DEVELOPMENT OF AN *ALU*-BASED REAL-TIME PCR ASSAY FOR THE QUANTITATION OF GENOMIC (HUMAN) DNA

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Most forensic laboratories use slot-blot probe hybridization technology to quantify genomic DNA. There are several drawbacks to this method: it requires significant technician "hands-on" time, and quantitation can be imprecise, inaccurate, or mis-interpreted. We have developed a real-time PCR assay to accurately and reproducibly quantitate human DNA samples. Primer and TaqMan probe sequences specific for primate *Alu* were designed using Primer Express (Applied Biosystems). *Alu* sequences are present in excess of 500,000 copies in the human genome, and amplification of these sequences will provide a rapid and sensitive method of detection for human DNA. Using an Applied Biosystems 7000 Sequence Detection System, we were able to amplify and detect *Alu* repeats using two detection systems: SYBR Green, a non-specific DNA intercelating fluorescent dye, and a TaqMan probe designed to hybridize specifically to the amplified product. In initial studies, SYBR Green was found to successfully detect the *Alu* amplicon DNA over a wide linear range of 10pg to 100ng of input DNA, but a non-specific product was observed in later cycles. The TaqMan method reduces this late non-specific product formation. Compared to slot blot quantitation methods, our real-time PCR methods provide more accurate and sensitive assays, have a greater linear range, are faster, and use less operator time. We will also report on population, species, and micro-organism interference studies.