

**A HIGHLY INFORMATIVE MULTIPLEX PCR AND LINEAR ARRAY PROBE PANEL
TARGETING 61 POLYMORPHIC SITES DISTRIBUTED THROUGHOUT THE
MITOCHONDRIAL GENOME**

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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 or degraded samples. While these regions are highly polymorphic, some HVI/HVII sequences are quite common, specifically in the Caucasian population. To increase the informativeness of mtDNA analysis, additional sequence polymorphisms outside the HVI/II regions need to be targeted in addition to the HVI/HVII regions.

We have developed a highly sensitive, easy to use 5-plex and 10-plex PCR and linear array assay for analysis of polymorphic regions within the mitochondrial genome. This assay targets 61 polymorphic sites distributed throughout the mitochondrial genome using 15 primer pairs and a total of 105 sequence specific oligonucleotide probes. 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.

We present here population to illustrate the increased discrimination power of this assay. We have greatly increased the discrimination power beyond that of the HVI/II linear array assay (18 sites) for all populations by targeting an additional 43 polymorphic sites. We also present data here which shows that the new expanded array is more informative than sequencing the HVI/II regions for individuals in the US Caucasian population. This newly expanded 5-plex and 10-plex PCR and linear array assay allows for both high throughput analysis and increased discrimination and can be easily adopted by any forensic DNA laboratory.