Internal Validation of the AmpFSTR® Identifiler® Plus PCR Amplification Kit and Comparison to Identifiler® for the Boston Police Department Crime Laboratory

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Abstract
An internal validation of the AmpFSTR® Identifiler® Plus PCR amplification kit was performed at the Boston Police Department (BPD) Crime Laboratory. The kit produced reliable, reproducible, and robust results throughout various validation studies. Identifiler® Plus also demonstrated an overall greater sensitivity and an improved performance on degraded samples as compared to the Identifiler® amplification kit currently in use.

Introduction
Amplification is an important step in forensic DNA analysis, and the amplification kit used can affect the quantity and quality of results obtained. Numerous validation studies were performed on the Identifiler® Plus amplification kit, including:

- Sensitivity Study: To determine an ideal range of DNA input to produce a reliable profile with limited stochastic effects
- Mixture Study: To demonstrate the kit’s ability to resolve mixtures
- Precision Study: To show reliable and precise typing based on allele size deviation over different runs
- Concordance Study: To show accurate typing of the kit
- Reproducibility Study: To demonstrate reproducible genotyping over time
- Contamination Study: To examine all negative controls to ensure no contamination was present

Comparison studies between the Identifiler® Plus kit and the Identifiler® kit, currently in use at the BPD, were performed to determine if the Identifiler® Plus kit should be implemented for forensic casework. The following studies were performed:

- Touch DNA Study: To compare kit performance on low level samples
- Degraded Sample Study: To compare kit performance on degraded samples

Materials and Methods
- Known control blood sample extracts provided by the BPD
- Touch DNA samples collected by analysts at the BPD
- AmpFISTR® Identifiler® PCR amplification kit
- AmpFISTR® Identifiler® Plus PCR amplification kit
- GeneAmp® PCR System 9700 Thermal Cycler- 28 Cycle Amplification
- Applied Biosystems 3130x/Genetic Analyzer
- Applied Biosystems GeneMapper® ID v3.2.1

Results and Discussion

Validation Studies
- Sensitivity Study: A range of 0.125-1.0 ng of DNA input was established, with an optimum of 0.75 ng.
- Mixture Study: Mixtures were detected when the minor component comprised 5% or more of the mixture. Samples with minor components comprising 25% or less of the total DNA input rarely crossed the stochastic threshold.
- Precision Study: Precise typing can be performed due to the low standard deviation (less than 0.15 basepairs) of the alleles of the ladder over several runs.
- Concordance Study: Allele calls were concordant with the Identifiler® kit allele calls. The Identifiler® Plus amplification kit is accurate.
- Reproducibility Study: See Figure 1. The kit was shown to be reproducible in allele calls and peak heights up to 2 weeks after amplification
- Contamination Study: All negative controls were clean. No contamination was present in the kit, capillaries, or pipettes.

Comparison Studies

- Additional alleles called
- DNA Target Input (ng)
- Above Stochastic Threshold
- Below Stochastic Threshold

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<thead>
<tr>
<th>Identifiler® Plus</th>
<th>Identifiler®</th>
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<tbody>
<tr>
<td>DNA Target Input</td>
<td>0.75 ng</td>
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<tr>
<td>Full Profiles To</td>
<td>0.125 ng</td>
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<tr>
<td>Peak Height Ratios</td>
<td>&gt;70%</td>
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<tr>
<td>Amplification Times</td>
<td>2.5 hours</td>
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Conclusions

- Identifiler® Plus was successfully validated for use at the BPD, as it was found to be reliable, reproducible, and robust.
- Identifiler® Plus was more sensitive and demonstrated improved performance on degraded samples compared to Identifiler®
- Identifiler® Plus will be implemented for use at the BPD Crime Laboratory for forensic casework.

References
- Coble MD, PhD. 2011. Design and execution of validation studies for establishing DNA mixture interpretation procedures, NFSTC DNA Mixture Interpretation Workshop; 2011 March 15-17; Large, Florida.

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