AMPFlSTR™ PCR Amplification Multiplex Systems

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Short tandem repeat (STR) polymorphisms are rapidly becoming the worldwide standardized tool for forensic science applications in DNA analysis. The AmpFlSTR™ multiplex systems are PCR amplification kits which coamplify (multiplex) several STR loci. In particular, the AmpFlSTR Profiler™ and Profiler Plus™ kits each multiplex nine STR loci along with amelogenin, a gender informative marker.

In developing these kits, many levels of optimization were considered to assure robust performance and consistent, reliable results on forensic and database samples. Key aspects of primer design and enzyme optimization will be presented. Experiments demonstrating the necessity for optimal primer design to maximize yield and amplification specificity will be presented.

The AmpFlSTR™ multiplex systems were developed for use with Ampli*Taq* Gold® DNA Polymerase as this enzyme provides an "invisible" hot start. Ampli*Taq* Gold® is a chemically modified form of AmpliTaq® DNA polymerase, which renders the enzyme inactive. Upon thermal activation, the modifier is permanently released, resulting in active enzyme. Consequently, Ampli*Taq* Gold® improves specificity and allows for flexibility in automation. Experiments illustrating the parameters critical for efficient activation of Ampli*Taq* Gold® will be presented.

In addition, results from forensic validation studies on the Profiler Plus™ kit will be presented. These will focus mainly on single locus versus multiplex amplifications and adjudicated casework evidence samples. In general, results indicate that the amplification efficiency of the Profiler Plus™ loci is equivalent or better when amplified in multiplex as compared to singleplex amplifications. Casework results indicate that the Profiler Plus™ kit is robust. Furthermore, to assess the optimization level of the Profiler™ and Profiler Plus™ kits with different sample preparations, the performance of the multiplex systems has been evaluated using genomic DNA samples extracted from organic phenol/chloroform and Chelex methods. Different formats of sample preservation were also examined, including FTA™ paper. PCR results demonstrated efficient amplification in each case and will be shown.

In addition to these systems, the newly released AmpFlSTR Cofiler™ kit will be introduced. The Cofiler™ and Profiler Plus™ kits, together, include the 13 STR loci on which North America has standardized. Moreover, another multiplex system is currently under development which includes those loci in the Second Generation Multiplex along with three additional new loci (in total, nine STR loci plus amelogenin). This new system will be described further and preliminary data will be presented.