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Human autosomal SNP profiling using fully automated electrospray ionization time of flight mass spectrometry

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ABSTRACT

Single nucleotide polymorphisms (SNPs) represent a simple yet powerful tool for individual identification. Efforts by Pakstis and Kidd et al. 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 and a more-recent highly unlinked 45-SNP panel analyzed across 44 global populations. A fully automated PCR/Electrospray ionization mass spectrometry (ESI-MS) assay that genotypes the first 40-marker SNP panel has been developed for the Ibis PLEX-ID™ platform and developmentally validated. A 64-SNP assay that incorporates the union of the two SNP panels has been developed for the Ibis PLEX-ID™ and is undergoing validation. Concordance with standard TaqMan assays for a panel of samples has been demonstrated for all loci. The assay has been characterized for sensitivity, reproducibility, species specificity, and the ability to detect when genotyping results indicate a pure sample or a mixture/contaminated sample. Validation studies suggest sensitivity close to 100 pg DNA per reaction. A convenient software interface has been developed for visual review of automated data analyses. The Ibis PLEX-ID™ ESI-MS platform is capable of running Y-STR, autosomal STR, mitochondrial DNA, and SNP analyses on a single instrument within the same automated run.

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1. SNP genotyping assay

Single nucleotide polymorphisms (SNPs) offer a powerful tool for molecular human identification [1,2]. The stability of single nucleotide positions compared to STRs [3,4] makes SNPs attractive for relationship testing, where germline mutations must be considered routinely. Pakstis and Kidd et al. have identified panels of human autosomal SNPs with a uniform distribution of heterozygosity across global populations, making them ideal for forensic applications [5–7].

Ibis Biosciences has developed SNP genotyping assays for the PLEX-ID™ mass spectrometry-based platform according to the panels identified by Pakstis and Kidd, et al. [5–7]. The Ibis platform fully automates analysis of PCR-amplified products and processes up to 15 assay plates in a single run with multiple assays, including mtDNA profiling, SNPs and STRs [8–10]. PCR primers were grouped into eight 5-plex (40-marker assay) or 8-plex (64-marker assay) reactions per sample in pre-fabricated 96-well assay "kit" plates, such that 12 samples can be analyzed on each plate. After thermal cycling, assay plates were placed on an Ibis PLEX-ID™ or T5000™ instrument for automated electrospray ionization mass spectrometry (ESI-MS) analysis [8]. Mass spectra were automatically deconvolved using a proprietary algorithm designed for complex ESI-MS spectra of nucleic acids and SNP identities of PCR products were assigned automatically using integrated analysis software (Fig. 1).

2. Sensitivity

Five independent DNA templates were analyzed at 1000, 667, 444, 296, 198, 132, 88, 59, 39 and 26 pg/reaction in duplicate to assess assay sensitivity. Sensitivity was between 88 and 132 pg/reaction for the 40-marker assay, and 198 and 667 pg/reaction for the 64-marker assay.

3. Species specificity

The 40-SNP assay has been assessed for cross reactivity with a 10-fold excess of *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, *Aspergillus oryzae*, Canis familiaris, *Felis catus*, *Callithrix jacchus* (marmoset), *Saimiri sciureus* (squirrel monkey), Chlorocebus aethiops (African green monkey), and *Macaca mulatta* (rhesus macaque) DNAs. Cross reactivity was seen with primate DNA, but

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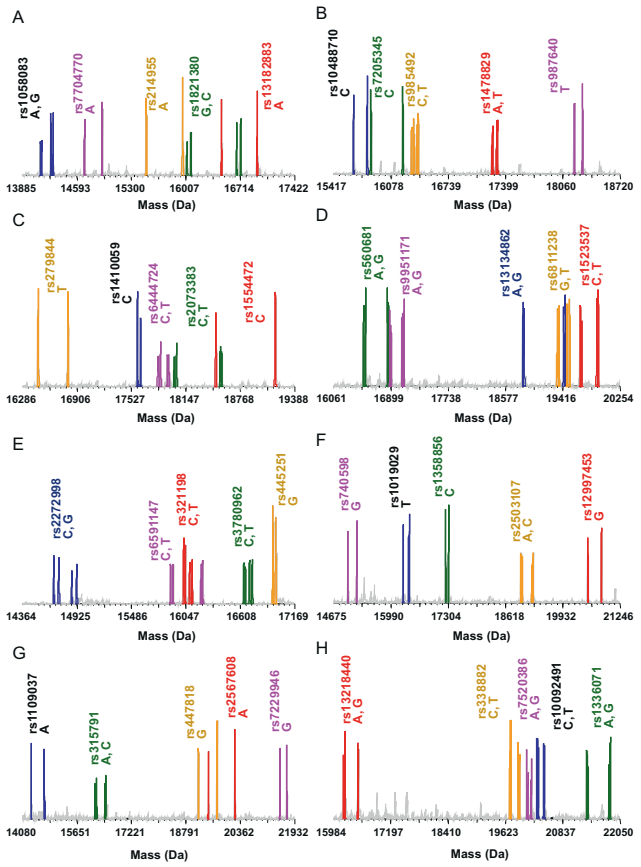

Fig. 1. Deconvolved mass spectra for eight 5-plex PCR reactions used to genotype 40 human autosomal SNPs. Each locus is labeled according to rs# and corresponding observed genotype for a representative DNA sample derived from human blood (1 ng per reaction).

only African green monkey and *rhesus macaque* directly interfered correct profile development at a 10-fold exogenous DNA excess. Sensitivity to human DNA was unaffected by a 100-fold excess of *E. coli* DNA.

4. Reproducibility

For 50 trials each of three templates at 500 pg/reaction, fully automated analyses produced 5998/6000 correct genotype assignments for the 40-marker SNP assay. The average accuracy of mass measurements for 31,030 independent DNA product assignments was 12.4 ± 11.4 parts per million (ppm).

5. Mixture detection

The ability to detect when a profile represents a single DNA source or two or more DNA contributors is essential to analyze a DNA sample of unknown origin. Based on a global distribution of heterozygosity of >0.4 for all loci [6,7], and quantitative inter-allelic signal output from the mass spectrometer, a scoring system was devised that indicates when an assay result is consistent with a single contributor DNA or appears to be explainable only by the presence of more than one template. The scoring is intended to judge the usefulness of a profile as a single-contributor profile, and no attempt is made to separate components of a mixture into individual profiles. For 10 mixtures of pairs of individuals analyzed in duplicate in the 40-SNP assay at ratios of 10:90, 25:75, 50:50, 75:25 and 90:10, all cases of profiles that contained alleles from more than one contributor scored >1.0. For 198 analyses of 81 independent single-source DNA templates, all profiles produced scores <1.0 (data not shown).

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Funding was coordinated through Ibis Biosciences for assay development. There was no external funding for this project to be disclosed.

Conflict of interest

Thomas Hall, Christine Marzan and Steven Hofstadler are salaried employees of Ibis Biosciences with financial interest in human forensics.

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