REAL-TIME QUANTITATIVE PCR ASSAY FOR QUANTIFICATION OF MITOCHONDRIAL DNA

Mark F. Kavlick[1], Helen S. Lawrence[1], R. Travis Merritt[2], Constance Fisher[2], Alice Isenberg[2], James M. Robertson[1], and Bruce Budowle[3]
[1]Counterterrorism and Forensic Science Research Unit, FBI Laboratory
[2]DNA Analysis Unit 2, FBI Laboratory
[3]Forensic Analysis Branch; FBI Laboratory, Quantico VA

Mitochondrial DNA (mtDNA) sequence analysis has become a valuable analytical tool in instances where nuclear DNA analysis of forensic specimens is not possible due to insufficient template or DNA degradation. Knowing the quantity and quality of mtDNA in purified DNA samples prior to forensic mtDNA analyses would be useful in order to assess the likelihood of generating mtDNA profiles from such samples. This knowledge could result in less sample consumption, thus conserving samples for additional analyses if necessary. Described is a highly sensitive real-time quantitative PCR (QPCR) assay which was developed to accurately quantify mtDNA for these purposes.

The target sequence for the assay is located within the mtDNA NADH dehydrogenase subunit 5 gene and possesses minimal sequence homology to the mtDNA of other forensically-relevant species. The primers and probe utilized in the assay are designed to enable the amplification and quantification of all human mtDNA sequence types as they hybridize to sequences which are to date monomorphic. The small amplicon generated (105 bp) facilitates amplification even for degraded samples. The assay is based on absolute quantification and exhibits high sensitivity and a large dynamic range to enable the quantification of as few as 10 copies to as many as 108 copies of mtDNA (0.17 fg - 1.7 ng of mtDNA, respectively).

We observed that when using plasmid standards template access to primers and probe can be restricted and result in inefficient amplification and inaccurate quantification. To address this phenomenon, a novel, synthetic DNA standard was employed for the QPCR assay. The synthetic standard was designed with a signature sequence to enable its identification if it were part of a contamination event. Benefits of a synthetic standard include high quality control, high purity, low cost, high yield, and rapid production. Additional assay features include minimal sample consumption (2µl), rapid run times of 40 minutes, and the option of an internal positive control to detect PCR inhibitors.

The QPCR assay described is reliable, robust, and reproducible and will enable the accurate and precise quantification of mtDNA for use in downstream analysis.