B142 Developmental Validation of a New 17-Y-STR Multiplex System: The AmpF/STR® Yfiler™ PCR Amplification Kit

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After attending this presentation, attendees will learn about the validation of a new Y-STR muliplex PCR assay.

This presentation will impact the forensic community and/or humanity by providing a new tool available for DNA testing of the Y chromosome.

The goal of this presentation is to summarize the results of the developmental validations studies for the AmpF/STR Yfiler™ PCR Amplification Kit. This kit was developed in accordance with the guidelines of the forensic community, as defined by SWGDAM and the DNA Advisory Board's (DAB) Quality Assurance Standards.

Analysis of DNA sequence variation on the Y chromosome has become an increasingly important method in forensic casework, especially in sexual assault samples where there is a relatively small amount of male DNA and a large amount of female DNA. A multiplex PCR amplification system targeting Y-specific loci can simplify interpretation of complex male/female mixtures and yield a high degree of confidence that only the male contributor(s) is being analyzed.

The authors have developed and validated a 17-locus Y-STR multiplex system that has a higher discriminatory capacity than the European minimal haplotype. The Y-STR multiplex includes all the loci in the "European minimal haplotypes;" DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, the SWGDAM recommended Y-STR loci; DYS438, DYS439, and six highly polymorphic loci; DYS437, DYS448, DYS456, DYS458, DYS635 (Y GATA C4), and Y GATA H4. By incorporating the automated 5-dye DNA fragment analysis technology and nonnucleotide linkers small amplicon sizes (100bp-326bp) can be maintained and ensure no overlap between allele ranges. 6-FAM™ labeled primers detects DYS456, DYS389I/II, and DYS390. VIC® labeled primers detects DYS458, DYS19, and DYS385 a/b. NED™ labeled primers detect DYS393, DYS391, DYS439, DYS635, (Y GATA C4), and DYS392. PET® labeled primers detect Y GATA H4, DYS437, DYS438, and DYS448. The fifth dye, LIZ® is used to label the GeneScan-500 Size Standard. All loci are coamplified simultaneously in a single tube and analyzed in a single capillary injection. The Yfiler™ kit includes an extensive allelic ladder containing the most common 137 variants observed at each locus.

To test the sensitivity of the multiplex male DNA was serially diluted from 4 ng to 31 pg. Complete male profiles above a 50-rfu threshold were reproducibly obtained at 125 pg. Partial to complete profiles could also be obtained at 62.5 and 31 pg. Testing a panel of domestic and farm animals, bacterial species, and female DNA, the kit demonstrated primate and male specificity. No cross reactivity was seen with the various animal or bacterial species and no reproducible peaks above 50-rfu were detected in the presence of 500 ng of female DNA. Complete male profiles were reproducibly obtained at 1000:1 female to male mixtures and the minor contributor was reproducibly distinguishable in male-to-male mixture ratios of up to 10:1. Haplotype concordance was 100% when tested with the NIST standard SRM#2395 and 600 population samples. A database for this new 17 Y-STR multiplex has been created for determining haplotype frequency using population samples from North American, European and Asian population groups. The data are accessible via the internet for haplotype searches.

Results demonstrate that the AmpF/STR Yfiler™ PCR Amplification Kit is a sensitive, valid, and robust multiplex system for Y chromosome STR analysis. The system can be used in conjunction with Applied Biosystems Thermal Cyclers, and ABI PRISM Genetic Analyzers, and provides the forensic scientist with a complete set of tools for Y chromosome analysis.

Multiplex PCR, Y Chromosome, STR

 ** Presenting Author*