B187 Application of an Optimized SNP Detection System for Human Identification: Comparison With STR Profiling Methods

Manohar R. Furtado, PhD, Rixun Fang, PhD, Fiona Hyland, PhD, and Cristin O'Shea, BS, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404*

After attending this presentation, attendees will learn about a novel method for multiplexed detection of SNPs that employs PCR followed by an oligonucleotide ligation reaction and separation by capillary electrophoresis. They will be presented with comparative data on a set of samples genotyped using STRs and SNPs.

This presentation will impact the forensic community and/or humanity by demonstrating the capability to detect 48 SNPs from < 1 ng of biological DNA and indicate that SNP and STR based genotyping methods can provide comparable discriminatory power.

The hypothesis was that SNP detection methods will be comparable to STR analysis, provided that a large enough set of SNPs are tested. Currently, multiplexed analysis of short tandem repeats (STRs) provides the best solution with high power of discrimination and rapid analysis speed. However, single nucleotide polymorphism (SNP) profiling is also very powerful for human ID testing purposes especially in instances where DNA is highly degraded, as with disaster victim identification (DVI), and in typing mitochondrial DNA. SNPs have also been used for paternity testing, genealogical studies and more recently in population based stratification of patient groups in clinical trials. A detection system for identification of known mutations and SNPs was optimized. The method involves a multiplexed PCR amplification of ~ 48 regions in the human genome, followed by detection of mutations or SNPs in these amplicons using an oligonucleotide ligation assay (OLA). Optimization of the multiplex PCR was achieved using primer titration and minimizing primer-dimer formation. The ligated products were hybridized to coded sequences with mobility modifiers and detected by capillary electrophoresis. Direct detection by CE following ligation is also possible. The SNPs used were based on a study by the SNPforID consortium, who selected a set of highly discriminative SNPs suitable for forensic analysis based on criteria described in Sanchez et al., (Electrophoresis 2006,27:1713). A gender specific deletion marker was designed.

To test the accuracy of the method and to compare relative utility of SNP analysis to STR analysis for human identification and paternity testing, a panel of 41 individuals from 3 different CEPH families spanning three generations was genotyped. The test samples were genotyped using 49 SNPs with the SNP detection system, and also with Identifiler™. To determine accuracy, the SNPs were also genotyped using the TaqMan® allelic determination method to yield 100% concordance. The results indicated that the overall informativeness is comparable, with about 45 SNPs being equivalent to Identifiler. The SNP panel was more informative for identity but less informative for paternity exclusion. In this data set, the SNP panel exhibited greater number of genotypic differences (mean ~19) compared to Identifiler (9) between closely related individuals. The SNP panel also exhibited a greater number of genotype differences (mean ~ 28) compared to Identifiler (mean ~ 14) between any two unrelated individuals. This trend was consistent in both full sibs and unrelated samples. And, while Identifiler™ has a higher probability of paternity exclusion, (about 10^{-7} for Identifiler and 10^{-5} for the SNPs when the mother's genotype is known); the SNP panel was better able to distinguish between close relatives for paternity identification. The SNP panel was also tested with degraded DNA and DNA from blood, semen, and saliva samples.

The results indicate that carefully selected SNPs can be as useful as STRs in human ID testing and related applications. The development of highly multiplexed SNP detection systems enabling lower cost, higher automation and higher throughput will result in increased use of SNP profiling in a number of applications.

SNPs, Genotyping, Human Identification

Copyright 2007 by the AAFS. Unless stated otherwise, noncommercial *photocopying* of editorial published in this periodical is permitted by AAFS. Permission to reprint, publish, or otherwise reproduce such material in any form other than photocopying must be obtained by AAFS. ** Presenting Author*