Development of a Multiplex SNP Detection System, GenPlex™ HID, Human Identification Applications

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Multiplexed analysis of short tandem repeats (STRs) is well accepted genotyping methodology used in the human identification (HID) laboratories. However, highly degraded samples and complex paternity cases provide challenges to STR systems, and may benefit from the analysis of additional genetic markers. Single nucleotide polymorphisms (SNPs) can provide a highly effective, complementary system to existing STR methods. SNPs are the most abundant genetic variations in the human genome, which gives many candidates for selection of markers. Further, SNPs have low mutation rates, can be genotyped using very short DNA fragments (40 – 60 bp), and contain alleles at each locus that are the same length. Therefore, a reliable and easy-to-use SNP genotyping system would be an important tool for various challenging Human Identification situations. Major constraints in the development of a SNP genotyping system have been sensitivity and the development of an easy-to-use detection platform.

We have developed a high throughput system, GenPlex™ HID, for detection of SNPs, which offers high quality data, speed, flexibility, and compatibility with existing capillary electrophoresis platforms. This system involves a multiplexed PCR amplification of genomic target regions and detection of SNPs in these amplicons using an oligonucleotide ligation assay (OLA). Regions containing specific SNP loci are first amplified by multiplex PCR. SNPs in the amplified regions are then detected by OLA reaction using multiple sets of locus and allele specific ligation probes. The ligated products containing universal ZipCode™ oligonucleotide sequences are then hybridized to corresponding fluorescent ZipChute™ reagents, which contain sequences complementary to the ZipCode™ oligonucleotide sequences and unique mobility moieties. The ZipChute™ reagents are subsequently eluted, separated by capillary electrophoresis, and genotyped by association with target SNPs using the GeneMapper® Software.

The GenPlex™ HID system has been configured to genotype up to 48 autosomal SNPs, plus a gender-determining marker, in single reaction. The system can process more than 90 DNA samples per run, with time-to-result turn around time less than 5 hours. Optimization of the multiplex PCR, while on-going, has included titrating primer concentrations and minimizing primer-dimer formation *in silico*. To test the accuracy and reliability of the method, Sanger sequencing and or TaqMan® SNP genotyping was performed accordingly to check the SNP genotype concordance. The ability of the system to genotype the SNPs in degraded DNA samples was also evaluated. We have applied this system to different SNP genotyping applications, such as autosomal SNP genotyping for Human Identification, and Y chromosomal and mitochondrial coding SNPs for human lineage and migration studies. The result from the method development, on-going optimization studies, and evaluation studies will be discussed.