VALIDATION OF THE PLEX-ID™ MTDNA ASSAY FOR USE IN FORENSICS

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When investigating very challenged biological samples such as bones, hairs, and teeth, mitochondrial DNA (mtDNA) analysis can often provide results where more traditional nuclear DNA markers fail. The most widely employed method of analyzing mtDNA has been to amplify the genetic material via the polymerase chain reaction (PCR) and subsequently sequence the amplicons using the Sanger method of fluorescent dideoxynucleotide incorporation and cycle sequencing. While this procedure has been used successfully for many years, it has a number of limitations. First, the process is time-consuming, labor-intensive, and costly. Second, the analysis of sequencing results may be impeded by artifacts resulting from heteroplasmy, electrophoretic issues, and the presence of mixtures. The Abbott PLEX-ID™ instrument enables analysis of mtDNA amplicons via electrospray-ionization mass spectrometry (ESI-MS), and produces comparable accuracy and sensitivity while offering a faster and less expensive alternative to Sanger sequencing. Unlike Sanger sequencing, this system is capable of quantifying DNA species, and thus may be exploited for evaluating heteroplasmy and, possibly, mixture deconvolution. This presentation will summarize and explain the findings of a validation study of the PLEX-ID™ mtDNA assay. The samples used in this study included human sources of buccal, blood, bone, and hair tissues, surface swab DNA, and a series of animal DNA. The DNA was extracted using a variety of protocols and kits, and quantified using the Quantifiler™ Human DNA Quantification Kit. Amplification was performed using PLEX-ID™ mtDNA Assay v2.0 plates. Each well of these 96-well plates is pre-filled with a triplex reaction mix containing 3 non-overlapping primer pairs designed to anneal to specific regions in the non-coding portion of the mtDNA genome. 5 µL of normalized, extracted mtDNA were added to each of the 8 wells in a single column of the thawed assay plate for a total of 24 primer pairs per sample, per analysis. The plates also included positive (HL-60) and negative (DNAse/RNAse-free distilled water) controls. This validation included a sensitivity study, wherein a dilution series was created for each sample at concentrations of 20, 10, 5, 2, 1, 0.2, 0.1, and 0.02 pg/µL. At 5 µL per well, these concentrations equate to 100, 50, 25, 10, 5, 1, 0.5, and 0.1 pg per well (or reaction), respectively. A reproducibility study was performed, as well as an animal cross-reactivity study. The latter included samples from guinea pig, Rhesus monkey, ferret, pig, horse, mouse, chicken, cow, baboon, rabbit, deer, cat, goat, dog, and rat. A mixture study involving 10 pairs of individuals was performed, at contributor ratios of 1:1, 1:2, 1:5, 1:10, 1:20, 20:1, 10:1, 5:1, and 2:1. Studies were also conducted using samples from the Caucasian, Hispanic, and African American population groups, and mock samples commonly encountered in forensic casework (e.g., bloodstains, hairs, and touch DNA samples). The results of this research confirmed that the PLEX-ID™ is highly sensitive and capable of yielding reproducible results. Samples commonly encountered in a forensic setting, as well as population samples, were typed correctly. The animal cross-reactivity portion of the study revealed that many of the pre-extracted animal samples obtained for validation work were contaminated with human mtDNA. This presentation will emphasize the need for caution and care in the scientific community when preparing such samples, as the development of more sensitive systems allows for the detection of low background contaminating DNA. Finally, it was discovered that the PLEX-ID™ mtDNA assay detects chimeric product formation, which may confound the mixture deconvolution process under certain scenarios. These findings and their implications will be discussed. This oral presentation will convey the findings that the PLEX-ID™ mtDNA assay yields reliable results for single-source samples, which are the same sample types currently examined in forensic laboratories via Sanger sequencing, at a level that meets or exceeds that of the current method. ⌘