

VALIDATION OF THE LINEAR ARRAY™ MTDNA HVI/HVII REGION-SEQUENCE TYPING KIT FOR USE IN A MISSING PERSONS DNA PROGRAM

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The LINEAR ARRAY™ mtDNA HVI/HVII Region-Sequence Typing Kit from Roche Applied Sciences (Indianapolis, IN) is a simple, fast, and highly sensitive tool for determining a mitochondrial genotype. The kit can function as an effective screening tool, and as a sensitive method for confirmation of heteroplasmy. This system utilizes a duplex PCR amplification of mtDNA hypervariable regions I and II and hybridization of PCR products to sequence specific-oligonucleotide probes immobilized in lines on a nylon membrane. The LINEAR ARRAYS target 19 polymorphic sites within 10 regions of the human mitochondrial genome. These polymorphic sites are among the most commonly observed variable sites in the mtDNA control region. Heteroplasmy is observed frequently at two sites and these are referred to as 'hotspots'. We performed a validation study with the kit to determine its effectiveness for use as a screening tool in a missing persons DNA program.

Reproducibility: DNA extracted from bloodstains, hairs, buccal swabs, nail clippings, and non-probative samples showed reproducible mtDNA types when tested multiple times. Identical typing results were obtained from thirteen hair samples from one individual with the exception of one hair, which displayed apparent heteroplasmy. The hairs were DNA sequenced, which confirmed a position change for the one hair that displayed heteroplasmy with the LINEAR ARRAY™.

Sensitivity: Optimal intensity of probe signals on the linear arrays was observed when roughly 1.0 pg or more (total genomic DNA) was amplified for 34 cycles. Typing of less than 0.5 pg of DNA sample required 38 cycles of amplification to obtain enough amplicon for optimal signal with the array. Stochastic effects were observed for some samples that were amplified for 38 cycles with 0.05 pg of template DNA.

Degradation: A time course digestion of template DNA with DnaseI enzyme resulted in DNA fragments of various average lengths. Typing results showed no loss of signal for DNA fragments of average length of 500 base pairs or more at amplification of 34 cycles. However DNA fragments of 500 base pairs or less required amplification for 38 cycles for optimal typing results.

Mixtures: Two DNA samples that differ at six positions were mixed in defined ratios and typed. The results indicated that the minor component was detectable down to a 10:1 ratio.

These and other results suggest that the LINEAR ARRAY™ mtDNA HVI/HVII Region-Sequence Typing Kit is a useful and reliable tool for human mtDNA genotyping.