Real-time mtDNA Specific Quantitation

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Several methods for detecting unamplified nuclear DNA are employed currently in forensic laboratories. When these assays are utilized in mitochondrial DNA (mtDNA) analysis, the amount of nuclear DNA present in a sample is used to estimate the amount of mtDNA. Several factors indicate a need for specific mtDNA quantitation. Since mtDNA can be successfully analyzed from samples with little to no detectable nuclear DNA, extracts often are processed for mtDNA even if the nuclear DNA specific assays are inconclusive. In addition, the slot blot hybridization technique that is employed by many laboratories to quantitate unamplified DNA is time-consuming and relies on subjective interpretation of the quantity of DNA. Alternatively, real-time quantitative PCR (QPCR) provides an objective estimate of DNA quantity, is amenable to automation and provides the opportunity for custom quantitation. This work focuses on development of a real-time QPCR assay based on the TaqMan system (ABI Prism), for quantitation of unamplified mtDNA.

This work focuses on a target that lies in the control region of the mitochondrial gemone. A search of the mtDNA database was performed to assess areas of polymorphisms in the control region and an area was selected which contains few differences from rCRS. Primers and probes were designed based on these results and BLAST searches were performed to confirm human specificity. It was established that the primers and probes bound to the correct region of the mtDNA, the PCR reactions were optimized and an internal positive control was added to the reaction. Once the conditions for optimal amplification were determined, sensitivity and reproducibility studies were carried out under these conditions.

A set of human mtDNA specific primers and probe were designed to target a conserved portion of the control region of the mtDNA genome. Optimization studies were performed and an internal positive control was added to the assay. Once the conditions for optimal amplification were determined, sensitivity and reproducibility studies were carried out under these conditions. This assay accurately detects 100 to 10^6 copies of a plasmid that contains a mtDNA control region insert with good precision (coefficient of variation $\leq$0.01) and is capable of detecting fewer than 100 copies of this plasmid. In addition, the effect of PCR inhibitors (humic acid, hematin, denim and EDTA) has been evaluated. Similar concentrations of these inhibitors comparably reduce real-time and standard amplification. DNA purification by Microcon-100 filtration or Qiagen column methods removes inhibitory concentrations of hematin, humic acid, EDTA and denim and reverses the effects of these inhibitors. A validation study on evidentiary-type samples, including buccal swab, blood, hair, and bone, is underway. Finally, this mtDNA quantitation assay has been designed to run concurrently with a nuclear QPCR

assay so that the amount of nuclear and mtDNA in a sample can be determined simultaneously. This will allow an analyst to determine whether nuclear DNA or mtDNA analysis should be performed. Since mtDNA testing is a lengthy, tedious and labor-intensive procedure, any improvements that reduce assay time and enhance casework results are desirable. Incorporation of a mtDNA specific quantitation step into mtDNA sequence analysis could be extremely beneficial.

A validation study on evidentiary-type samples is underway. DNA extracted from buccal, blood, hair and bone samples are quantitated by TaqMan and the slot blot method. A total of 100 pg of DNA is amplified based on the values provided by each instrument and cycle-sequenced according to standard protocol. In addition, the effect of PCR inhibitors (humic acid, hematin, denin and EDTA) on this assay has been evaluated.

Mitochondrial DNA testing is a lengthy, tedious and labor-intensive procedure. Any improvements that reduce the assay time and enhance casework results are desirable. In addition, improvements in mtDNA typing will facilitate transfer of this assay to the broader forensic community. Development of a mtDNA control region TaqMan assay into mtDNA sequence analysis could be extremely beneficial to the forensic community.