B111 SNP Analysis of the Y Chromosome Using SNaPshot™

Kerri Dugan, PhD, and Elizabeth Olivastro, PhD, Counterterrorism and Forensic Science Research Unit, Federal Bureau of Investigation, FBI Academy, Quantico, VA; and Bruce Budowle, PhD, Forensic Analysis Branch, Federal Bureau of Investigation, Washington, DC*

Attendees of this presentation will learn about design and development of an assay for Y SNP analysis.

Analysis of single nucleotide polymorphism (SNP) markers is a valuable tool that offers a mechanism to augment conventional forensic DNA analyses, such as tandem repeat and mitochondrial DNA typing. Moreover, SNP analysis may be useful for evaluating DNA from substantially degraded samples. In addition, SNP analysis is amenable to automation and could enable higher sample throughput. It is predicted that approximately 10,000,000 polymorphisms are dispersed throughout the genome on the nuclear chromosomes and mitochondrial DNA molecule. This presentation will focus on SNPs that are located on the Y chromosome. Since the Y chromosome is uniparentally inherited and has a large non-recombining region, Y chromosome analysis could be valuable for forensic analysis in some cases of paternity, missing persons, mass disasters, and violent crimes.

Several platforms are available to perform SNP analysis, including a variety of single base extension reactions, microarray analysis and pyrosequencing. The focus of this presentation will be analysis of Y SNPs using the SNaPshot system. After PCR, single base extension of primers is performed using dideoxynucleotides that are differentially labeled with fluorescent dyes. The single base extended primers are resolved using capillary electrophoresis and detected using laser induced fluorescence. The SNaPshot kit has been designed to be analyzed using ABI Prism 310 or 3100 instrumentation already in use by a large portion of the forensic community. The SNaPshot system can be a rapid, multiplex, high-throughput platform for SNP analysis but requires careful planning for SNP selection and assay design at the onset.

Before beginning SNP analysis, it is important to identify markers that will be useful in forensic analyses. More than 250 SNP sites on the Y chromosome have been discovered. Because of the population substructuring associated with genetic markers on the non-recombinant portion of the Y chromosome, the Y SNP markers can be evaluated using phylogenetic analysis. The phylogenetic tree has been examined for SNP sites that potentially could be useful in forensic identity analyses. The approach that has been used in this study is to 1) identify the Y haplogroups that predominantly comprise relevant, major U.S. population groups and 2) select markers that facilitate differentiation between individuals within haplogroups. The markers lie within haplogroup defining branches of the tree rather than at the base branch points. A preliminary set of 20 Y SNP markers has been selected to provide discrimination of individuals within each forensically relevant haplogroup.

Multiplex SNP assays are needed in order to obtain the most information from limited amounts of DNA in as few assays as possible. Since the degree of multiplexing directly impacts the ability to generate a sensitive, high-throughput system, primer-target and primer-primer interactions must be considered in primer design. Initially, amplification primers were designed to have similar thermal melting temperatures (Tm). The second step in the design of the amplification primers included evaluations of potential primer-primer interactions and possible interactions of the primers with non-target areas of the genome. Primers that had the potential to interact significantly with other primers or with other non-target chromosomes were modified or have not been included in further studies. In addition to Tm compatibility, G/C content was a critical factor in designing the primer extension primers. For research purposes, both forward and reverse primer extension primers were constructed when molecularly feasible. This provides a redundant control at each site, allows the effect of G/C content to be tested, and provides data on which primer is more effective. For example, at a given SNP site, the forward and reverse primers may have substantially different G/C contents but, due to length differences in the primers, will have similar Tm. Analysis of these extension primers will enable a deeper understanding of effective primer design that can be applied to subsequent studies. The effect of primer length, Tm, and G/C content on the effectiveness of primer extension primers will be presented.

Studies involving singleplex SNP analysis also will be presented. Using DNA purified from cell lines as well as evidentiary-type sources, amplification and sequencing were used to determine the correct sequence at each SNP site. Data will be shown confirming that primer extension using the designed primers provides the correct typing of the SNP site. Critical positive and negative controls will be described. For example, DNA isolated from female sources was considered as a potential negative control.

Y chromosome analysis may become an importance source of information to complement STR analysis and mitochondrial DNA sequencing. Selection of Y-chromosome SNP sites, primer evaluation and SNaPshot primer extension results will be discussed as well as the applicability of this system for use in forensic analyses.

Y Chromosome, Single Nucleotide Polymorphism, Primer Extension

 ** Presenting Author*