



B64 Validation and Implementation of Applied Biosystems AmpF/STR® MiniFiler for Challenging Samples Caveats and Impacts

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The goal of this presentation is to provide an introduction to the first commercially available miniSTR kit from Applied Biosystems. Upon completion of this presentation attendees will have been provided an overview of BCIT's validation studies of this new kit as it was integrated into our laboratory system and an overview of MiniFiler's strengths and weaknesses through casework examples.

This presentation will impact the forensic science community by introducing BCIT's latest validation data and case examples related to the newly released MiniSTR kit. This new system will push the boundaries of usable forensic DNA evidence for human identification.

Custom mini-short tandem repeats (miniSTR's) have been reported over the last several years and have shown great promise for forensic DNA applications on highly degraded and/or inhibited DNA. A commercially available miniSTR multiplex (AmpFISTR Minifiler) was released in the winter of 2007 by Applied Biosystems that will facilitate a standardized approach and wide spread adoption by the forensic community. The Minifiler™ system amplifies eight autosomal STR loci (D7S820, D13S317, D21S11, D2S1338, D18S51, D16S539, FGA, CSF1PO) and amelogenin using 5-dye technology and spanning an amplicon size of 70 to 283 basepairs. This multiplex is designed to augment and add genetic information to standard STR systems by redesigning primer sets to produce smaller amplicons.

This paper will outline BCIT's validation results for this new system as it was integrated into our standard operating procedure for forensic DNA analysis on "challenging" samples. In our laboratory, samples are processed as follows: extraction with one of two techniques (organic extraction followed by Amicon filtration or Promega's DNA-IQ paramagnetic resin system), quantification with Applied Biosystems Quantifiler™, amplification with Applied Biosystems AmpFISTR Profiler Plus (and/or now MiniFiler™), genetic analysis using an ABI-310 interfaced to Genemapper ID and statistical analysis using EasyDNA_In_A_Minute. In our validation studies very reliable results can be obtained routinely with 200pg of input DNA (at 1/2 reaction volume) although the adoption of Minifiler™ has extended the range of usable samples down to the 62pg range. At this low end of input DNA, however, one must interpret the results cautiously due to an increased rate of allelic dropout and higher peak imbalance and, therefore, we recommend running samples in duplicate to address these stochastic effects. These results along with additional validation results will be presented to highlight the utility of this new kit within a system designed to analyze low copy number or degraded samples. BCIT's laboratory has also done extensive validation of the two DNA extraction methodologies on a wide range of sample types in order to assess the effectiveness of the DNA-IQ system and these results will also be presented.

Ultimately the goal of any validation study is to prepare a new system for use in forensic casework. In this presentation some case examples will also be presented to highlight the integration of MiniFiler™ into this laboratory. For example, in the mid-1970's a man disappeared while piloting his single engine aircraft in southern BC. The wreckage and associated remains were recently discovered by hikers in a heavily wooded area to the northeast of Vancouver, BC. Even after decades in this moist environment sufficient nuclear DNA was recovered to proceed with STR analysis, however, in this case the standard Profiler Plus procedure showed signs of significant degradation and allelic dropout. Minifiler™ augmented the usable data which resulted in a full panel of 12 STR loci plus amelogenin. By comparison to a daughter, the deceased's sole living relative, the combined likelihood ratio was extended from an estimated 118 (Profiler Plus® data only) to approximately 5000 (Profiler Plus® and MiniFiler™ data combined).

AmpFISTR Minifiler™, MiniSTR, Degraded DNA