A49 Human Autosomal SNP Profiling Using Fully-Automated Electrospray Ionization Time of Flight Mass Spectrometry

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The goal of this presentation is to demonstrate the use of electrospray-ionization mass spectrometry (ESI-MS) system in the automated analysis of forensically-relevant human autosomal SNP markers.

This presentation will impact the forensic science community by using an automated assay with sensitivity, reliability, convenience, and ease of use/analysis suitable for forensics applications has been developed for the Ibis PLEX-IDTM and developmentally validated.

Single nucleotide polymorphisms (SNPs) represent a simple yet powerful tool for individual identification. Efforts by Pakstis and Kidd[1] to produce an ideal panel of genetically unlinked binary SNPs with high heterozygosity, low population bias, and uniform distribution over global populations have resulted in a 40-SNP panel analyzed across 40 global populations. A fully-automated PCR/electrospray ionization mass spectrometry (ESI-MS) assay capable of genotyping these 40 SNP markers has been developed for the Ibis PLEX-ID™ platform and has been developmentally validated.

The 40-SNP assay consists of a pre-fabricated kit containing all components necessary to analyze a sample except for template DNA. Eight five-plex PCR reactions per sample are arranged in columns of a 96-well assay plate such that up to 12 samples may be analyzed on a single assay plate. After adding DNA template to the appropriate wells of an assay plate and thermal cycling, all downstream analytical steps through data processing are fully automated. A novel ESI-MS deconvolution algorithm deconvolves multiplexed PCR product charge state distributions into individual masses while retaining relative output signal amplitude estimates in proportion to relative input DNA concentrations, allowing evaluation of inter- and intra-locus product balance to aid interpretation of potential mixtures/contaminated profiles. Forward and reverse strands of each PCR product are measured with high mass accuracy, such that base compositions (A, G, C and T nucleotide counts) can be directly calculated for each product, allowing accurate assignment of nucleotide identity at the SNP position. The average mass measurement deviation for 31,030 independent DNA strand assignments was 12.4 ± 11.4 parts per million (ppm), corresponding to ~0.2 Da for PCR products of the average size amplified in the assay.

The 40-SNP assay has been characterized for concordance to existing methodology, sensitivity, reproducibility, species specificity, and the ability to detect when genotyping results indicate a pure sample or a mixture/contaminated sample. Concordance has been demonstrated for all loci using individual TaqMan® assays for each locus for 20 samples and comparing results to those obtained with the Ibis SNP assay. All loci displayed 100% concordance to TaqMan® results. Dilution studies suggest that reliable genotyping results can be obtained at template input levels close to 125pg of DNA per reaction.

Species specificity analysis indicated no interference using a 10-fold excess of exogenous cat, dog, *E. coli*, *S. aureus*, *C. albicans* or *A. oryzae* genomic DNA. Non-primate DNA did not produce PCR products recognizable as SNP locus products, and did not interfere with correct typing results when in excess in the presence of human DNA. Bacterial DNA at a 100-fold excess over human DNA did not interfere with correct genotyping results. Primate DNA (African green monkey, squirrel monkey, rhesus macaque, and marmoset monkey) did produce products and interfered with genotyping results to different degrees.

Fully-automated data analysis produced 5998/6000 correct genotype assignments (99.97%) in reproducibility studies utilizing three samples each analyzed 50 consecutive times at a constant DNA template input. A convenient software interface has been developed for visual review of data analyses. The Ibis PLEX-ID™ ESI-MS platform is capable of typing the full spectrum of forensically-relevant markers, Y-STR, autosomal STR, mitochondrial DNA, and SNPs on a single instrument within the same automated run.

Reference:

[1.] Pakstis, *et. al. Hum. Genet.* 121: 305-317.

Autosomal SNPs, Single Nucleotide Polymorphisms, Mass Spectrometry

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