IMPROVED DISCRIMINATION OF MITOCHONDRIAL DNA ANALYSIS TARGETING 61 POLYMORPHIC SITES

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The hypervariable regions I/II (HVI/II) of the mitochondrial genome are routinely targeted by forensic DNA laboratories for the analysis of limited and/or degraded samples. However, there are limitations to targeting only the hypervariable regions I/II independent of the method of analysis. The power of discrimination is limited for all population groups as a result of a few common HVI/II sequences. Most notably, 7% of Caucasians share the same common HVI/II sequence. To increase the informativeness of mtDNA analysis, additional sequence polymorphisms outside the HVI/II regions need to be targeted.

To address this issue, we have developed a highly sensitive, easy to use 5-plex and 10-plex PCR and linear array assay for simultaneous analysis of polymorphic regions in the non-coding and coding regions. This assay targets 61 polymorphic sites distributed throughout the mitochondrial genome using 15 primer pairs and a total of 105 sequence-specific oligonucleotide probes immobilized in lines on a nylon membrane. The 5-plex PCR is used to amplify 5 regions ranging in size from 314-444 bp; the 10-plex PCR is used to amplify 10 regions ranging in size from 102- 183 bp. The 5-plex probe panel consists of 59 probes and targets variation at 14 HVI sites, 11 HVII sites, 8 coding region (CR) sites, and 2 variable region I (VRI) sites. The 10-plex probe panel consists of 46 probes and targets 17 CR sites, 4 VRI sites and 5 VRII sites.

A population study (674) was conducted in order to determine the power of discrimination for the new expanded HV+ array. The discrimination power was greatly increased for all populations and a significant increase in the power of discrimination was observed for both the US Caucasian (0.9946 from 0.9768) and the US Hispanic (0.9893 from 0.9449).

In order to determine the assay limitations, a developmental validation study was conducted. A sensitivity study was conducted and typeable results were observed with ~5 pg of DNA input for the 5-plex at 34 cycles and ~1 pg input for the 10-plex. At 38 cycles, ~0.5pg of DNA input yielded typeable results for both the 5-plex and 10-plex assays. A mixture study was conducted and it was shown that a minor component present at ~10% is reliably detected with the 5-plex assay and ~5% with the 10-plex assay.

Lastly, a procedure was validated for automating the typing assay and scanning and interpretation software were modified for use with the 5-plex and 10-plex assays. Up to 48 samples can be typed manually or automated in less than 2 hours and the data can now be quickly analyzed using the Strip Scan Mitotyper software.