# Internal Validation of AmpFlSTR® MiniFiler™ PCR Amplification kit for Forensic Casework

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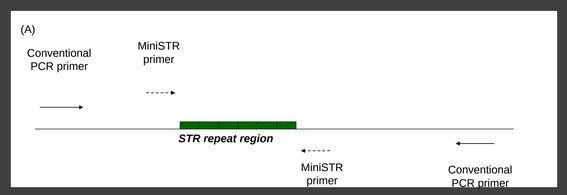
## Abstract

In Forensic Laboratories, DNA typing of degraded and inhibited samples is a challenging task using conventional Short Tandem Repeat (STR) amplification kits such as AmpFlSTR® Identifiler® and Promega PowerPlex®16 amplification kits. The larger loci included in these kits do not amplify in degraded or inhibited samples. Thus, Applied Biosystems introduced AmpFlSTR® MiniFiler™ PCR Amplification kit that includes eight larger loci with reduced amplicon sizes, which increases the success of producing genotypes from challenging samples. MiniFiler™ , which is used to supplement standard multiplex amplification kits, is more sensitive than conventional kits and generates profiles which other STR kits fail to produce.

## Introduction

STR markers are most commonly used in forensic analysis for DNA typing and human identification purposes. STR's are highly polymorphic regions which are amplified by the Polymerase Chain Reactions (PCR), to generate a DNA profile. Conventional kits such as Identifiler® and PowerPlex® 16 include 15 STR loci and Amelogenin, which is a sex-determining marker. These kits amplify all loci at the same time; hence they are called 'multiplex' systems. The amplicons generated by these kits range from 100-400 nucleotides in size. The primers are located in conserved regions adjacent to repeat and flanking regions and spread 100-200 base pairs on each side of the repeated sequence. The larger loci can fail due to their high molecular weight in degraded or inhibited samples.

MiniFiler™ is a multiplex system which uses the same five-dye chemistry as Identifiler® . The kit includes eight of the larger loci and Amelogenin that are included in the Identifiler® amplification chemistry . These loci include D7S820, D13S317, D16S539, D2S1338, D18S51, D21S11, CSF1PO, and FGA. The size range of the amplicons is reduced by moving primers to conserved regions immediately adjacent to the repeat regions, which results in a size range of 70-283 base pairs(bp) compared to the 100-400bp range in Identifiler®. Due to this smaller amplicon size range, larger loci are efficiently amplified by MiniFiler™.



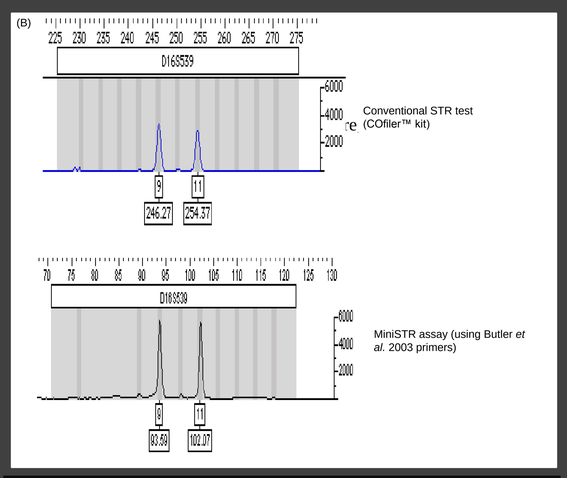




**Fig. 1. MiniFiler™ primer positioning**

## Methods and Results

### Sensitivity Study

• Purpose: To determine the target DNA concentration of a target value at which reliable and complete profiles are observed.

• Methods and materials: Two DNA sample were run at concentrations:2ng,1ng,0.8ng,0.6ng,0.5ng,0.4ng,0.25ng,0.125ng,0.062ng, and 0.031ng.

• Results: Full profiles were observed at the 400pg input concentration. The optimal range was determined to be 0.4ng -1.0ng, and the optimal target DNA concentration was 0.6 ng.

### Stochastic Effect

• Purpose: To determine the concentration at which allelic dropout is observed.

• Methods and Materials: 19 samples were amplified at target DNA concentration of 0.1ng.

• Results: Allele dropout was observed in 4 of 19 samples. The Peak Height Ratio(PHR) range was 13%-97%.

### Reproducibility Study

• Purpose: To observe similar results using MiniFiler™ as observed using Identifiler®.

• Methods and materials: 70 samples were amplified using 0.6ng target DNA concentration.

• Results: All Identifiler® alleles were observed with MiniFiler™ resulting in genotype concordance.

### Heterozygous PHR

• Purpose: To determine the peak height balance in heterozygous loci.

• Methods and materials: 70 samples from the reproducibility study were used and PHR was calculated by dividing the height of the smaller peak by the height of the larger peak, and multiplying the value by 100.

• Results: The average PHR was calculated to be 0.83 with a standard deviation of 0.11.

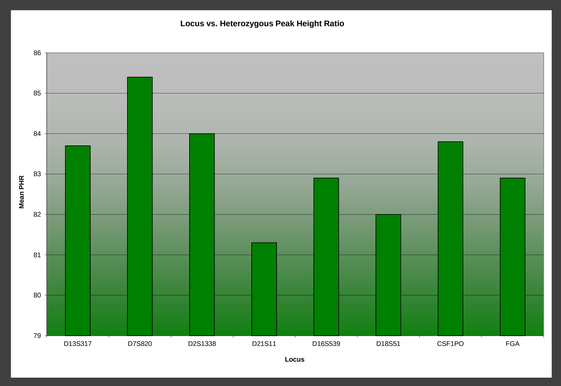



**Fig.2: Heterozygous Peak Height Ratio of 70 samples typed with MiniFiler™**

### Stutter

• Purpose: To record the level at which an amplification artifact was observed at the n-4 position of a true allele.

• Methods and materials: All samples from the reproducibility study were used and stutter was calculated by dividing the peak height of an n-4 allele by the peak height of the associated genuine allele and multiplying the value by 100.

• Results: The stutter percentages for all loci were calculated to be less than those recommended by Applied Biosystems.

| Locus | Range % | Mean % | SD | Upper Range * % | MiniFiler Marker Specific Stutter % |
|---|---|---|---|---|---|
| D13S317S317 | 11.9-1.9 | 6 | 1.9 | 12 | 14 |
| D7S820S820 | 8.8-1.8 | 5.1 | 1.7 | 11 | 11 |
| D2S1338S1338 | 12.8-4.8 | 8 | 1.8 | 14 | 18 |
| D2S13381S11 | 12.6-3.6 | 8.2 | 1.4 | 13 | 16 |
| D16S539 | 13-3.7 | 7.2 | 2.0 | 14 | 15 |
| D18S51S51 | 16.5-3.1 | 7.9 | 2.3 | 15 | 18 |
| CSF1PO | 14-3.2 | 6.4 | 1.7 | 12 | 14 |
| FGA | 11.6-3.3 | 6.7 | 1.6 | 12 | 15 |

**Table 1: Stutter percentages of samples typed with MiniFiler™**


**Fig.3: MiniFiler™ stutter Percentages**

### Precision

• Purpose: To determine bin position for each allele in the MiniFiler™ ladder.

• Methods and materials: 40 ladders were run with four different ladder preparations in separate wells. Each was injected ten times.

• Results: The calculated standard deviation of each allele was calculated to be less than 0.1bp, while the mean range was 0.21bp.

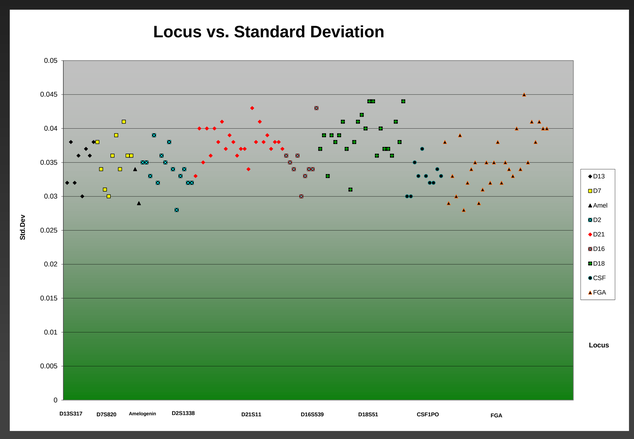



**Fig.4: Precision study using 40 MiniFiler™ ladders**

### Mixture Study

• Purpose: To observe the major and minor contributors of mixture samples different ratios.

• Material and Method: Mixture ratios of 20:1,10:1,5:1,2:1,1:1,1:2,1:5,1:10, and 1:20 were run with 0.6ng target DNA concentration.

• Results: Full profiles were observed at ratios up to 2:1/1:2. Allelic dropout was observed at the 5:1 ratio.

### Inhibition Study

• Purpose: To determine how samples with unknown inhibition are typed with the MiniFiler™ kit.

• Methods and materials: Two samples known to be inhibited samples were quantified with the Quantifiler® DUO, a real time quantitation kit, and run with a 0.6ng of target DNA concentration. Three sets of samples were run, including neat samples, 1:10 dilution, and the samples with additional BSA and Taq Polymerase. All three sets were run using the Identifiler® chemistry and a 1.2ng concentrations. The results were compared between the two kits.

• Results: For 1:10 diluted samples, full profiles were observed with MiniFiler™ , while a full profile and partial profile were observed with Identifiler®. For samples with additional BSA and Taq, partial profiles were observed for both samples with MiniFiler™ , while no peaks were observed using the Identifiler® kit. For neat samples, no alleles were observed with MiniFiler™ or Identifiler®.

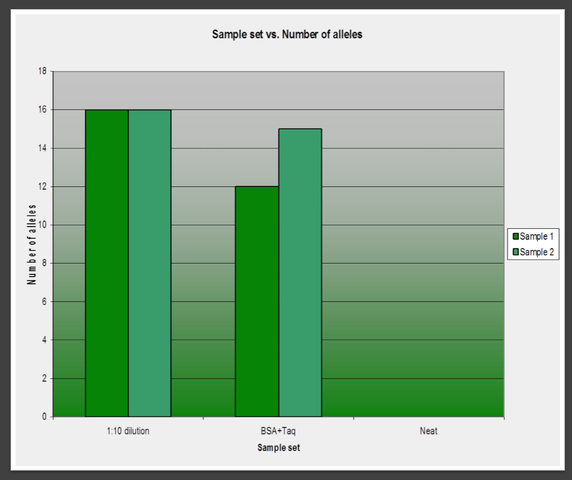



**Fig.5. Observed alleles out of 16 using MiniFiler™**

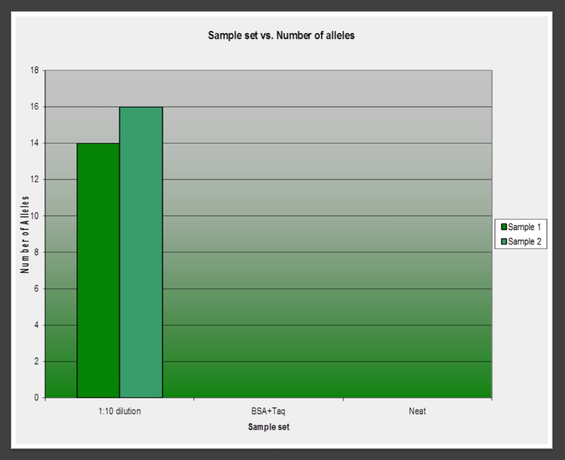



**Fig.6. Observed alleles out of 16 using Identifiler®**

## Conclusions and Future Directions

From this internal validation, we can conclude that MiniFiler™ is a more sensitive than Identifiler®. This kit is highly reproducible and sensitive up to 400pg. The ladder bins ranges were found to be within the manufacturer's recommendations, so this kit can be confidently used in our lab. For mixtures, caution should be taken when using this kit for ratios above 2:1/1:2 because allelic dropout is likely. Allelic dropout were observed at the 0.1ng input. For inhibited samples, the 1:10 dilution was the most effective target. Full profiles were observed with inhibited samples.

There are some disadvantages to using the MiniFiler™ chemistry, however. Several minor peaks were observed in single source profiles, most likely attributable to n+4 stutter, which is rarely seen in other commercial kits. These n+4 stutters could be due to the sensitivity of the MiniFiler™ kit or the two additional cycles added in the thermal protocol. Furthermore, contamination can also play a role in these extraneous peaks. Hence, according to this research MiniFiler™ is best suited for compromised, single source samples rather than mixtures. These compromised samples can be from natural disaster sites, aerial crashes, or missing persons investigations.

Further studies need to be performed in order to incorporate this kit into the laboratory. The studies include known and probative evidence, match criteria, contamination, and qualifying tests. If the extraneous peaks can be eliminated by manufacturer's modification, more labs are likely to adapt this kit due to its sensitivity.

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