APPLICATIONS OF THE LINEAR ARRAY™ MITOCHONDRIAL DNA HVI/VHII REGION – SEQUENCE TYPING KIT

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Analysis of sequence variation in the two hypervariable segments (HVI and HVII) of the mitochondrial genome has been applied to the study of human populations and used to resolve questions of human identification. The most widely used method of analysis for forensic and human identification applications involves PCR amplification of mitochondrial DNA (mtDNA) extracted from biological samples followed by direct sequence analysis. Over the past several years, we have been developing a rapid method of analysis of sequence variation in HVI and HVII utilizing the established technologies of PCR amplification and immobilized probe hybridization. The current version of the HVI/HVII linear array assay consists of two primer pairs for co-amplification of HVI and HVII PCR products and 33 probes immobilized in 31 lines for detection of sequence variation at 18 positions spanning both hypervariable regions. The linear array assay can be performed in approximately 6 hours, including amplification time, on up to 48 samples. Positive signals are detected as blue lines following a color development reaction and interpretation of the probe reactivity patterns (mitotypes) can be done either visually or by scanning. In addition to being rapid and informative, the linear array assay consumes only one-half to one-quarter the amount of extracted sample as sequence analysis because the HVI and HVII regions are amplified simultaneously rather than in two or four separate reactions. Also the PCR products amplified for the linear array assay can be used for sequence analysis.

To determine the value of the expanded HVI/HVII linear array assay as a screening method, we typed 689 samples from four different populations as well as 105 Croatians and 200 US Georgians. We also sequenced the HVI and HVII regions of many of the samples. We found that this panel of SSO probes captures a significant level of genetic diversity within the control region in all of these populations. In addition to the samples from the Georgia population database, samples from multiple cases submitted to the GBI also were typed using the SSO linear array and sequence analysis. Cases in which the suspect had been excluded by STR typing were chosen for this study to allow us to assess the value of the mtDNA linear array assay as a screening tool for the exclusion of individuals. In all but one case, linear array typing was sufficient to exclude the suspects who had been excluded by STR analysis. In this particular case, the suspect excluded by STR analysis had the same SSO mitotype as well as the same HVI and HVII sequence as the donor of the semen stain. Prior to mtDNA typing, it was though that the suspect was a brother of the donor of the semen stain based on STR analysis. The mtDNA analysis was consistent with this conclusion. Several additional cases will be summarized, along with the mitotype frequencies of the individuals in these cases obtained from the Georgia database and the US database.

An initial version of this assay analyzing only the HVII region also has been tested on over 200 samples taken from more than 20 cases in Sweden. Using the HVII region probes alone, over 70% of these samples could be excluded, therefore greatly reducing the number of samples that needed to be sequenced. Sequence analysis of HVI and HVII of these samples resulted in the exclusion of only seven additional samples as originating from the suspects and showed that further exclusions could have been made using our current HVI/HVII linear array. More recently, the current HVI/HVII linear array has been used in cases and the results will be summarized. Clearly, as a result of using the linear array assay to screen samples, the sequencing effort in this Swedish laboratory could be directed toward the most probative samples, resulting in a significant decrease in casework turnaround time. 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.