

DEVELOPMENT OF A REAL TIME PCR ASSAY FOR HUMAN MITOCHONDRIAL DNA QUANTIFICATION IN FORENSIC SAMPLES

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Mitochondrial DNA (mtDNA) analysis is a reliable alternative for challenging forensic samples, but its success depends on the amount and integrity of the targeted DNA on the sample. Commercially available human DNA quantification kits usually have autosomal and/or Y chromosome DNA as targets. Their quantities are not always well correlated to the amount of mtDNA in the samples, particularly in samples from highly degraded forensic specimens. Our main goal is to develop a human specific mtDNA quantification assay in order to better guide downstream applications. In this work, we have developed a real-time quantitative PCR (qPCR) assay in order to estimate the number of amplifiable copies of human mtDNA in forensic samples. The assay uses a 77-base pair amplicon which spans part of the segment I of the human hypervariable region (HVI). For amplification we have validated the use of GoTaq[®] qPCR Master Mix (Promega) and very sensitive detection was observed, even at very low-copy numbers and in the presence of PCR inhibitors usually found in forensic samples. The assay encompasses a wide range of detection (10 to 10⁵ copies). Our results indicated that a quantification result of approximately 500 copies of mtDNA is sufficient for downstream applications, such as DNA sequencing and SNP detection. The assay is being validated with a wide range of forensic samples, such as highly decomposed skeletal remains and hair shafts.

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