SNP GENOTYPING OF FORENSIC SAMPLES USING MEGAPLEX PCR AMPLIFICATION AND LINEAR PROBE ARRAYS

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An alternative to STR analysis of forensic specimens is SNP (single nucleotide polymorphism) genotyping. 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, to be of greatest value to the forensics community, 50 SNPs should be co-amplified from a few nanograms of DNA and the genotyping results should be accurate and quantifiable. We have been developing SNP-based genotyping assays for the cardiovascular and inflammatory diseases research communities. At present, we routinely co-amplify 50-55 SNPs in a single reaction. Accurate results can be obtained from less than 5 ng and from as much as 200 ng DNA extracted from blood and cell lines. Following megaplex PCR amplification, SNP detection is performed using and 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. The hybridization instrument is being modified so that an image of the strips can be automatically generated and transferred for analysis without removing the strips from the trays.

To evaluate the performance of megaplex PCR and the SNP linear arrays on samples likely to be encountered by forensic laboratories, we amplified and genotyped DNA extracted from buccal swabs, sexual assault samples, tissues, bones, and hairs. In addition, samples from adjudicated cases previously types using STR markets were genotyped using our linear array assay. Two features of our megaplex SNP linear array technology make it particularly attractive for the analysis of casework samples and remains. First, amplification of smaller PCR products enables analysis of some samples that cannot be typed using STR markers. Second, greater tolerance of DNA input levels results in unambiguous genotyping results even when quantitation methods are inaccurate, whereas with STR markers, incorrect input amounts can lead to inconclusive results. Based on the results of these studies, we concluded that by choosing a different panel of SNPs and further optimizing the megaplex PCR conditions, this assay could be valuable for human identification applications.