

EVALUATION OF THE LINEAR ARRAY MTDNA HVI/HVII REGION-SEQUENCING TYPING KIT ON AFRICAN AMERICANS FROM THE SWGDAM MTDNA POPULATION DATABASE

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Analysis of the first and second hypervariable segments (HVI and HVII) of the mitochondrial DNA (mtDNA) control region is a useful tool in the forensic investigation of biological samples that are highly degraded or have limited quantities of nuclear DNA. Currently, forensic mtDNA analysis is conducted by the sequencing of approximately 610 nucleotide base pairs (HVI: nucleotide positions 16024-16365, and HVII: nucleotide positions 73-340). Although nucleotide sequencing affords a complete complement of information, it is labor intensive compared to other typing techniques. The LINEAR ARRAY mtDNA HVI/HVII Region-Sequencing Typing Kit can be used to screen casework samples, potentially increasing the throughput and casework turn-around time.

The LINEAR ARRAY mtDNA HVI/HVII region-sequence typing kit, developed by Roche Molecular Systems, targets 19 polymorphisms across HVI and HVII via 33 sequence-specific oligonucleotide probes immobilized on a nylon membrane in 31 lines. Probes complementary to the most informative and highly polymorphic regions of mtDNA are covalently linked to a nylon membrane in bands. HVI and HVII are simultaneously amplified with biotinylated primers, resulting in two biotinylated PCR products, which are then hybridized to the membrane. Blue precipitate forms on the membrane where complementary probe-mtDNA hybrids have formed following a reaction with streptavidin-Horseradish peroxidase (SA-HRP) and chromogen.

The purpose of this study was three-fold: 1.) To determine whether information obtained by nucleotide sequencing could be used to predict the mitotypes that would be obtained from LINEAR ARRAYS for a highly polymorphic population. We predicted the LINEAR ARRAY mtDNA type and haplogroup of 100 African American samples based on previously sequenced samples in the Scientific Working Group on DNA Analysis Methods (SWGDM) mtDNA database, 2.) To compare the discrimination power of the sequencing method versus the LINEAR ARRAY method, and 3.) To use mtDNA population databases developed from the SWGDAM mtDNA population database for each method to compare the number of occurrences obtained from LINEAR ARRAY versus nucleotide sequencing techniques.

The utility of this assay is best illustrated in cases of mass disasters where it can be used for the rapid screening of large numbers of samples. This simple technique can save time and sample material as full sequencing can then be conducted on the same amplification product as that used for hybridization.