B138 Development and Characteristics of a Novel Y-STR Multiplex PCR Amplification System

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After attending this presentation, attendees will understand the characteristics of a Y-STR multiplex amplification system suitable for use in forensic analysis of samples containing mixtures of male: female DNA.

This talk will acquaint the forensic community with a novel Y -STR multiplexed kit that will provide reliable and robust Y haplotypes from forensic samples. This will be able to be used in conjunction with their existing instrumentation currently used for autosomal STR analysis.

Analysis of the Y chromosome is useful for tracing human evolution through male lineages and in a variety of paternity and forensic applications. In a forensic setting, Y chromosome tests provide the ability to separate and analyze the male DNA component from samples containing mixtures of female and male DNA. If autosomal short tandem repeat (STR) markers are used, preferential amplification of the major component of the mixture can mask the genetic profile of the minor contributor. A multiplex PCR amplification system containing Y– STR loci can enhance the detection of low levels of male DNA in these types of samples. Y - STR loci show moderate levels of polymorphism when compared to autosomal STR's currently used in forensic analysis due to the haploid nature of the Y chromosome. The addition of recently described Y - STR loci to the European minimal haplotype and Scientific Working Group on DNA Analysis Methods (SWGDAM) recommended loci increases the possibility of obtaining sufficiently discriminative haplotypes for use in forensic investigations.

A multiplex PCR amplification system in development at Applied Biosystems has been designed to include the complete European minimal haplotype and SWGDAM loci of DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS438, and DYS439, along with a number of additional loci chosen for their power of discrimination and allele size range. To ensure no overlap between allele ranges, loci are labeled with 6 - FAM™, VIC®, NED™ and PET® dyes. Thermal cycling conditions are optimized for the GeneAmp® PCR Systems, with subsequent amplified products being run on ABI PRISM® genetic analysis instruments in conjunction with G5 and G5v2 dye sets or modules (Applied Biosystems).

Male DNA samples and female DNA samples were quantified by using a real - time PCR quantification system, the Quantifilerä Y Human Male DNA Quantification Kit, or the Quantifiler™ Human DNA Quantification Kit respectively (both Applied Biosystems, currently under development at the time of writing this abstract). These reactions were amplified and analyzed on an ABI PRISM® 7000 Sequence Detection System (Applied Biosystems). From these data, the quantity of amplifyable DNA in each sample was calculated and this value used to prepare test samples. These test samples included serial dilutions ranging from 2ng to 0.0625ng, along with male:male mixtures in ratios of up to 10:1, and female:male mixtures in ratios of up to 200:1. Using these samples, the amplification conditions were optimized for signal strength, color balance and to minimize stutter and –A artifacts. Cross reactivity studies showed no consistent peaks for male animals and prokaryotes, although some reproducible peaks were seen with DNA samples from higher primates. Haplotypes produced from the male DNA samples were consistent across thermal cyclers and instruments used throughout the development of the kit.

This Y - STR PCR amplification system and the Quantifiler™ Y Human Male DNA Quantification Kit, used in conjunction with instrumentation from Applied Biosystems, are designed to produce reliable and accurate Y - haplotypes and provide the forensic scientist with a robust set of tools for Y - chromosome analysis.

DNA Testing, Y-STR, PCR Amplification

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