B16 Analysis of DNA Mixture Samples: Integration of the Quantifiler™ Real-Time PCR Kits and the AmpF/STR®Yfiler™ Kit

Chien-Wei Chang, PhD, Julio J. Mulero, PhD, Robert L. Green, BA, and Lori Hennessy, PhD, Applied Biosystems Inc., 850 Lincoln Centre Drive, Foster City, CA 94404*

After attending this presentation, attendees will that sensitive, reproducible and reliable methods are available to quantify and genotype mixture DNA samples containing relatively low proportions of male DNA. This presentation will impact the forensic community and/or humanity by demonstrating advances in mixture DNA sample analysis for quantitation and STR analysis.

This presentation will discuss the incorporation and utility of the Quantifiler™ Real-Time PCR Kits and the AmpFlSTR®Yfiler™ Kit into the analysis of mixture samples typically encountered in forensic casework.

Forensic samples from sexual assault cases frequently contain a mixture of genotypes in which the DNA of the male contributor, present only in a very small amount, needs to be discerned from a high background of female DNA. Physical separation of the two genotypes using the differential extraction method may sometimes be possible. However, the technique is laborious, time-consuming and is limited to certain sample sources. With current autosomal short tandem repeat (STR) typing systems, male DNA in mixture samples can be correctly interpreted only when it comprises 5% or more of the DNA in the mixture. Although the degree of male contribution can be estimated by the analysis of the amelogenin locus or by estimating the quantity of male DNA from the number of spermatozoa detected, genotyping results of mixture samples may require a significant degree of interpretive skill from the analyst. With the aim to improve the ability to obtain useful genotypic information from mixture samples containing relatively low proportions of male DNA, recent advances in DNA analysis of forensic samples have focused on developing sensitive, reproducible and reliable methods to quantify and genotype mixture DNA samples.

The AmpF/STR®Yfiler™ PCR Amplification kit is a STR multiplex assay that co-amplifies 17 Y chromosome STR loci in a single PCR reaction. The authors have evaluated the efficacy of the AmpF/STR®Yfiler™ typing system combined with the Quantifiler RealTime PCR Kits to determine the range of ratios of mixture DNA within which positive detection and accurate genotyping of a minor male contributor DNA could be obtained. First, mixtures of purified male and female DNAs were prepared and analyzed with the Quantifiler™ Y and Quantifiler™ Human assays to determine the relative contributions of male and total human genomic DNA in samples, respectively. Mixtures with ratios ranging from 1:1 to 1:8000 (male:female) were then processed with the AmpF/STR®Yfiler™ kit. The characteristics of Yfiler profiles produced from DNA sample mixtures were studied on the basis of specificity, signal quality and intensity. These experiments indicated that full profiles of minor male contributors were obtained from male-female mixture samples with ratios up to 1:4000. In contrast, the male components were not detectable by standard autosomal STR typing methods. The resulting signal intensity of the Yfiler assays directly correlated with the concentrations of male DNA determined by the Quantifiler™ Y assays. In addition, it was shown that the AmpF/STR®Yfiler™ typing system allows alleles from multiple male individuals in mixtures sample to be easily determined in the presence of excess female DNA. Results clearly illustrate that the use of the Quantifiler™ Y Human Male DNA Quantification Kit in conjunction with the AmpF/STR®Yfiler™ Kit allows the detection, quantification and genotype determination of the male DNA component specifically without interference from female DNA.

Mixture DNA Sample, STR, Y Chromosome

 ** Presenting Author*