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TECHNICAL NOTE

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Validation of the AmpFℓSTR® MiniFiler™ PCR Amplification Kit for Use in Forensic Casework*

ABSTRACT: The AmpFℓSTR® MiniFiler™ PCR Amplification Kit is designed to genotype degraded and/or inhibited DNA samples when the AmpFℓSTR® Identifiler™ PCR Amplification Kit is incapable of generating a complete genetic profile. Validation experiments, following the SWGDAM guidelines, were designed to evaluate the performance of MiniFiler. Data obtained demonstrated that MiniFiler, when used in conjunction with Identifiler, provided an increased ability to obtain genetic profiles from challenged samples. The optimum template range was found to be between 0.2 and 0.6 ng, with 0.3 ng yielding the best results. Full concordance was achieved between the MiniFiler kit and Identifiler kit except in a single case of a null allele at locus D21S11. Numerous instances of severe heterozygous peak imbalance (<50%) were observed in single source samples amplified within the optimum range of input DNA suggesting that caution be taken when attempting to deduce component genotypes in a mixture.

KEYWORDS: forensic science, DNA typing, miniSTR, D13S317, D7S820, D2S1338, D21S11, D16S539, D18S51, CSF1PO, FGA, polymerase chain reaction, validation, AmpFℓSTR

The AmpFℓSTR® MiniFiler™ PCR Amplification Kit (Applied Biosystems, Foster City, CA) is designed to function as an adjunct DNA typing kit to current commercial short tandem repeat (STR) typing kits, such as the AmpFℓSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems), in order to obtain a more complete genetic profile of an individual. MiniFiler is the first commercially available 9-plex miniSTR amplification kit for use on forensic casework when other DNA typing kits have proven to be unsuitable for the genotyping of highly degraded and inhibited DNA samples (1). The MiniFiler kit contains primers that amplify eight of the Identifiler kit's largest loci: D13S317, D7S820, D2S1338, D21S11, D16S539, D18S51, CSF1PO, FGA, and the sex-typing locus amelogenin (2).

STR typing systems are useful tools for individualizing biological specimen in forensic casework. The advantage that MiniFiler has over other commercial STR typing kits is the shorter amplicons (miniSTRs) that are produced during the polymerase chain reaction (PCR). MiniSTRs are the result of relocating the PCR primers as close as possible to the STR repeat region, reducing the flanking region and thus the overall size of the STR marker, consequently resulting in an increased success rate for obtaining a genetic profile from degraded DNA samples (3–7). Higher molecular weight STR markers fail to amplify when a sample is highly degraded, resulting in an incomplete Identifiler profile. MiniFiler generates amplicons that span a range between 70 and 283 nucleotides, whereas current commercially available multiplex STR kits produce amplicons in the range of 100–450 nucleotides (2,8). MiniSTRs were designed to complement the current commercial STR megaplexes in order to maintain database compatibility with CODIS (8,9). In addition to shorter amplicons, robust profiles are obtained from challenged samples due to the optimization of the amplification reaction, which aids in overcoming PCR inhibition, and the increased number of PCR cycles (30 cycles as compared to 28 cycles for the Identifiler kit), which increases the sensitivity and reduces the optimum DNA template requirement (1).

When DNA has undergone extensive template fragmentation or the sample contains PCR inhibitors, amplification generally results in sub-optimal results at the larger STR loci. The use of miniSTRs provides a greater likelihood of successfully typing a degraded or inhibited sample (9,10). Extensive DNA template fragmentation can be a result of several environmental factors including humidity, elevated temperatures, microorganisms, soil pH, and ultraviolet radiation (3,4,7). Thus, when cases arise where the DNA is either degraded or inhibited, miniSTRs have proven useful in achieving a more complete genetic profile. Such events include the identification of victims of the World Trade Center attacks, mass fatality incidents, missing persons cases, and cases that have remained unsolved for many years (3,11,12).

The goal of this validation study was to develop a protocol and interpretation guidelines before implementing the AmpFℓSTR® MiniFiler™ PCR Amplification Kit as a new technology to be used in forensic DNA casework analysis. Nine studies were performed to examine the success of the kit in recovering complete DNA profiles from challenged samples that are often encountered in forensic casework, and to verify that the kit would perform reliably and yield robust results. The validation work, carried out according to the Scientific Working Group on DNA Analysis Methods (SWGDAM) guidelines, provides the basis for the experiments conducted: (i) sensitivity, (ii) stutter percentage determination, (iii) stochastic effects, (iv) heterozygous peak height ratio, (v) mixture, (vi) reproducibility, (vii) precision, (viii) mock casework and challenged samples, and (ix) concordance (http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm).

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Materials and Methods

Extraction and Quantitation

DNA obtained from buccal swabs from 17 individuals was extracted and purified using the BioRobot EZ1 (Qiagen, Valencia, CA), which employs a silica-based magnetic particle technology (13). Following the San Diego Police Department (SDPD) protocols each reference swab sample was first pre-treated for extraction with 190 μL of digest buffer (10 mM Tris–HCL, 10 mM EDTA, 50 mM NaCl, 2% SDS) and 10 μL of 10 mg/mL proteinase K. Subsequently, each sample was purified and eluted into 200 μL of TE buffer using the Trace protocol on the BioRobot EZ1. These samples were then quantified with the Quantifiler™ Human DNA Quantitation Kit (Applied Biosystems) using the manufacturer's recommended protocol on the ABI Prism 7500 Sequence Detection System (Applied Biosystems).

PCR Amplification

The PCR reactions were prepared according to the manufacturer's recommended protocol using the AmpFℓSTR® MiniFiler™ PCR Amplification Kit unless otherwise stated, using a template DNA amount of 0.3 ng in an Applied Biosystems 96-Well GeneAmp® PCR System 9700 (Applied Biosystems).

Detection of Alleles

Following PCR amplification, both the ABI PRISM® 310 Genetic Analyzer (Applied Biosystems) and the ABI PRISM® 3130 Genetic Analyzer (Applied Biosystems) were employed for electrophoretic separation of amplified products. For ABI 310 sample preparation, 24.5 μL Hi-Di™ Formamide (Applied Biosystems), 0.5 μL GeneScan™ 500 LIZ® Size Standard (Applied Biosystems), and 1.5 μL of PCR product or AmpFℓSTR® MiniFiler™ Allelic Ladder (Applied Biosystems) were added to each sample tube. For ABI 3130 sample preparation each well on the sample plate contained: 8.7 μL Hi-Di™ Formamide, 0.3 μL GeneScan™ 500 LIZ® Size Standard, and 1.0 μL of PCR product or AmpFℓSTR® MiniFiler™ Allelic Ladder. The reaction plate and tubes were heated at 95°C for a 3-min denaturation step, immediately snap-cooled on a freezer block for 3 min, and then subjected to capillary electrophoresis. PCR product separation was performed according to the manufacturer's recommended protocol.

Data obtained from the runs were collected using either the 310 Genetic Analyzer Data Collection software v3.1.0 (Applied Biosystems), or the 3130 Genetic Analyzer Data Collection software v3.0 (Applied Biosystems). Data were analyzed using a peak detection threshold of 75 relative fluorescence units (RFU) for all dyes, with GeneMapper® ID v3.2.1 (Applied Biosystems) using the panels and bins provided by Applied Biosystems.

Sensitivity

The sensitivity study was carried out to determine the recommended template DNA range, to be used with the MiniFiler kit. Two separate amplifications were performed in order to achieve the optimum target DNA range that would produce complete, reliable, and artifact-free DNA profiles. The protocol for PCR amplification using the MiniFiler kit was followed as stated in *PCR Amplification*, with the exception of a range of template amounts distributed between sample tubes. For the first round of amplification, the MiniFiler positive control DNA (007) was amplified using template amounts of 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 1.5, and 2.0 ng, and a reference sample was amplified at template amounts of 0.0156, 0.0312, 0.0625, 0.125, 0.25, 0.5, 1.0, 1.5, and 2.0 ng. To narrow the target template DNA range a second round of amplification was performed in which the MiniFiler positive control DNA (007) and a reference sample were amplified at template amounts of 0.2, 0.3, 0.4, 0.5, and 0.6 ng. In addition, 17 reference samples were amplified at 0.3 ng, the amount determined to be the optimum target. Capillary electrophoresis was performed on all samples using both the ABI 310 and 3130.

Stutter Percentage Determination

Due to slippage of DNA polymerase during amplification, stutter peaks tend to be one repeat unit less than the true allele (14). Identifying the effects of stutter on a new DNA typing kit is imperative in order to help distinguish between a minor contributor and a stutter product of an adjacent allele (15). Stutter percentages for the MiniFiler kit were calculated using data obtained from the sensitivity study from both the 310 and 3130 Genetic Analyzers, for stutter peaks which occurred at 4 basepairs less than the true peak. This study demonstrated the effectiveness of the manufacturer's stutter filter values in efficiently filtering most stutter peaks. Stutter was assessed for the samples at optimum template amounts of 0.2–0.6 ng. The percentage stutter was calculated by dividing the height of the stutter peak by the height of the adjacent parent allele peak. Stutter data for peaks with a paired allele one repeat larger were not used in this study because their peak heights are artificially higher due to the stutter contribution from the larger allele.

Stochastic Effects

When amplification is performed with few template molecules, peak height imbalance can occur and may result in difficulties in determining the correct genotype due in part to allelic dropout or incorrect assessment of allele homozygosity (16). When trace amounts of DNA are amplified, this can often result in allelic dropout and the potential of falsely interpreting homozygosity. The homozygous peak threshold is defined as the RFU level of peaks below which an analyst can expect that one of a pair of heterozygous peaks may drop below the detection threshold. To identify the RFU level below which allelic dropout begins to become of concern using the MiniFiler kit (and thus the homozygote peak threshold), a total of 11 samples were amplified using a DNA template range of 15–36 pg that would produce peak heights that straddled the detection limit. The homozygote peak threshold, used as a guideline for determining the confidence of a homozygote genotype determination, was determined by assessing the RFU level where allelic dropout was observed to occur from samples run on both the ABI 310 and 3130.

Heterozygous Peak Height Ratio

Peak height ratios (PHRs) are often used in interpreting DNA profiles to identify possible mixtures and elucidate possible contributing genotypes of a mixture. This study was aimed at determining the expected peak height ratios between heterozygous pairs when optimal amounts of template DNA were used. The peak height data used was obtained from the samples from the sensitivity study, and contained peak heights that were produced from amplifying 17 reference samples at a template DNA range of 0.2–0.6 ng. In addition, peak height ratios were compared between the ABI 310 and 3130 instruments. The peak height of the smaller peak in the heterozygous pair was divided by the peak height of the larger peak.

Mixture

In forensic DNA casework, mixed samples consisting of DNA from two or more individuals are routinely encountered. It is therefore imperative to be able to properly interpret DNA mixtures when typing forensic casework samples. This study assessed the level at which a mixture could be detected in a sample using various mixture ratios of two components, since the likelihood of detecting a minor allele at a locus is decreased as the mixture ratio increases. The effect on peak balance from having DNA from more than one source was also investigated. Two sets of mixtures were prepared, with each set containing DNA from previously extracted reference samples of two different individuals. Each mixture set was chosen for comparison purposes due to the minimal number of overlapping alleles at each locus between the two individuals. The following sample ratios were prepared for each mixture: 20:1, 15:1, 10:1, 5:1, 3:1, 1:1, 1:3, 1:5, 1:10, 1:15, 1:20. In the 1:1 mixtures, *c.* 0.3 ng DNA from each contributor was targeted by normalizing the peak heights obtained from previous amplifications of the samples. The lesser components of each mixture were obtained through a titration of the original 0.3 ng sample. These mixtures were then amplified with MiniFiler and run on the ABI 3130.

Reproducibility

Multiple DNA analysts independently typed all reference samples used in this study on the 3130 and 310 Genetic Analyzer instrument platforms on different days. In addition, the nonprobative casework and casework-like samples assessed in the mock casework and challenged sample study were also analyzed

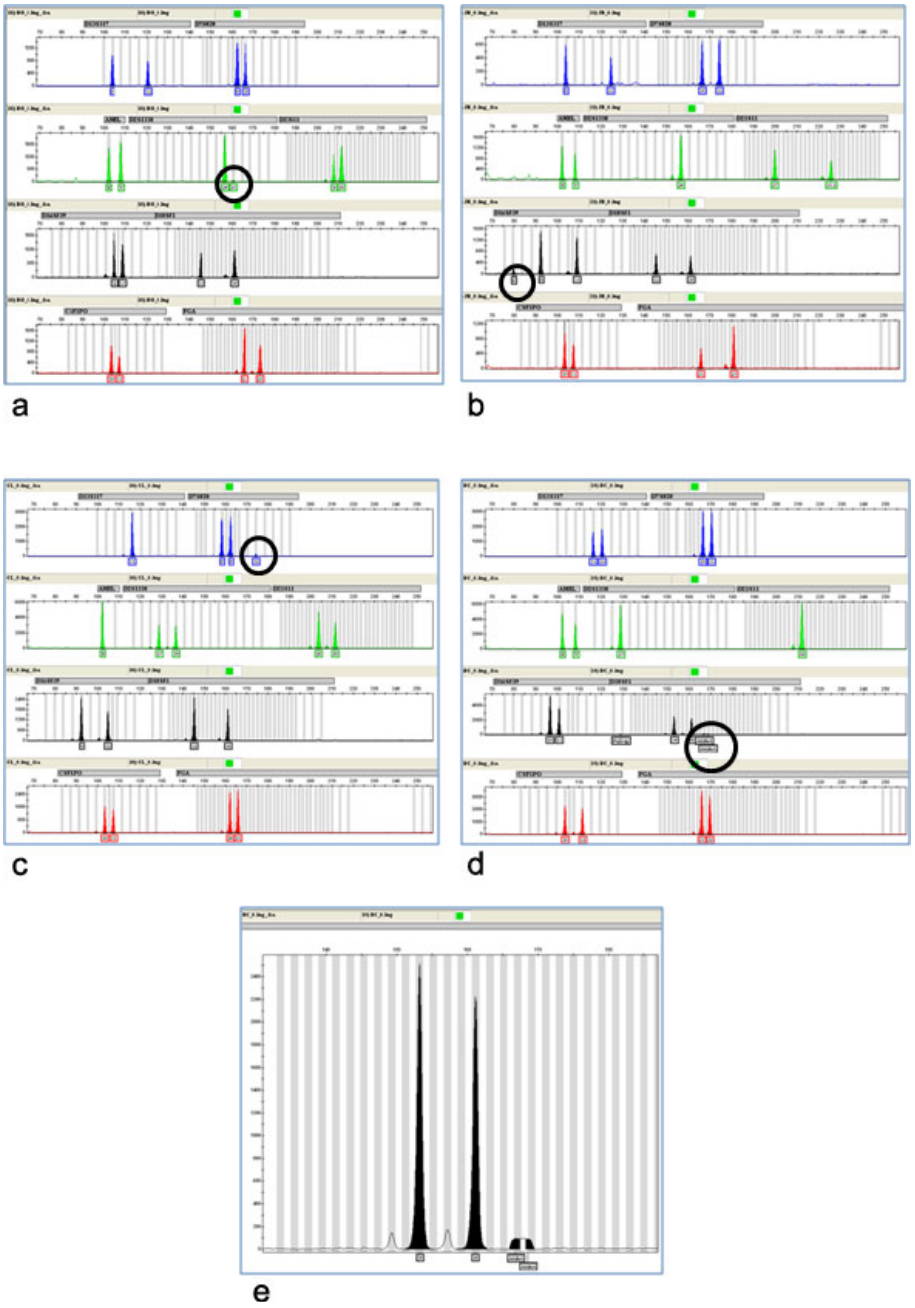

FIG. 1—*Artifacts observed by amplifying samples at the 0.3 ng level. (a) Possible* n + *4 stutter. (b and c) Extraneous peaks that could not be confidently identified as artifacts. (d and e) Baseline artifacts that could not be confused with DNA peaks.*

independently by at least two DNA analysts. The data from each independent analysis were compared to determine that consistent allele calls were obtained from all samples.

Precision

The reliability of the MiniFiler kit and the methodology, when analyzing samples, was demonstrated by evaluating intra-assay precision. The consistency of sizing was assessed by looking at the average base size and standard deviation of all alleles for each sample injected, in which multiple injections of the MiniFiler allelic ladder and two previously amplified reference samples were performed. Using the 310 Genetic Analyzer, three ladders and two reference samples were injected four times each. Six ladders and two reference samples were run on the ABI 3130. The first two ladders were injected once, and the remaining four ladders and two samples were injected three times each.

Mock Casework and Challenged Samples

In accordance with the validation requirements in the SWGDAM guidelines any novel DNA typing kit must be tested using casework-like samples or samples from nonprobative casework before it can be implemented in forensic casework. As such, a comparison was made of the results achieved from the typing of seven previously extracted samples using MiniFiler to previous typing performed using the Identifiler kit. These challenged samples were selected from previously analyzed nonprobative samples and included degraded and inhibited DNA from bone samples recovered from a 1992 helicopter crash in the high desert of Baja California, degraded DNA from a bloodstain from a T-shirt in a 1977 homicide case, and a sample spiked with Pepsi® (Pepsi [or some component of it] has been identified as inhibiting PCR reactions in casework at the SDPD) acting as a PCR inhibitor. All samples in this experiment were amplified and subsequently run on the 3130 Genetic Analyzer.

Concordance

Evaluating the concordance between typing kits that are designed to be complementary, yet contain different PCR primers, is crucial in determining both the reliability of the typing kits and the application of databasing the results from the kits. Nonconcordance between STR typing kits has previously been established (17–19) and is a result of differences or changes in primer binding sites between kits. These instances of nonconcordance where an allele may fail to be amplified, and therefore not detected, generally result from primer binding site mutations on the template region of interest rendering the primer unable to bind (3). Nonconcordance can also be the result of the primer not binding as well, resulting in a lower peak height due to less amplicon production. The potential allelic dropout due to a mutation, such as an insertion or deletion in the flanking region of the miniSTR, was narrowly assessed by comparing the DNA profiles obtained from amplification of 20 previously extracted reference samples using the MiniFiler kit to the DNA typing results of the same samples using the Identifiler kit.

Results and Discussion

Sensitivity

For the first amplification round of the sensitivity study partial DNA profiles, in which allelic dropout (peaks occurring below the detection threshold) was exhibited at one or more loci, were obtained at a DNA range of 0.0156–0.0312 ng (data not shown). The lowest template amounts at which full profiles (no allele dropout) were obtained were 0.05 ng for the MiniFiler positive control DNA (007) and 0.0625 ng for the reference sample. Off-scale peaks and baseline artifacts were observed to occur at template amounts of 1.0 ng and higher. For the second round of amplification full DNA profiles were obtained for each sample amplified using a range of 0.2–0.6 ng with off-scale alleles appearing at 0.5 and 0.6 ng for the MiniFiler control DNA (007) at the amelogenin locus (data not shown). Full DNA profiles were also obtained for each of the 17 reference samples amplified at 0.3 ng. Since complete profiles were achieved with minimal amounts of off-scale data or artifactual peaks it was established that 0.2–0.6 ng of DNA, with an optimum target of 0.3 ng, should be employed for the optimal typing of DNA samples. These levels are in contrast to the template DNA range of 1.5–2.5 ng employed at the SDPD Crime Laboratory when using the AmpFℓSTR® Identifiler™ PCR Amplification Kit, and as such MiniFiler offers increased sensitivity. Possible extraneous DNA types or unexplainable artifacts were observed in four of the 17 samples amplified within the optimum template range (Fig. 1). These extraneous DNA types were possibly artifacts caused by the 30 amplification cycles, low level contamination, or the genetic analyzer fluorescence intensity being exceeded by the linear dynamic range for detection. In two of the samples the extraneous peaks occurred in $n + 4$ positions that could possibly be stutter artifacts or were baseline artifacts that clearly did not resemble DNA peaks. Only two instances of possible DNA peaks in nonstutter positions were observed and their presence could not be confidently attributed to a specific type of known artifact (spike, pull-up, etc.). In both instances these peaks were observed on the instrument with the highest sensitivity, and would not be confused for being part of the predominant DNA profile as they occurred at less than 12% of the predominant DNA types at the locus. Sensitivity differences between the ABI 310 and 3130 instruments used at the SDPD were apparent in this study. In contrast to the ABI 3130, two ABI 310 instruments demonstrated larger peak heights and an increased amount of pull-up, artifactual peaks, and a few other unexplainable peaks at template DNA amounts of 0.4 ng and higher. In our laboratory, the sensitivity of the ABI 310 Genetic Analyzers was observed to be greater than

TABLE 1—*Observed % stutter for MiniFiler loci for samples amplified using 0.2–0.6 ng of DNA.*

Locus	Range (%)	Mean (%)	SD	Upper Range* (%)	MiniFiler Marker-Specific Stutter† (%)	Identifiler Marker-Specific Stutter‡ (%)
D13S317	2.9–7.0	5.4	1.1	8.7	14.0	8.0
D7S820	2.3–8.7	6.0	1.4	10.2	11.0	8.2
D2S1338	5.1–13.5	8.3	2.0	14.3	18.0	11.1
D21S11	6.2–15.3	8.5	1.6	13.3	16.0	9.4
D16S539	3.5–10.2	6.7	1.9	12.4	15.0	10.4
D18S51	4.5–16.7	8.5	2.6	16.3	18.0	17.0
CSF1PO	5.1–9.9	7.2	1.3	11.1	14.0	9.2
FGA	5.3–11.9	8.3	1.6	13.1	15.0	14.7

SD, standard deviation.

*Upper Range % Stutter = Mean % Stutter + 3 (SD).

†MiniFiler marker-specific stutter values obtained from the Applied Biosystems AmpFℓSTR® MiniFiler™ PCR Amplification Kit User Guide (2).

‡Identifiler marker-specific stutter values obtained from the Applied Biosystems AmpFℓSTR® Identifiler™ PCR Amplification Kit User's Manual (20).

that of the ABI 3130 in the detection of fluorescently labeled miniSTRs.

Stutter Percentage Determination

Stutter values were assessed for data obtained from the ABI 310 and 3130 as demonstrated in Table 1. The range of stutter percentages obtained from each locus was found to lie below the MiniFiler marker-specific percentage stutter cut-off provided in the AmpF*ℓ*STR® MiniFiler™ PCR Amplification Kit User Guide. In addition, a comparison was made between the marker-specific stutter percentages of the MiniFiler kit and the marker-specific stutter percentages of the Identifiler kit (2,20). Figure 2 demonstrates the stutter percentages for the different alleles at each locus for the combined ABI 310 and 3130 data. As revealed in Fig. 2, stutter values increased as the number of repeats in each allele increased (2). This study demonstrated that the manufacturer's stutter filter values can be effectively used in forensic casework interpretation to efficiently filter most stutter peaks.

Stochastic Effects

Allelic dropout was observed as a result of stochastic amplification due to a low quantity of template DNA being amplified for a particular allele. Figure 3*a* illustrates the instances of allelic dropout when the samples were run on two different 310 Genetic Analyzers, while Fig. 3*b* demonstrates the instances of allelic dropout on the ABI 3130 Genetic Analyzer. Assessing stochastic effects

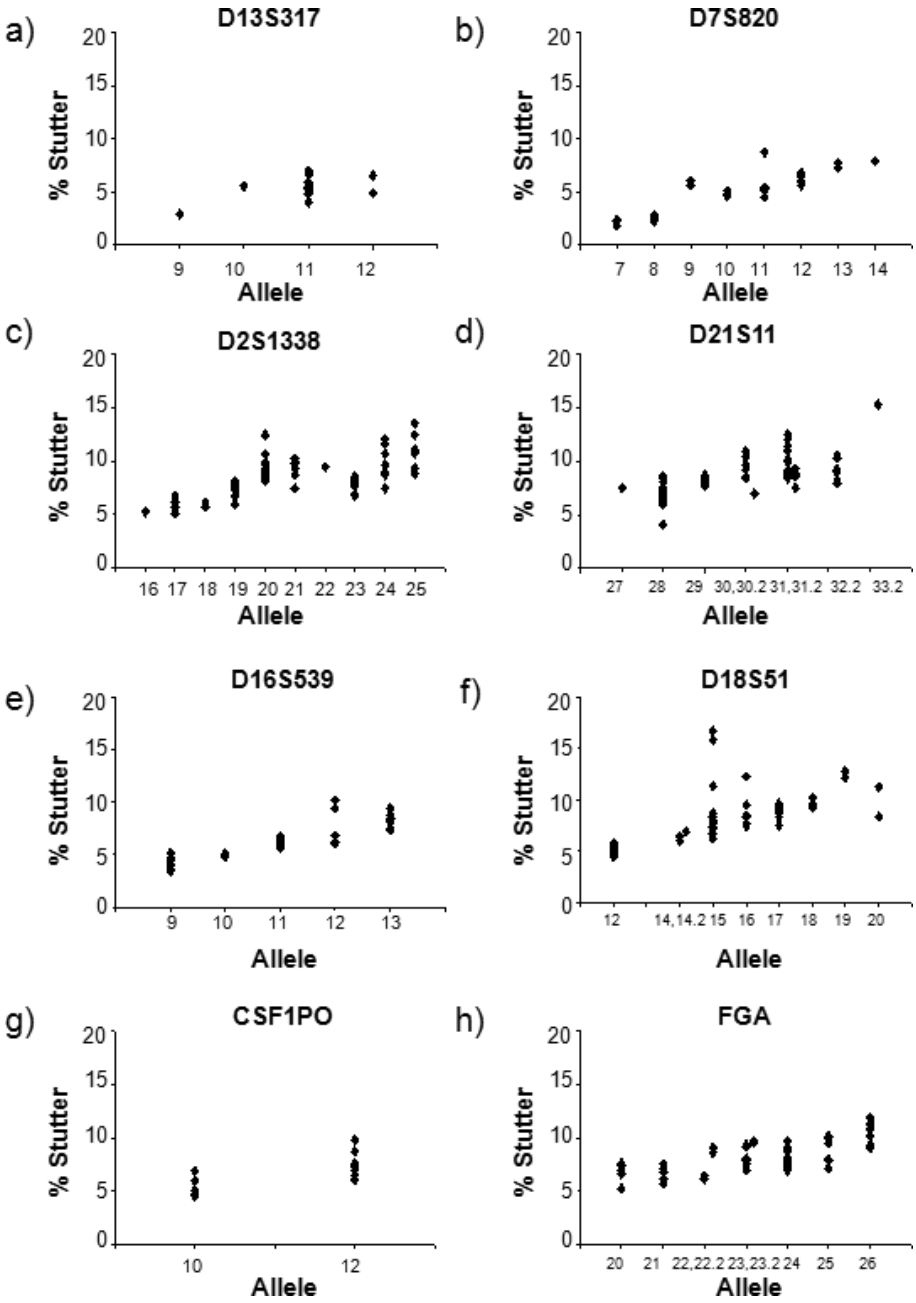

FIG. 2—*Observed stutter percentages at each locus included in the MiniFiler kit for samples amplified using 0.2–0.6 ng of template DNA. Data is combined for the ABI 310 and ABI 3130 instruments. (a) D13S317 = 30 data points, (b) D7S820 = 43 data points, (c) D2S1338 = 81 data points, (d) D21S11 = 75 data points, (e) D16S539 = 33 data points, (f) D18S51 = 70 data points, (g) CSF1PO = 19 data points, (h) FGA = 75 data points.*

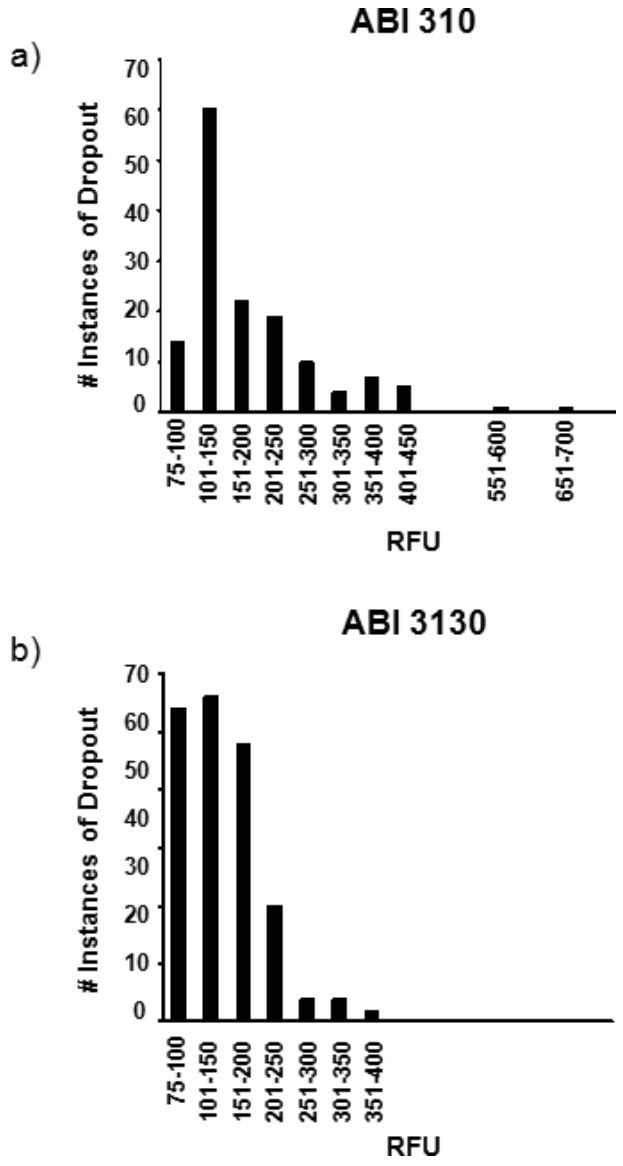

FIG. 3—*Instances of allele dropout observed for samples with low amounts of target DNA (15–36 pg) using the MiniFiler kit and two ABI 310 units (panel a) or one ABI 3130 unit (panel b). The data presented were generated from single amplifications of the low level samples.*

between both instrument models, it was observed that the majority of allelic dropout occurred with peaks below 450 RFU. However, there were two instances out of 421 peak pairs (0.4%), where dropout occurred on one of the ABI 310s with peaks above 450 RFU: a 570 RFU peak at the D21S11 locus and a 680 RFU peak at the D16S539 locus. Both instances had the second peak of the heterozygous pair below the 75 RFU detection threshold, but still clearly visible on the electropherogram. Despite these two instances of dropout, it was determined that a reliable homozygote genotype determination could be made with a high degree of confidence for both the 310 and 3130 Genetic Analyzers utilizing a homozygote threshold of 450 RFU.

Heterozygous Peak Height Ratio

Peak height ratios were found to range from 0.35 to 1.00 when samples were amplified within the optimum DNA template range of 0.2–0.6 ng. A minimal difference between peak height ratios of the ABI 310 and 3130 was observed. The average peak height ratio when the taller of the peaks was between a certain RFU range was calculated. Combining the data for the 310 and 3130 Genetic Analyzers, the peak height ratio between the two heterozygous peaks averaged 77% for 356 heterozygous peak pairs (Table 2). However, peak height balance as low as 36% was observed in peak height pairs between 500 and 4000 RFU (Table 3), with amelogenin being the locus most susceptible to imbalance. Caution should be exercised when using peak height ratios to elucidate if a sample is a mixture, as single source samples may have peak height ratios as low as 36%.

Mixture

The DNA profile of a major contributor was assigned with confidence in the 10:1 (and 1:10) mixtures of DNA (data not shown). As the mixture ratio approached 1:3 the determination of major and minor genotypes became increasingly difficult. At a mixture ratio of 3:1 (and 1:3) a majority of the alleles from both individuals were present, and at a ratio of 1:1 all alleles from both individuals were present. As the mixture ratios increased, allelic dropout from the lesser component increased. As expected, the 20:1 and 1:20 mixture ratios contained the most allelic dropout of the minor components. However, the minor component was still observed at one or more loci for both sets of mixtures. This study concluded that MiniFiler is suitable for assessing casework samples that contain DNA from two or more individuals.

Reproducibility

The results from the multiple analyses of the reference samples and the casework-like and nonprobative casework samples showed full allele concordance when independently run on the 3130 or 310 instrument platforms (data not shown). The absence of any detected discordance between independent analyses demonstrates that the

TABLE 2—*Combined data for the ABI 310 and 3130 when observing peak height ratios for the MiniFiler kit per locus.*

Locus	No. Observations	Mean PHR	Median PHR	Minimum PHR	Maximum PHR
CSF1PO	40	0.79	0.82	0.43	0.98
D2S1338	38	0.82	0.87	0.35	0.99
D7S820	42	0.79	0.85	0.38	1.00
D13S317	34	0.71	0.75	0.45	0.96
D16S539	40	0.75	0.73	0.52	1.00
D18S51	36	0.77	0.75	0.49	1.00
D21S11	38	0.76	0.79	0.40	0.92
FGA	44	0.80	0.81	0.46	1.00
Amel	44	0.75	0.80	0.37	0.99

PHR, peak height ratio.

TABLE 3—*Combined data for the ABI 310 and 3130 when observing peak height ratios of up to 4000 RFU.*

Peak Height Range	Mean PHR	SD	Minimum PHR	Maximum PHR
<500	0.74	0.19	0.35	0.94
500–1000	0.78	0.17	0.36	0.99
1000–2000	0.77	0.16	0.38	1.00
2000–3000	0.76	0.15	0.37	0.99
3000–4000	0.82	0.16	0.57	1.00

PHR, peak height ratio; SD, standard deviation.

MiniFiler kit and accompanying procedure is reliable and reproducible.

Precision

The standard deviation of the allele size for multiple injections of the same sample was at the highest 0.070 base, which was observed at the D21S11, CSF1PO, and D18S51 loci (data not shown). As a result, miscalled alleles due to sizing imprecision was not a concern. The MiniFiler kit, used in conjunction with the ABI 310 and 3130, demonstrated a high degree of precision proving that reliable results can be obtained by performing capillary electrophoresis on samples amplified with MiniFiler.

Mock Casework and Challenged Samples

The odds of obtaining a more complete genetic profile were found to be greater when MiniFiler was used in conjunction with Identifiler in the genotyping of challenged samples exhibiting DNA degradation or inhibition. At many of the loci in which no alleles were detected using Identifiler, with the use of MiniFiler a complete DNA profile was achieved on all samples typed (selected data shown). Figure 4 illustrates the success of MiniFiler on typing the sample containing degraded DNA from a bloodstained T-shirt of a 1977 homicide case, while Fig. 5 also demonstrates the increased sensitivity of the MiniFiler kit as compared to that of the Identifiler kit in its ability to achieve complete DNA typing results on the bone sample from a 1992 helicopter crash.

Concordance

DNA profiles of 20 samples, previously amplified using Identifiler, were compared to the genotyping results using MiniFiler to assess the concordance between the two kits. In one instance, a null allele was discovered in a sample when amplified with MiniFiler which was previously detected using the Identifiler amplification kit

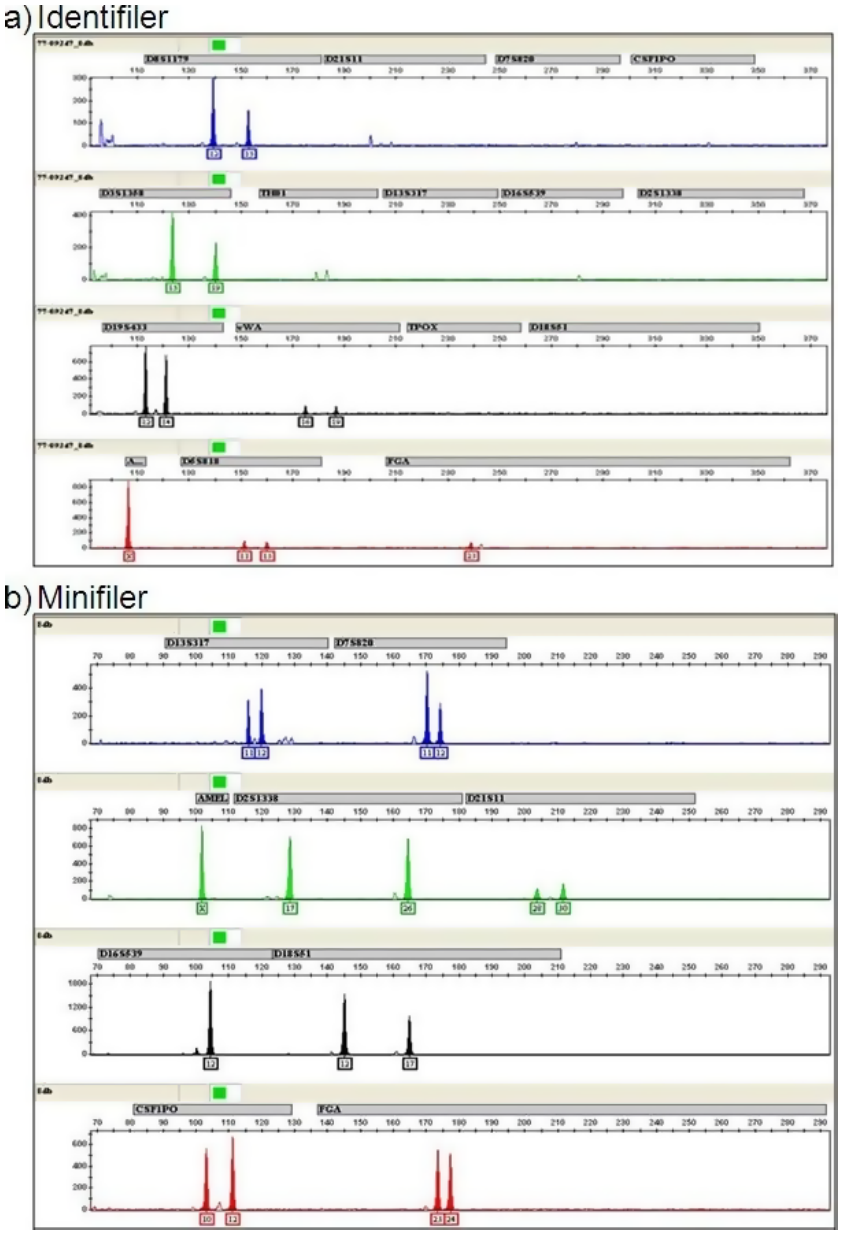

FIG. 4—*(a) Initial Identifiler profile obtained from the 1977 homicide case. (b) MiniFiler profile obtained from the same sample.*

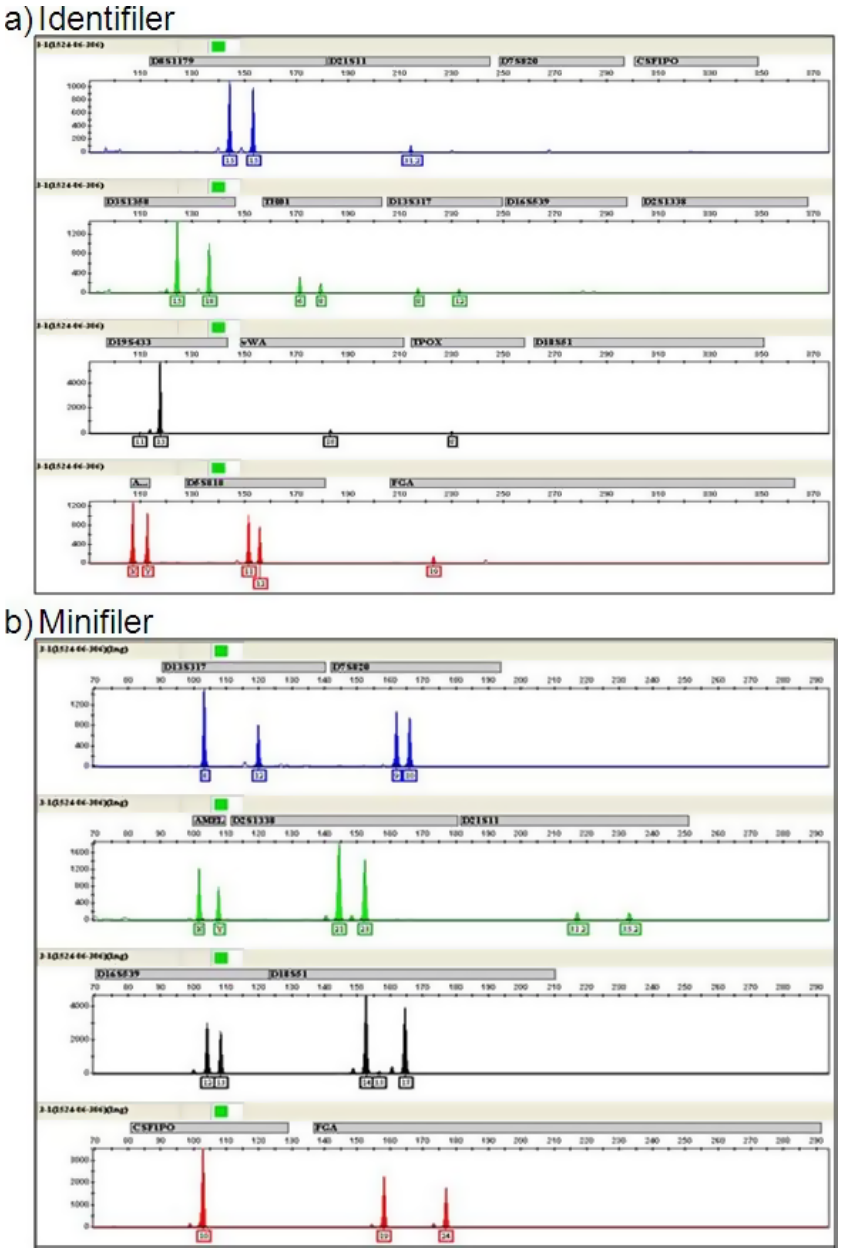

FIG. 5—*(a) Initial Identifiler profile obtained from the bone sample recovered from the helicopter crash site. (b) MiniFiler profile obtained from the same sample.*

(Fig. 6). In this instance a 29.1 microvariant at the D21S11 locus was not detected with the MiniFiler kit. The 29.1 allele is not an allele contained within the Identifiler allelic ladder and has only been reported five times in the variant allele report on the Short Tandem Repeat DNA Internet Database (http://www.cstl.nist.gov/div831/strbase/). This instance of nonconcordance was most likely due to a primer binding site mutation for this 29.1 allele that may have prevented the MiniFiler primer from binding to the DNA, resulting in no amplification of this allele. Although a previous study was conducted comparing the concordance between the Identifiler and MiniFiler kits, there was no detection of a null allele at locus D21S11 (21). This sample has since been sent out for sequencing to confirm the cause.

Conclusions

An internal validation following the SWGDAM guidelines composed of nine experimental studies was conducted in order to assess the reliability and performance of the AmpFℓSTR® MiniFiler™ PCR Amplification Kit for use on forensic casework. A single instance of nonconcordance at the D21S11 locus was observed between the kits in which a 29.1 allele that was detected with Identifiler failed to be detected with the MiniFiler kit. This instance of nonconcordance resulted from the amplification of a sample with a rare microvariant allele. Amplification of all other samples resulted in full concordance across the two typing kits. It was found that caution should be taken in deducing component genotypes, given that it was determined that heterozygous peak balance could be as low as 36% in a single source sample amplified with an optimum amount of input DNA. Another area that warrants consideration is in the interpretation of homozygote genotypes, largely due to the increase in sensitivity of the MiniFiler kit that enhances the possibility of observing stochastic effects. In our laboratory the homozygous threshold for MiniFiler could be effectively set at 450 RFU, while in comparison that value for the Identifiler kit is 200 RFU. In contrast to the 0.5–

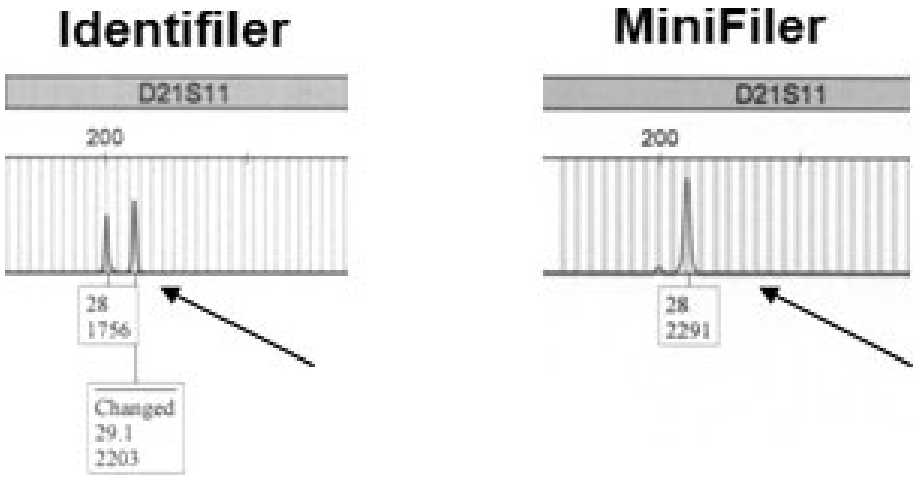

FIG. 6—*Nonconcordance between the MiniFiler kit and Identifiler kit. The arrows point to a null allele detected at locus D21S11 using the MiniFiler system.*

0.75 ng of template DNA recommended by Applied Biosystems for the MiniFiler kit, it was determined that complete profiles could be obtained using a template DNA amount of 0.3 ng. The 0.3 ng threshold represents approximately a six-fold increase in the sensitivity when compared to the 1.8 ng optimum input for Identifiler testing in our laboratory. This study demonstrates that the MiniFiler kit is successful in generating robust and reliable DNA profiles from samples exhibiting DNA degradation or PCR inhibition, and can be used in association with the Identifiler kit to obtain complete DNA profiles from challenged samples.

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