Loci Recovered**	**Percent Recovery**	**Additional Loci Recovered**	**Percent Add'l Loci Recovered**	**STR Loci Recovered**	**Percent Recovery**
0	10	4	3.1%	0	0.0%	0	0.0%	4	2.9%
0.001-0.005	14	43	23.6%	4	3.6%	3	1.5%	46	23.5%
0.005-0.01	6	29	37.2%	22	45.8%	15	17.9%	44	52.4%
0.01-0.1	11	51	35.7%	41	46.6%	30	19.5%	81	52.6%
0.1-1.15	7	56	59.3%	51	91.1%	32	32.7%	88	89.8%
Totals	**48**	**183**	**29.3%**	**118**	**30.7%**	**80**	**11.9%**	**263**	**39.1%**

Lab E also conducted several concordance studies on seven challenging samples and mock casework samples. Based on the results from DNA quantification using Quantifiler®, five samples were then processed using Identifiler® and MiniFiler™ to test for concordance and the ability of MiniFiler™ to enhance partial profiles obtained or overcome inhibition. Two of the samples were concluded to be mixtures from at least two contributors, a case in which the laboratory would need additional reference samples to establish attributable sources for the samples (see Lab E Table 1). In all samples MiniFiler™ was able to provide missing loci to incomplete profiles generated with Identifiler® (see Lab E Table 2).

Lab E Table 1. Comparison of Bone Sample Profiles vs. Reference Sample Profiles from Relatives.

Locus	Combined Data from Sample 2-1	Reference Sample (KR)	Combined Data from Sample 2-2	Reference Sample (MB)	Combined Data from Sample 3-1	Reference Sample (DK)
D8S1179	13	13,14	13	14,15	13,15	13,15
D21S11	29,30,31	29,30	29,30,31,32.2	30	31.2,35.2	29,31.2
D7S820	11,12	10,11	11,12	11,12	9,10	9,10
CSF1PO	11,12	9,11	11,12	10,12	10	10,11
D3S13358	15,18	15,17	15,16	15,16	15,18	18
TH01	6	6,8	6	6,9.3	6,8	6,9.3
D13S317	9,11,12,13	11,13	9,11,12,13	12	8,12	11,12
D16S539	11,12,13,14	11,12	11,12,13,14	11,13	12,13	12
D2S1338	17,20,24	23,24	17,20,24	17,20	21,23	21,23
D19S433	12,13,14	14,15.2	12,13,14	12,12.2	11,13	13
vWA	14	14	18	16,17	18	16,18
TPOX		8		8,11	8	8,11
D18S51	13,15	15,16	13,15	13,16	14,17	13,14
Amel	X,Y	X	X,Y	X	X,Y	X,Y
D5S818	9	9,11	9,13	10,12	11,12	11
FGA	19,23,25	19,23	19,23,25	25,26	19,24	20,24

Lab E Table 2. Concordance Between Identifiler® and MiniFiler™ kits on Non-Probative Samples.

Kit	Sample	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	Amel	D5S818	FGA
MiniFiler™	AC		28	8	11, 12			8, 12	9, 11	17, 19				9, 19	X, Y		20, 23
Identifiler®	AC	12, 13	28, 29.1	8	11, 12	15	6, 8	8, 12	9, 11	17, 19	14	15, 16	8, 11	9, 19	X, Y	13, 14	20, 23
MiniFiler™	P		28, 30, 32.2	8	12			8, 11	9, 10	17, 19				14, 20	X, Y		21, 22
Identifiler®	P	11, 12, 13	30, 32.2	8, 10, 12	12	15	6, 9	11	9, 10	17, 19	12, 13	15, 18	8, 11	14, 20	X	11, 12	21, 22
MiniFiler™	84b		28, 30	10, 12	10, 12			11, 12	12	17, 24				12, 17	X		23, 24
Identifiler®	84b	12, 15		11, 12		15, 19					12, 14	16, 19			X	11, 13	23
MiniFiler™	27		28, 30		10, 12			9, 12	11, 14	16, 24				12, 17	X, Y		21, 23
Identifiler®	27	13, 15		8, 10		15, 17	8, 9	9, 12			14, 15.2	19	8		X, Y	9, 11	
MiniFiler™	(2-1)		29, 30, 31		11, 12			9, 11, 12, 13	11, 12, 13, 14	17, 20, 24				13, 15	X, Y		19, 23, 25
Identifiler®	(2-1)	13		11, 12		15, 18	6				12, 13, 14	14			X, Y	9	
MiniFiler™	(2-2)		29, 30, 31, 32.2		11, 12			9, 11, 12, 13	11, 12, 13, 14	17. 20, 24				13, 15	X, Y		19, 23, 25
Identifiler®	(2-2)	13		11, 12		15, 16	6				12, 13, 14	18			X, Y	9, 13	
MiniFiler™	(3-1)		31.2, 35.2		10			8, 12	12, 13	21, 23				14, 17	X, Y		19, 24
Identifiler®	(3-1)	13, 15	31.2	10		15, 18	6, 8	8	12		11, 13	18	8		X, Y	11, 12	19, 24

Electropherogram analysis from the five samples showed improved signal strength for longer amplicons using MiniFiler™ (such as FGA , CSF1PO and D18S51 alleles) and slightly improved peak-height ratios at some heterozygous loci . The reference samples all had profiles that were in concordance between Identifiler® and MiniFiler™, serving as a positive internal control that MiniFiler™ can produce accurate results comparable to those obtained with a currently validated and widely used kit. One obstacle in data interpretation continued to be the range of peak-height ratios observed for heterozygous loci.

Overall it is seen that MiniFiler™ does perform well in enhancing incomplete profiles obtained from samples that are compromised either by degradation or PCR inhibition. MiniFiler™ is also useful in instances of low copy number DNA, in which preservation of the sample itself remains of utmost importance for the possibility of future testing. However, the use of MiniFiler™ must come with a caveat that analysts using the kit must be well-versed in its sensitivity to the amount of input DNA used, as well as possible obstacles in data interpretation. These include the observances of -4 and +4 stutter, amplified artifacts and variable amplification imbalances. Peak-height ratios at heterozygous loci had a wide range both within each laboratory and between the five labs, indicating that data interpretation may be difficult at times during routine casework. Call thresholds may have to be altered to account for low signal strength from loci with extreme peak-height ratio imbalances, possible primer binding mutations or that undergo unpredictable amplification events. Together, these findings indicate that MiniFiler™ is best used on single-source samples to enhance incomplete profiles due to degradation, low copy number or inhibition.

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