B112 Y-SNP Analysis by Pyrosequencing

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The goal of this presentation is to inform the forensic community about Y-SNP analysis and the use of pyrosequencing.

Y chromosome specific typing systems are increasingly being employed in forensic science to augment or, in some cases, supplant the use of autosomal STR markers. For example, they may be the only means of obtaining the genetic profile of the male donor in mixed male/female stains in those instances where the female component is present in overwhelming quantities. Most Y chromosome typing systems currently use STR loci due to their good discrimination potential, ease of analysis and ability to incorporate into multiplex assays.

STR loci suffer from one serious drawback that may limit their longterm use. Future genetic typing systems are likely to require the ability to perform massively parallel, automated analysis and to be miniaturized for incorporation into point-of-use devices. STRs are extremely difficult to automate or to incorporate into micro-electrical mechanical systems (MEMS) devices or lab-on-a chip format. This is in contradistinction to single nucleotide polymorphism (SNP) loci, which due to their abundance in the genome and pivotal importance for gene discovery are currently the subject of a massive capital investment program to improve their analytical efficacy by automation and miniaturization.

The applicability of SNP systems in forensic analysis remains to be demonstrated. One particular disadvantage of autosomal STRs is the perceived difficulty in resolving and interpreting body fluid mixtures, which are often encountered in forensic casework. Since SNPs are biallelic in nature, an individual who is heterozygous at a number of loci may be difficult to distinguish from a mixture of DNA from 2 individuals. The situation with Y-SNPs is simplified due to hemizygousity (lack of heterozygotes) and these SNP loci are excellent candidates for exploring their potential use in forensic science, particularly from the technological standpoint. Studies are also needed to determine whether Y-SNP loci possess sufficient discriminatory ability for forensic utility. The non-independent segregation of Y-SNPs in which a haplotype of widely spaced but physically linked markers are transmitted unchanged from father to son, results in reduced genetic diversity.

Through a variety of public sources, including the SNP consortium, dbSNP (National Center for Biotechnology Information, NCBI) and the primary literature, a number of candidate Y-SNP loci have been identified for further evaluation as to their forensic suitability. Primer extension assays were developed for the candidate loci and used to confirm or, in some cases, determine the degree of polymorphism in African-American and Caucasian populations using DNA from 20 individuals from each racial/ethnic group. Suitable loci were then subjected to pyrosequencing analysis, which is a novel, semi-automated mini-sequencing method for SNP typing. Pyrosequencing employs an enzyme cascade to detect the release of pyrophosphate that occurs when the appropriate complementary dNTP is incorporated into the newly synthesized polynucleotide. Appropriate SNP pattern recognition software facilitates the detection and typing of the polymorphism.

This presentation will describe the forensically relevant Y-SNP loci identified so far, their individual degree of polymorphism and the overall haplotype diversity of various SNP loci combinations in different racial/ethnic groups. For example, as few as 5 of the characterized Y-SNPs are able to distinguish 6 different haplotypes in 32 AfricanAmerican and Caucasian individuals. Results indicate the potential use of Y-SNPs for the racial/ethnic differentiation of individuals and the implications thereof will be discussed. The potential general utility of pyrosequencing technology for SNP analysis in forensics will also be described.

Y-SNP, Pyrosequencing, Y-Chromosome Haplotype

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