



A22 The Evaluation of Eight Commercially Available STR Kits

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After attending this presentation, attendees will have a better understanding of the performance of eight commonly used STR amplification kits.

This presentation will impact the forensic community by providing information on the key performance measures of each amplification kit enabling the laboratory to make more informed decisions when choosing the kit(s) that best suit their needs.

A number of commercially available short tandem repeat (STR) amplification kits are available for use in forensic DNA laboratories. While it is the responsibility of each laboratory system to evaluate and choose the analytical methods that best suit its needs, it is important that forensic DNA analysts have a general understanding of the performance of commonly used STR amplification kits. In an effort to assist in this process, the National Forensic Science Technology Center (NFSTC) conducted a study to evaluate the performance of eight STR amplification kits: Applied Biosystems' AmpfSTR® Profiler Plus® kit, Cofiler® kit, Identifiler® kit, Minifiler™ kit, Yfiler® kit, Promega's PowerPlex® 16 system, PowerPlex® Y system, and the PowerPlex® S5 system.

The performance of each STR amplification kit was assessed based on a defined set of criteria: sensitivity, peak ratios at heterozygous loci, baseline noise, stutter ratio, and amplification artifacts. These criteria were determined through the analysis of single source human DNA samples. A mixture series was prepared and analyzed to assess the percent contribution necessary to detect a minor contributor in a two donor mixture for each STR amplification kit.

Two separate known human DNA standards were prepared utilizing a standard organic extraction method in conjunction with the Millipore Microcon® 100 centrifugal filter device. The samples were quantitated using the Applied Biosystems Quantifiler® Human and Y Human Male DNA Quantification Kits on an Applied Biosystems 7500 Real-Time PCR System. To minimize variation, a large volume (1000µl) of each sample was prepared and used for the dilution and two person mixture series. Samples were amplified on an Applied Biosystems GeneAmp® PCR 9700 thermal cycler following manufacturer's specifications. The samples were then separated and detected using an Applied Biosystems 3130x/ Genetic Analyzer and the data was analyzed using GeneMapper® ID Software v3.2 using a threshold of 75 rfu.

A serial dilution was performed on a known human DNA sample to yield target concentrations of 1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, and 0.0078 ng. The data obtained from each of these samples was used to assess the sensitivity and peak ratios at heterozygous loci for each of the eight STR amplification kits. In addition, the observation of any reproducible amplification artifact(s) in the data was noted. The baseline noise was assessed by evaluating the data from ten injections of amplification negative controls for each of the eight STR amplification kits.

A two donor mixture experiment was performed to evaluate the percent contribution necessary to detect a minor contributor for all eight STR multiplexes. Two separate known human DNA standards were systematically combined to create the following mixture ratios: 1:20, 1:15, 1:12, 1:10, 1:8, and 1:5. The DNA profile from the minor contributor was evaluated at a 75 rfu threshold and the peak ratios at heterozygous loci was calculated and noted at all loci where the alleles were 75 rfu or higher.

There are various commercially available STR multiplex kits available to the forensic science DNA community that are designed to address the ever changing needs of crime laboratories. A primary goal of this study is to provide an overview of key performance measures of these eight commercial available STR kits. The demand on forensic DNA laboratories to employ methods that meet their system's needs is a continual challenge and is compelling DNA technical leaders and laboratory management to acquire relevant information that will aid in making these crucial decisions.

Kit Comparison, Amplification Kits, STR Kits