APPLICATIONS OF REAL-TIME MITOCHONDRIAL AND NUCLEAR DNA QUANTIFICATION

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We have developed a highly sensitive, rapid and reliable system for quantification of nuclear and mitochondrial DNA copy numbers that consumes a minimum of the valuable DNA sample. The system is based on the real-time 5'exonuclease detection assay, using the ABI PRISM® 7700 instrument (TaqMan). Two specific probes, labeled with different dyes, enables simultaneous quantification of the nuclear Retinoblastoma 1 gene and the mitochondrial tRNA Lys gene. This assay has proven very useful for quantification of various forensic evidence materials in casework analysis. In addition to aid in the selection of the optimal analysis method (mtDNA or nDNA) for a specific sample, it is used to estimate the optimal amount of DNA to be used for a successful amplification and to avoid allelic dropout or preferential amplification. The quantification system has been used to determine the DNA copy numbers available in hairs, a type of evidence material frequently found at a scene of a crime. Shed and plucked head hairs have been quantified to evaluate inter- and intra-individual differences in mtDNA and nDNA copy numbers. In a comparison of plucked hairs from different individuals an up to 45-fold difference was seen in the root part. In distal shaft parts at the same length a 2 - 6 fold difference was observed and among hairs form the same individual an up to 30-fold difference in mtDNA content between roots was found. The DNA content in plucked and shed hairs has also been compared. Moreover, different types of body hairs were evaluated displaying large differences in DNA quantity. The quantification assay has also been used to quantify nuclear and mitochondrial DNA after whole genome amplification reactions (WGA). In cases where the DNA material is very limited, WGA can be used to enhance the DNA content prior to specific PCR amplification. The WGA-methods Primer Extension Pre-amplification (PEP) method and a kit for WGA, GenomiPhiTM, have been tested for use in forensics. The PEP method is based on an extension reaction from random pentahexanukleotides, which amplify 80 % of the genome in a cell to a minimum of 30 copies, approximately 1000 bp in length. GenomiPhiTM employs the unique biochemical properties of Phi29 DNA polymerase, a highly processive enzyme with excellent strand displacement activity, in combination with random-sequence hexamer primers to amplify DNA. Microgram quantities of DNA are produced overnight from nanogram amounts of starting material. Both WGA methods amplified DNA from both forensic and control samples, however not equally efficient. The quantification assay has, furthermore, been used to evaluate and compare different DNA extraction methods. Silica based, organic and salting out procedures have been evaluated for DNA recovery on a sample collection of bone specimens found in a grave from the 1000-century in Sigtuna in mid Sweden.