## D51 Reduction or Elimination of Observed Reproducible Artifacts in the AmpF*l*STR® Identifiler® PCR Amplification Kit

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After attending this presentation, attendees will have an increased understanding of the continual efforts of Applied Biosytems to improve the quality of their products used by forensic community at a technical level.

This presentation will impact the forensic community and/or humanity by demonstrating how the forensic community will benefit from an understanding of both the steps taken to improve the performance of the AmpF*l*STR® Identifiler® PCR Amplification Kit, and the subsequent studies to validate the kits produced with the updated manufacturing procedure.

This presentation will discuss the reduction or elimination of observed VIC® and PET® artifacts in the AmpFISTR® Identifiler® PCR Amplification Kit, and the subsequent validation study of the updated kit. The Identifiler® kit is a STR multiplex assay that amplifies 15 tetranucleotide repeat loci and the gender determining marker, Amelogenin in a single PCR amplification. Loci are genotyped using the Identifiler® Allelic Ladder after running samples on an ABI PRISM® Genetic Analyzer. The Identifiler® kit is widely used in both forensic and paternity applications.

Within the Identifiler® Kit, a VIC® dye labeled artifact was observed at approximately 120 bps, within the range of the D3S1358 loci. PET ®dye labeled artifacts were observed between the Amelogenin and the D5S818 loci at approximately 110 to 130 bps. These artifacts were reproducible and could be detected as labeled alleles or off ladder alleles (OL Alleles) during data analysis. In order to reduce or eliminate these artifacts, modifications were made in the manufacturing process of the Identifiler® Kit (PN 4322288) effective from lot number 0310018 onwards. These modifications reduced the artifacts observed in analyzed samples amplified with the PET® dye and VIC® dye labeled primers, without compromising the performance of the Identifiler® Kit.

In order to address the PET® dye artifacts, a modification in the manufacturing process of the PET® primers was introduced, which importantly, did not alter the PET® dye primer sequences. Non-nucleotide linkers, which enable reproducible positioning of the alleles to facilitate inter-locus spacing, are used in primer synthesis between the primer oligonucleotides and the dye. The PET® dye artifacts were observed to be correlated with the use of a particular non-nucleotide linker and therefore it was possible to diminish the appearance of the artifact by using a different non-nucleotide linker during synthesis of the PET® dye labeled primers.

In order to address the VIC® dye labeled artifact, an additional step was introduced into the purification process of the VIC® dye labeled primers. This resulted in the significant reduction in the observation of VIC® dye artifacts.

For the Identifiler® Kit produced with the updated manufacturing steps (lot number 0310018 onwards), validation studies were performed according to the DNA Advisory Board's "Quality Assurance Standards for Forensic DNA Testing" and the Scientific Working Group on DNA Analysis Methods "Revised Validation Guidelines: (July 10, 2003)" (Forensic Science Communications July 2004, V. 6, No.3). These studies specifically addressed sensitivity, stability, reproducibility, precision, and accuracy. In each instance the Identifiler® kit produced with the updated manufacturing steps performed comparably to the previous Identifiler® kit.

These minor modifications to the manufacturing process of the Identifiler® Kit, which were introduced in response to customer feedback, either significantly reduced or eliminated the observed reproducible artifacts. This facilitates the use of the Identifiler® kit for forensic casework involving mixed specimen samples and has led to an overall increase in customer satisfaction.

**STR, Genotyping, Identifiler®**

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