VALIDATION OF THE LINEAR ARRAY™ MITOCHONDRIAL DNA HVI/VHII REGION - SEQUENCE TYPING KIT

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Over the past several years, we have developed and optimized a rapid method for the analysis of sequence variation in the HVI and HVII regions of the human mitochondrial genome utilizing the established technologies of PCR amplification and immobilized probe hybridization. The final version of this assay consists of two primer pairs for co-amplification of HVI and HVII regions and 33 probes immobilized in 31 lines for detection of sequence variation within 10 segments of HVI and HVII. Using this rapid informative assay, samples can be quickly screened to identify the most probative samples. The remaining PCR product generated for the linear array assay can be used for sequence analysis if necessary. Additionally, the LINEAR ARRAY™ Mitochondrial DNA HVI/HVII Region-Sequence Typing Kit consumes 50-75% less sample extract than sequence analysis because the HVI and HVII regions are amplified simultaneously rather than in two or four separate reactions.

Ten laboratories have tested the assay as part of a beta study and the results will be summarized here. A subset of these laboratories have begun collaborative projects and the progress will be reported here. In addition, validation studies, including sensitivity, mixture, population and species specificity studies, will be summarized. Sensitivity studies show successful amplification of <1pg of DNA using the duplex PCR primer and reaction mix included in the LINEAR ARRAY™ Mitochondrial DNA HVI/HVII Region-Sequence Typing Kit. Also, linear array typing was found to be a more sensitive method of detecting sequence polymorphisms within mixtures than sequencing as well as more accurate at reflecting the true ratio of two sequences. Consequently, the linear array not only provides for a rapid screening tool, but also aids in overcoming interpretation challenges posed by mixtures of mtDNA sequences, attributable to either heteroplasmy or a secondary source. To determine the discrimination power of the final panel of HVI/HVII probes, several population studies were conducted, including typing and sequencing 689 samples from four different populations, and the results will be reported. We concluded from these studies that the linear array assay is a simple, rapid screening tool for casework analysis because it is robust and provides a high degree of discrimination in a relatively short period of time.