Assessment of DNA Extracted from Forensic Samples Prior to Genotyping

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ABSTRACT: Quantification of human DNA has been an integral part of forensic DNA analysis. Hybridizationbased methods such as Quantiblot[®] kits were used extensively in the 1990s. These methods fulfilled the need at the time, since their sensitivity range was similar to the genotyping methods in use, such as restricted fragment length polymorphism. Later, the development of robust and more sensitive megaplex genotyping systems such as short tandem repeat profiling, mitochondrial DNA sequencing, and single nucleotide polymorphism typing, created the need not only for quantification of DNA at the picogram level but also for assessment of the quality of the DNA extract to make informed decisions to ensure the success of downstream analysis. Real-time PCRbased quantification methods fulfilled this need. The different real-time PCR methods developed range from singleplex reactions for quantification of human or mitochondrial DNA to multiplex systems that enable analysis of up to four targets for quantification of human DNA, human male DNA, mitochondrial DNA, detection of PCR inhibitors, or determination of the extent of DNA degradation. Incorporation of these assays into the workflow enables selection of appropriate genotyping systems and increases the first-pass success rate for obtaining a genotype using a minimal amount of evidence sample. The real-time PCR methods described here would also be useful as DNA assessment tools prior to other genotyping methods like copy number variation, insertion/deletion, and Alu dimorphism analysis as well as sequencing, etc., that are currently being investigated as additional informative tools for human identification purposes.

KEY WORDS: Assessment of DNA extract, assessment of forensic samples, DNA analysis, DNA quantification, DNA typing, human DNA, human male DNA, real-time PCR.