Analysis of DNA Forensic Markers Using High Throughput Mass Spectrometry

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We present recent results on a novel DNA forensics platform based on fully automated high throughput electrospray ionization mass spectrometry (ESI-MS). The approach is based on using ESI-MS to "weigh" DNA forensic markers with enough accuracy to yield an unambiguous base composition (i.e. the number of A's, G's, C's and T's) which in turn can be used to derive a DNA profile for an individual. Importantly, these base composition profiles can be referenced to existing forensics databases derived from mtDNA sequence or STR profiles.

We have performed numerous blinded validation studies with this approach in collaboration with the FBI, AFIP/AFDIL, and NIST to evaluate the platform for both STR and mtDNA typing. Importantly, the same platform is used for both types of analyses and in both approaches MS offers distinct advantages over the conventional approach.

With respect to mtDNA typing, the ESI-MS based approach facilitates the analysis of samples containing sequence and length heteroplasmy in the HV1, HV2, and HV3 regions without degradation of information; in fact the method captures the extent and type of heteroplasmy in situations that are not amenable to sequencing. Based on the base compositions derived from a 24-primer pair tiling panel which covers nucleotide positions 15924-16428 and 31-576, this approach is shown to be more resolving than traditional sequencing approaches covering 16024- 16365 & 73-340. Owing to the quantitative nature of ESI-MS, mixtures of different mtDNA types can be detected and resolved into distinctive components. Where mixtures of mtDNA types analyzed by sequencing often lead to uninterpretable results, analysis through ESI-MS yields a quantitative data that maximizes the informative value of evidence.

With respect to STR markers, because base compositions are used to derive specific alleles, the MS-based method picks up SNPs within STR regions that go undetected by conventional electrophoretic analyses. For example, all "allele type 13"s for the D5S818 marker are not equivalent; some contain a G to T SNP which distinguish them from individuals containing the "normal" allele type 13. Similarly, individuals which are typed as homozygous for this allele may in fact be "same-length-heterozygotes" containing alleles 13 and 13G/T_SNP. A preliminary survey of samples from different population groups suggests that there may be some population bias with respect to the frequency and types of SNPs observed in the STRs; interestingly the frequency of SNPs in several of the CODIS loci is in excess of 20%.

Both the ESI-MS based mtDNA and STR assays are fully automated (post-PCR) and have a sample throughput of 6 minutes/sample.