B37 Detection of Sequence Variation in the HVI and HVII Regions of the Human Mitochondrial Genome in 889 Individuals and Casework Samples Using Immobilized Sequence-Specific Oligonucleotide Linear Array Assay

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The attendee will gain knowledge of the frequencies of distinct SSO mitotypes and the level of discrimination of the current HVI/HVII SSO linear array as well as gain appreciation of the usefulness of this technology as a screening tool for casework samples.

The immobilized SSO probe linear array technology has proven to be a rapid, sensitive method for detecting sequence polymorphism within the mtDNA genome and is a useful screening tool for various biological samples submitted as evidence material. Detection of sequence variation in the HVII region of the human mitochondrial genome in 689 individuals from four population groups using a panel of 17 sequence-specific oligonucleotide probes immobilized on a nylon membrane has been previously reported by Reynolds et al. (2000) in the *J Forensic Science*. Since that report, the linear array assay has been expanded to include additional probes in 4 regions of the HVI region and for positions 16093 and 189. Also, several probes from the original HVII assay have been redesigned and the HVII E region ("C-stretch") probe has been removed. Currently, both the HVI and HVII regions are co-amplified simultaneously rather than separately. In order to evaluate the performance of the new array and to obtain population frequencies for a database, 689 unrelated individuals were typed (200 U.S. Caucasians, 200 African Americans, 200 U.S. Hispanics, and 89 Japanese) with the current panel of 31 SSO probes spanning the HVI and HVII regions. As with the HVII linear array, one of four categories of probe signal within each probe binding region was observed for the HVI/HVII linear array: (1) a single probe is positive, (2) a single probe signal is visible but its intensity is weaker than a positive signal in other regions, (3) no probe signals are visible, or (4) two probe signals are visible. To characterize these categories, DNA sequence analysis was performed when blanks or "0" signals and weak signals were observed. In addition, samples in which mixtures of two sequences were observed by SSO typing were sequenced and the second sequence was either attributed to heteroplasmy or contamination. Also, the genetic diversity value for each population was calculated from the frequency data and the frequencies of distinct mitotypes in each group were determined and compared to the published values and frequencies obtained with the original HVII linear array.

The current HVI/HVII linear array was also used to generate a regional database for Georgia at the Georgia Bureau of Investigation (GBI). For this database, blood samples randomly collected from 100 Caucasians and 100 African Americans from individuals who resided in Georgia, previously used to generate their regional STR and AmpliType PM databases, were typed with the SSO linear array. The HVI and HVII regions of each of the 200 samples were sequenced as well. The genetic diversity values for each population was calculated from the frequency data for both typing methods and are compared to each other as well as to the general U.S. population database. Also estimated was the frequency of heteroplasmy detected by both typing methods. 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 assess the value of the 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 thought 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 U.S. database.

Since the current method for reporting mtDNA frequencies is the counting method, a large database is necessary to increase discrimi- nation. Therefore, with this study as well as through collaborations with several crime laboratories, a substantial increase to the SSO population database is hoped. It is concluded that, based on the population data collected from these 889 unrelated individuals and the casework samples, the HVI/HVII immobilized SSO probe linear array typing system provides valuable, discriminating information and is an effective screening method prior to sequencing.

mtDNA, Population Database, Linear Arrays

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