SINGLEPLEX AND DUPLEX REAL-TIME QUANTITATIVE PCR ASSAYS FOR THE HUMAN NUCLEAR AND MITOCHONDRIAL GENOMES

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The accurate quantification of human DNA is an important step in the analysis of forensic samples. The commonly used slot-blot hybridization methods for quantification of human nuclear DNA provide excellent specificity and adequate sensitivity and accuracy, but require a significant amount of hands-on laboratory time and are not readily automated to increase throughput. Real-time, quantitative PCR (qPCR) has several features that make it an attractive alternative for quantification of DNA in forensic samples. Real-time qPCR assays can be very sensitive, offering the potential for quantification down to less than 10 copy numbers per genome of interest, as well as highly specific, providing the ability to quantify DNA in the complex mixtures encountered in forensic samples. By using suitable detection methods (e.g., TaqMan® or Molecular Beacon reporter probes), qPCR assays can be multiplexed to two (or more) different targets, offering the possibility to conserve DNA-limited samples. Finally, compared to slot-blot methods, qPCR protocols require less hands-on laboratory time, less analysis time, and can be automated for high throughput.

We have developed singleplex and duplex qPCR assays for quantification of the human nuclear and mitochondrial genomes, the nuclear assay for estimating the amount of template to be used in STR PCR multiplexes, the mitochondrial assay for estimating the amount of template to be used in PCR for sequencing and linear array genotyping applications. The development of these assays will be described. For the nuclear quantification assay, the HUMTH01 STR locus has been chosen as the target. This assay has been optimized for both SYBR® Green detection and TaqMan® detection, and has been characterized for specificity, sensitivity, and precision. For the mitochondrial assay, we have examined three targets for quantification; two developed in-house using the ND1 locus and one previously described (H.Andreasson, U.Gyllensten, and M.Allen, BioTechniques, 33:402-411(2002)). The mitochondrial assays have been optimized for SYBR® Green and TaqMan® detection. Quantification results will be described for a variety of sample types using both singleplex and duplex versions of the nuclear and mitochondrial assays.