MEGAPLEX PCR AMPLIFICATION AND PROBE HYBRIDIZATION FOR SNP-BASED ASSAYS

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There has been some interest expressed in having a highly discriminating SNP-based assay for forensic applications. Because SNPs have only 2 alleles compared to 5 or more alleles in STR loci, many more SNPs (~50) are required to provide a significant level of discrimination between individuals. Therefore, a SNP-based assay should be capable of co-amplifying 50 SNPs in a single PCR amplification and produce accurate, quantifiable signals to be of most value to the forensics community.

We have been developing SNP-based assays for the cardiovascular and inflammatory diseases research communities. At present, we routinely co-amplify 50-55 SNPs in a single reaction. To our knowledge, no other laboratory has demonstrated the ability to co-amplify this number of SNPs consistently. Consequently, other SNP-based assays require at least two PCR amplifications to obtain a sufficient number of SNPs for human identification applications. As the number of required amplification reactions increases, so do the chances of sample mix-up and contamination. The cost and set-up time of the assay are also greatly increased when more PCR amplifications have to be performed per sample. Perhaps of greatest significance to the forensics community is the fact that more extracted DNA is consumed for each additional PCR amplification that must be run.

In our assay, SNP detection is performed using an array of SSO probes immobilized in a line format on a strip of nylon membrane. Each strip can accommodate 58 probes and, using new instruments, up to 48 strips can be processed at a time. In the line-blot format, signals can be quantitated and we demonstrate that the two signals resulting from heterozygous loci are consistently balanced. As with STR markers, signal quantitation can be used to interpret mixtures more easily. Although some other SNP detection formats have higher throughput, none of them appears consistently to yield balanced signals for alleles at all loci. While a positive/negative result is sufficient for diagnostic applications, balanced signals are critical for the successful application of SNP analysis to the field of human identification.

Some general features of our megaplex SNP-based assay that make it attractive for casework and analysis of remains are listed below.

- Smaller product sizes: some samples that cannot be analyzed by STR markers will be amenable to SNP analysis.
- Greater tolerance of DNA input levels: DNA quantitation is not essential for the SNP assay because acceptable results can be obtained from input amounts that span an order of magnitude rather than a 5-10 ng range.
- Ability to transfer to microarray format: when the microarray probe hybridization technology is ready for applications that require quantifiable and consistent signals, an optimized panel of SNPs and probes will be available.