A133 Evaluation of the SNPlex Genotyping System for Screening Ancestry and Phenotype Informative SNPs

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The goal of this presentation is to introduce the attendees to the SNPlex™ methodology and its potential application to the screening of a high number of Ancestry Informative and Phenotype Informative Single Nucleotide Polymorphisms (AISNPs and PISNPs respectively).

This presentation will impact the forensic community by demonstrating the application of SNPs in criminal investigations. The selection of SNPs that can provide ancestry and phenotype information, combined with a high throughput cost effective method to type them, could benefit the forensic community in cases where a profile, obtained from crime scene samples, doesn't match an existing profile from a database or an identified suspect.

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Current forensic DNA testing for human identification (HID) purposes is based on the ability to generate a DNA profile from biological samples using STR markers. While STRs allow a determination of whether a sample matches an existing profile from a database or an identified suspect, the method is of limited use in solving a crime when no matches are found and no likely suspects have been identified. Further DNA analyses, targeted at inferring the phenotype and the ancestral origin of the donor, can provide information useful to the investigation by improving the ability to identify potential suspects. It is important to understand that such an assay cannot be thought of as something that will directly identify a single suspected individual but is intended as a tool to help prioritize suspect processing, corroborate witness testimony, and help determine the relevance of a piece of evidence to a crime.

The SNPlex™ Genotyping Systems (Applied Biosystems) allows the simultaneous typing of up to 48 SNPs in a single reaction. It uses an oligonucleotide ligation assay (OLA) followed by PCR, then hybridization of universal reporter probes to amplicons, and finally detection by capillary electrophoresis. The main advantages of this methodology are in the assay design, facilitated by the online tools provided by the manufacturer, and in its suitability for automation.

A review of the relevant literature on AISNPs and PISNPs was the basis for the selection of an initial battery of 60 autosomal and X-linked SNPs likely to provide information on ancestry and phenotype. For example, the Duffy (*DARC*) blood group identifies phenotypes associated with two proteins that appear on the outside of red-blood cells as a receptor; these play an important role in susceptibility to malaria infection. The Fy(a-b-) phenotype (rs2814778) represents an adaptation to living in malaria-endemic regions where mutations in the genes that produce these proteins result in this receptor not being expressed. This is a predominant feature in the African populations especially those from West Africa. Another example is the SLC24A5, a putative cation exchanger, which has been shown to be strongly involved in skin pigmentation. An A to G substitution at codon 111, which determines an Alanine to Threonine change, is a critical polymorphism within the sequence (rs1426654). The allele frequency for the Thr^{111} variant ranges from 98.7 to 100% among several European-American population samples, whereas the ancestral Ala^{111} allele has a frequency of 93 to 100% in African, Indigenous American, and East Asian population samples. Using the Reference Cluster ID (rs#) number all 60 SNPs were submitted to the manufacturer for assay design. Two assays were generated: one including 33 SNPs and another including 25 SNPs. Two SNPs failed the design process and were not included in either assay.

To date a total of 315 anonymous DNA samples (with self-defined ancestry), extracted from either whole blood or buccal swabs, were processed with the two SNPlex™ assays and analyzed on a 3130 Genetic Analyzer (Applied Biosystems): 80 Caucasian, 81 African American, 46 Asian, 84 Hispanic, and 24 Native American. Data were then imported into GeneMapperÒ Software V 4.0 (Applied Biosystems) and analyzed with macros specifically tailored to the SNPlex™ methodology.

In each plate 4% to 30% of the SNPs failed to meet GeneMapperÒ's default quality standard values with an average of 16.9% per plate. As a consequence the genotype at these SNPs was not called by the software for all samples on that plate. Clustering of the SNPs passed by GeneMapperÒ was consistent with the reference data. At this time modifications to the protocol are being tested to increase the number of

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SNPs successfully typed and a valid statistical approach to analyze the generated data is being investigated.

The SNPlex™ Genotyping Systems is not an analytical tool that can be used directly on forensic samples but rather is potentially a valuable tool for high throughput SNP screening of samples to generate reference data, although further optimization is necessary. Once a panel of the most informative AISNPs and PISNPs is identified, user friendly and sensitive assays can be developed for use in the routine crime laboratory setting.

SNPlex™, Ancestry Informative SNPs, Phenotype Informative SNPs

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