DEVELOPMENTAL VALIDATION OF AN STR GENOTYPING ASSAY PROVIDING BASE COMPOSITION ANALYSIS BY PCR/ELECTROSPRAY IONIZATION MASS SPECTROMETRY

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An assay providing profiles for the thirteen core CODIS STR loci plus the sex-typing locus Amelogenin has been developed. In a highly automated process the assay markers are amplified and then analyzed on an electrospray ionization mass spectrometry (ESI-MS) platform. The resulting mass determinations are converted to base compositions specifying the number of dA, dG, dC, and dT present in each of the PCR amplicons. STR profiles are derived from the mass measurements and base compositions. The highly accurate mass determinations allow detection of SNP variants of STR alleles which are undetectable with mobility-based estimates of length determined with conventional electrophoretic analyses.

Here we describe the developmental validation of this assay. Non-human DNA from bacterial, fungal and mammalian sources gave no detections in the assay, and did not interfere with the generation of profiles from human DNA in mixtures, even in a 10:1 mass excess. The assay sensitivity on a per well basis was typical of PCR-based assays, and showed high reproducibility and accuracy. Profiles of 53 human DNA samples as determined by the PCR/ESI-MS assay were fully concordant with results obtained with the Applied Biosystems Identifiler assay. Allelic balance and interlocus balance were characterized with the set of 53 samples. Mixtures of DNA were analyzed to define the range of proportions of source DNA where a sample could be identified as a mixture. A panel of parent-offspring trio samples was profiled and results demonstrated faithful transmission of polymorphic alleles.