SDPD Forensic Science Section **July 26, 2007**

MiniFiler™ Validation Study

Validation Summary

Introduction

The AmpF*l*STR MiniFiler™ Amplification Kit is designed for the genotyping of degraded and/or inhibited DNA samples when a complete DNA profile is unable to be obtained with the AmpF*l*STR Identifiler™ Amplification Kit alone. In a single PCR reaction MiniFiler amplifies eight autosomal STR loci: D7S820, D13S317, D2S1338, D21S11, D16S539, D18S51, CSF1PO, FGA, and the sex determining locus Amelogenin; targeting Identifiler's eight largest loci. Thus, MiniFiler is to be used in conjunction with Identifiler.

The advantage that MiniFiler boasts over Identifiler are the shorter amplicons produced as a result of the PCR reaction. Primers are placed closer in to the STR repeat region, resulting in these shorter amplicons (miniSTRs). As a result, the new primer pairs in the MiniFiler kit produce amplicons in the range of 70-283 basepairs.

Amplifying MiniSTRs is advantageous when extracted DNA is either degraded or contains PCR inhibitors. With larger STR amplicons, such as those produced by Identifiler, the likelihood of achieving a profile decreases due to loss of signal when the DNA is degraded, or amplified in the presence of PCR inhibitors. In addition to shorter amplicons, MiniFiler is able to overcome inhibition due to optimization of the thermocycling parameters with 30 amplification cycles, the concomitant reduction of optimum template requirement, as well as the additional Taq/BSA added to the reaction mix. Although miniSTRs are smaller, the database compatibility with commercial STR megaplexes is still maintained.

The following validation experiments were designed to assess the performance of MiniFiler: Sensitivity, Stutter, Stochastic, Mixture, Precision, Challenged Samples, Peak Height Ratio, and Concordance.

Sensitivity

The sensitivity study assessed two factors: the recommended template DNA range for Applied Biosystem's AmpF*l*STR MiniFiler kit, and the sensitivity between the 310 and 3130 genetic analyzers.

The lowest concentrations at which full profiles were obtained (no allele drop-out) were 0.05 ng for the positive control and 0.0625 ng for a reference sample. Off-scale peaks and baseline artifacts occurred at a template amount of 1.0 ng and higher. Thus, the ideal template DNA range in which to achieve full profiles with a minimal amount of off-scale data and artifactual peaks is 0.2-0.6 ng, with the optimum target amount being 0.3 ng.

Larger peak heights were obtained for samples analyzed on the 310 genetic analyzers as compared to the peak heights from the same samples analyzed on the 3130. From the results obtained from this study, the sensitivity of the 310 genetic analyzers is greater than that of the 3130.

Stutter

Stutter values were assessed for data obtained from both the 310 and 3130 genetic analyzers, for -4 bp stutter peaks. This study demonstrates that the manufacturer's stutter filter values can be effectively used to filter most stutter peaks, as the % stutter values observed in this study lie below the recommended stutter percentage filter values for each locus.

Twenty-three samples were analyzed for stutter analysis in this study, whereas 967 samples were used by ABI for their stutter determinations. Since more samples were utilized by ABI that cover many more alleles, the stutter values provided by ABI should be employed for casework interpretation.

Stochastic

Both the 310 and 3130 genetic analyzers were used to perform capillary electrophoresis on samples amplified with template DNA amounts that straddle the detection threshold of 75-100rfu. A peak detection threshold of 75rfu was employed to analyze the samples.

The majority of allelic dropout occurred with peaks below 450rfu when analyzed on the 310. All allelic dropout occurred with peaks below 450rfu when analyzed on the 3130. There were two instances on the 310 where dropout occurred with peaks above 450rfu: 570rfu at locus D21S11 and 680rfu at locus D16S539.

This study demonstrated that an accurate homozygote genotype determination can be obtained for both the 310 and 3130 genetic analyzers with a homozygote threshold of 450rfu. At this level, allelic dropout (in the majority of instances) is not often observed and can be employed for casework.

Mixture

MiniFiler was successful in detecting mixtures in the range of 20:1 to 1:20. At a ratio of 1:1, all alleles from both individuals were present, and at a mixture ratio of 3:1 (and 1:3), a majority of alleles from both individuals were present. As the mixture ratios increase, the amount of allelic dropout from the lesser component also increases.
Thus, the 20:1 (and 1:20) were the mixture ratios containing the most allelic dropout of the minor components, however minor component was still observed at one or more loci for both sets of mixtures.

According to the peak height ratio study using MiniFiler, if a heterozygous locus has a peak height ratio of less than 36% a mixture can be inferred, but caution must be taken in deducing the genotypes of the contributors. The DNA profile of a major contributor was assigned with confidence in 1:10 mixtures of DNA, however as the mixture ratio

approached 1:3 the determination of major/minor genotypes became increasingly difficult.

Precision

Precision was evaluated by assessing the average basepair size and standard deviation of all alleles for each sample injected. The size difference of the alleles for the same sample deviated from a hundredths to a thousandths of a basepair for each sample injected. The highest standard deviation observed for any alleles was 0.070.

Since the highest standard deviation of all the samples and ladders was 0.070, miscalled alleles due to sizing imprecision should not be a concern. Larger loci have higher variation due to the variable migration of the 340 bp peak of the ISS. Furthermore, this study demonstrates that reliable results will be obtained by performing capillary electrophoresis on samples amplified with MiniFiler. Thus, the MiniFiler kit used in conjunction with the 310 and 3130 genetic analyzers demonstrate a high degree of precision.

Challenged Samples

This study consists of a comparison of the results achieved from the typing of degraded or inhibited samples using MiniFiler versus previous typing performed using Identifiler. The benefits of using MiniFiler in conjunction with Identifiler are demonstrated in this study.

At loci where DNA typing results were unable to be achieved using Identifiler, in many cases MiniFiler was able to detect allele peaks at these loci. Improved peak detection is most likely an effect of using a lower target amount of DNA that allows for more dilution of PCR inhibitors as well as optimization of the amplification reagents and cycling parameters. The conclusion that the AmpF*l*STR MiniFiler kit is suitable for use on casework is supported by the results obtained in this study.

Peak Height Ratio

This study aims to determine the expected peak height ratios between heterozygote pairs when optimal amounts of template DNA are used. The knowledge gained is useful in interpreting mixed DNA samples. Minimal differences in peak height ratios were observed between the 310 and 3130 genetic analyzers.

A comparison of peak height imbalance was made between each locus containing PHRs ≤50%, in which the following results were obtained.

- D2S1338 & D21S11: peaks >1000rfu have >40% PHRs
- D7S820 & D13S317: peaks >1000rfu have >45% PHRs
- D18S51 & FGA: peaks >1000rfu have >48% PHRs
- CSF1PO: peaks >1000rfu have >50% PHRs
- Amelogenin is the most susceptible to imbalance

The peak height ratio between the two heterozygous peaks averaged approximately 78%. However, imbalance of as much as 36% was observed at all peak height pairs between 500 and 4000rfu. Caution should be exercised in relying on peak height ratios when attempting to elucidate component genotypes in a mixture.

Concordance

The DNA profiles of twenty reference samples that were previously amplified using Identifiler were contrasted to the DNA profiles achieved using MiniFiler. For 19 of the 20 reference samples there is consistency between the DNA typing results for both MiniFiler and Identifiler. A null allele occurring at one locus for one sample, when amplified with the MiniFiler kit, prevents full concordance between the two kits. A suspected mutation in the primer binding site for this 29.1 allele at the D21S11 locus may have prevented the MiniFiler primer from binding to the DNA, resulting in no amplification. To further understand the nature of this genetic variation, DNA sequence analysis should be performed. SEE ATTACHED NIST PAPERWORK.

A paper entitled "Concordance Study Between the AmpF*l*STR® MiniFiler™ PCR Amplification Kit and Conventional STR Typing Kits" was published July 2007 in the Journal of Forensic Science 52(4). Researchers conducting this concordance study between MiniFiler and Identifiler found that genotyping discrepancies between the two kits resulted in 27 discordant STR profiling results when 1,308 samples were analyzed. Full concordance was observed in 99.7% of the STR allele calls that were compared. Of the 1,308 samples analyzed in this study three samples contained a null allele, with two of the samples containing a null allele at the D16S539 locus (null allele 11), and one of the samples containing a null allele at the D13S317 locus (null allele 10). The authors of this study conclude that the genotyping discrepancies present at these loci are a result of Mendelian Inheritance of a MiniFiler primer binding site mutation. Locus D21S11 was not one of the loci at which non-concordance was observed in this study.

Due to a possibility of non-concordance, comparisons should be made between samples amplified with the same kit.

Conclusion

In terms of successful DNA typing using MiniFiler, a range of 0.2-0.6 ng DNA should be used, with an optimum template amount of 0.3 ng. As a result, reliable and artifact-free DNA profiles can be achieved.

Full concordance is achieved between MiniFiler and Identifiler except in the rare cases of individuals that have genetic variants of certain alleles which may result in null, or partial null, alleles at a particular locus.

Data obtained from the eight validation experiments evaluating the performance of the AmpF*l*STR® MiniFiler™ PCR Amplification Kit demonstrates that the MiniFiler kit, when used in conjunction with the Identifiler kit, provides an increased power to obtain genetic profiles from challenged samples. This validation study supports the use of the

MiniFiler kit on forensic casework samples that are degraded and/or contain PCR inhibitors.

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