B184 Forensic Application of the Affymetrix Human Mitochondrial Resequencing Array

Peter M. Vallone, PhD, and John J. Jakupciak, PhD, National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899; and Michael D. Coble, PhD, Armed Forces DNA Identification Laboratory, 1413 Research Boulevard, Building 101, Rockville, MD 20850*

In the field of forensic DNA testing, coding region polymorphisms in the mitochondrial genome can be useful for resolving individuals who have the identical HV1 and HV2 control region sequence. Sequencing regions of the mitochondrial genome is performed when insufficient genomic DNA is present for traditional autosomal short tandem repeat (STR) testing. Various methods and strategies have been established to interrogate coding region polymorphisms. These range from SNP assays probing sites most likely to differentiate individuals based on their HVI/HVII sequence to the use of mass spectrometry to pyrosequencing. The goal of this presentation is to evaluate the potential of the Affymetrix GeneChip® Mitochondrial Resequencing Array (ver 2.0) for forensic applications.

This presentation will impact the forensic community and/or humanity by providing the forensic community with an evaluation of a array-based method for full genome mitochondrial sequencing. At this time the mitochondrial resequencing array method requires more input DNA in contrast to traditional sequencing or even STR analysis. However, the array may find utility in full genome sequencing of family reference samples.

The GeneChip® Mitochondrial Resequencing Array is a means to perform full genome sequencing on an array-based platform. The amount of DNA needed for the resequencing array is much greater than that required for autosomal DNA typing (1 ng versus 10-30 ng). Because of this relatively high sample requirement the array may have limitations for running a limited quantity of casework sample. However the platform should have utility in running family reference samples for the elucidation of SNPs that will help resolve individuals. These array-determined polymorphisms found in reference sample can then be probed in the limited casework sample.

Materials and Methods: A set of 10 U.S. Caucasian samples found to contain the same control region sequence by traditional fluorescent sequencing were run on the GeneChip® platform. The reproducibility of GeneChip® experiments was evaluated by running samples in triplicate for two of the samples. A sensitivity study was also conducted in which a dilution series of 10 ng down to 0.3 ng of template DNA (nuclear) was amplified for the array experiments. Two challenging samples were also examined to test the array's ability to successfully call a relatively large number of sequence differences (46 and 63 respectively) compared to the revised Cambridge Reference Sequence. All results were compared to traditional dideoxy fluorescent full genome sequencing experiments.

Summary of Results: All 10 U.S. Caucasian samples were fully resolved after comparing coding region sequence data from the GeneChip®. A typical array experiment resulted in approximately 95% sequence coverage (the remainder being Ns or no calls). Comparisons between the GeneChip® and traditional sequencing indicated the array platform had difficulty calling insertions and deletions as well as some closely spaced polymorphisms.

Conclusions: At this time the mitochondrial resequencing array method requires more input DNA in contrast to traditional sequencing or even STR analysis. However, the array may find utility in full genome sequencing of family reference samples. Unique polymorphisms elucidated by the array can then be sequenced by traditional sequencing methods or typed using an appropriate SNP assay.

Mitochondrial DNA, Sequencing, Microarray

 ** Presenting Author*