A160 Comparison of Commercial Multiplex Kit Performance With Low Template DNA

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The goal of this presentation is to provide attendees with an understanding of the relative merits and disadvantages of several commercial STR multiplex kits when dealing with low template DNA, including the sensitivity of each and common artifacts at low input concentrations.

This presentation will impact the forensic science community by providing information that will aid in the selection of the most appropriate STR kit for amplification of quantified evidentiary extracts.

The Armed Forces DNA Identification Laboratory has validated the Promega PowerPlex® 16, Applied Biosystems AmpfℓSTR® Minifiler® and AmpfℓSTR® Identifiler® amplification kits, which provide the scientist with additional tools for evaluating evidentiary material. Extracts that quant at the low end of the sensitivity spectrum can be processed with Minifiler®, but the question remains whether or not it is worthwhile to attempt amplification with PowerPlex® 16 or Identifiler®, especially if the quant values are on the cusp of the sensitivity of these traditional kits. Minifiler® has proven to be a useful tool with degraded samples but its sensitivity makes it prone to artifacts and peak height imbalances. Though many laboratories are investigating methods of dealing with low copy number specimens, there is a dearth of information directly comparing different commercial kits, particularly at low template amounts.

This study was undertaken to examine the behavior of the Identifiler®, PowerPlex® 16, Minifiler® and PowerPlex® 16 HS commercial kits with low template DNA and provide information that will assist the analyst in choosing which method to pursue. The results of this study will also aid laboratories that are considering bringing another commercial amplification kit online.

Buccal swabs from two highly heterozygous individuals were extracted using a standard organic extraction protocol. A known human DNA standard was also utilized. All three known human DNA samples were quantitated using Quantifiler®.

The three known DNA samples were amplified in triplicate with the PowerPlex® 16, Identifiler® and Minifiler® kits at the following six DNA templates: 0.500, 0.250, 0.100, 0.050, 0.025 and 0.010ng. Amplification was conducted in accordance with validated protocols. One of the specimens was also amplified in triplicate at both 30 cycles and 32 cycles using PowerPlex® 16 HS at 1.000, 0.500, 0.250, 0.100, 0.050, 0.025 and 0.010ng input concentrations. All amplified products were typed and injected for 5 and 10 seconds at 3KV on the 3130*xl* Genetic Analyzer. The data was analyzed using GeneMapper® ID v.3.2 with a 40 RFU detection threshold.

The criteria used to assess the STR typing results for each of the kits included detection of profile alleles and the number of profile alleles above the laboratory-designated reporting thresholds of 75/150 RFU (heterozygotes/homozygotes) for the Identifiler® and Minifiler® kits and 100/200 RFU (heterozygotes/homozygotes) for the PowerPlex® 16 and PowerPlex® 16 HSkits. Peak height ratio was calculated for all heterozygous loci, and average peak height was calculated for both homozygous loci and heterozygous loci within and across replicates. The data accumulated from these examinations will be presented and discussed.

The decision of which commercial kit is appropriate to employ for amplification is crucial, whether on a small scale such as an analyst processing a single evidentiary sample or a technical leader deciding which amplification kits will be validated for use in the lab. Sensitivity to time and cost as well as the potential for limited extract volumes makes the choice of an appropriate amplification kit imperative.

The opinions and assertions contained herein are solely those of the authors and are not to be construed as official or as views of the United States Department of Defense or the United States Department of the Army.

STR Multiplex, LCN DNA, Sensitivity

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