

## DEVELOPMENTAL VALIDATION OF A Y-STR MULTIPLEX PCR AMPLIFICATION SYSTEM

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Current interpretation of sample mixtures that are analyzed with autosomal markers via PCR can suffer from competition between a relatively small amount of male DNA and a large amount of female DNA. These specimens require the forensic analyst to make a judgment call on which autosomal STR loci correspond to the major and minor contributor. A multiplex PCR amplification system targeting Y-specific loci yields a high degree of confidence that only the male contributor is being analyzed. We are currently in development and validation of a 17-plex Y-STR system with an increased discriminatory capacity over current commercial Y-STR assays. The Y-STR multiplex includes all the loci in the "European minimal haplotype"; DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, and the SWGDAM recommended Y-STR loci; DYS438, DYS439. Additionally, we have included six highly polymorphic loci DYS437, DYS448, DYS456, DYS458, DYS635 (Y GATA C4) and Y GATA H4. To ensure an overlap between allele ranges, loci were labeled with 6-FAM™, VIC®, NED™, and PET® dyes. We have also generated an extensive allelic ladder containing 137 alleles. The multiplex amplification conditions were optimized on the GeneAmp® PCR system 9600 and 9700 thermal cyclers. The amplified products are separated on Applied Biosystems Genetic Analyzer instruments that support G5 matrix analysis such as the ABI PRISM® 3100, 3100-Avant and 310 Genetic Analyzers.

Validation studies demonstrating the effectiveness of the Y-STR multiplex will be presented including male to female, male to male, sensitivity and species specificity studies.

The Y-STR PCR amplification system described, used in conjunction with the Quantifiler™ Y Human Male DNA Quantification Kit, produce reliable and accurate Y haplotypes from the types of samples seen in the forensic laboratory. The system can be used in conjunction with a number of Applied Biosystems Thermal Cyclers and ABI PRISM® Genetic Analyzers and provides the forensic scientist with a complete set of tools for Y chromosome analysis.