B183 Increasing Mitochondrial DNA Discrimination Using a Panel of 84 Immobilized SSO Probes Targeting Informative Sites Within the Mitochondrial Genome

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After attending this presentation, attendees will be introduced to a newly developed, highly sensitive multiplex PCR and LINEAR ARRAY assay used to detect sequence variation in the mitochondrial genome with a panel of 84 probes designed to increase the power of discrimination compared to the LINEAR ARRAY HVI/HVII assay as well as HVI/HVII sequencing.

This presentation will impact the forensic community and/or humanity by demonstrating how this newly developed assay will allow the practioner an alternate method of typing mtDNA with increased discrimination power compared to current mtDNA HVI/HVII sequencing and typing methods.

Mitochondrial DNA, particularly the hypervariable regions I and II (HVI/II), has proven to be a useful target for the forensic genetic analysis of limited and/or highly degraded samples. However, there are some inherent limitations to targeting only the HVI and HVII regions independent of the method of analysis; the power of discrimination is limited for all population groups as a result of a few relatively common HVI/HVII sequences. Most notably seven percent of Caucasians share the same common HVI/II sequence (differing from the Anderson reference sequence at 263G) and 13 additional sequences are shared among >0.5% of the population. Also, several common sequences can be found among Hispanic individuals. Therefore to overcome the limited power of discrimination, additional sequence polymorphisms in the variable and coding regions need to be targeted to increase the power of discrimination of mtDNA analysis.

To achieve this goal, additional polymorphic sites have been identified within the coding region as well as the HVII and Variable Regions (VR) of the mitochondrial genome that help further distinguish common HVI/HVII types. Primers and probes targeting these sites were designed to work under the existing amplification and typing parameters of the LINEAR ARRAY HVI/HVII mtDNA Sequence-Typing assay. Up to 53 probes will be added to the existing 31 HVI/HVII probe panel for a total of 84 probes. PCR products generated with a single multiplex PCR targeting the VR and coding region sites can be combined with PCR products generated with the HVI/HVII duplex PCR and can then be typed simultaneously with a panel of 84 probes immobilized on a nylon membrane. Once the samples are typed, the LINEAR ARRAY probe panels can then be scanned and typed using the newly developed StripScan Mitotyper 1.0 mtDNA typing software.

Additionally, results from a small population study will be reported here to illustrate the improved power of discrimination of the 84 probe panel assay compared to HVI/HVII mtDNA typing and sequencing. Preliminary results of a small Caucasian population study using the 56 probe panel indicate that the genetic diversity (h value) was increased from 0.973 with the HVI/HVII probe panel to 0.992 with the 56 probe panel, which is nearly as informative as the h value obtained with HVI/HVII sequencing (0.994). With the addition of 28 probes, the discrimination power of the 84 probe panel will be greater than that of HVI/HVII sequencing.

Mitochondrial DNA, Linear Array, Immobilized SSO Probes

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