MULTIPLEX PCR FOR RAPID QUANTITATION OF HUMAN NUCLEAR AND MITOCHONDRIAL DNA

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Human forensic casework requires sensitive quantitation of human nuclear and mitochondrial DNA (mtDNA) from complex biomaterials. Although many DNA quantitation systems are currently available to forensic laboratories, including real-time PCR assays for both nuclear and mtDNA quantification, most of these systems are not multiplex compatible and lack human specificity. Rapidly evolving DNA sequence databases facilitate large scale comparisons between the human genome and others. Here, we report the development of a human specific multiplex PCR assay for the quantitation of human nuclear and mtDNA from mixed sources. Using a TaqMan-MGB probe labeled with 5'-FAM for mtDNA and an intra-*Alu*Yb-lineage based TaqMan-MGB probe labeled with 5'-VIC for nuclear DNA, the effective linear quantitation range for this multiplex assay was 100 ng to 10 pg of starting genomic DNA template. Background cross-amplification with DNA templates derived from 3 other primate species and 10 additional mammalian species, was negligible prior to 30 cycles of PCR. The human mtDNA assay displayed no cross-amplification with DNA templates derived from a human-rodent somatic cell hybrid panel. The human-specificity of the assays was further demonstrated by the ability of the multiplex to accurately detect 0.05% human DNA from within a complex source of starting templates. We are currently in the process of adding a Y chromosome specific assay to this multiplex for the simultaneous quantification of human mitochondrial, nuclear and Y chromosome (male) DNA.