REAL-TIME PCR METHODS FOR QUANTITATION AND ANALYSIS OF FORENSIC SAMPLES

Janice A. Nicklas and Eric Buel,
Vermont Forensic Laboratory, Waterbury, VT

DNA isolated from crime scene samples must be quantitated to determine the amount of human DNA present. Recently the forensic community has become interested in developing faster, cheaper and more quantitative methods than the widely used slot blot method utilizing a D17Z1 probe. In most molecular diagnostic laboratories, PCR techniques have almost completely replaced Southern and slot blot methods. Thus, development of quantitative PCR methods to measure the amount of human DNA in the forensic setting is a logical extension of this progression. Obviously the sequence chosen must be human or at least primate specific and if present in multiple copies would allow more sensitive quantitation. *Alu* sequences are primate-specific and are found in ~800,000 copies in the human genome and make an excellent marker for human DNA.

Our initial assay used endpoint PCR quantitation with SYBR Green I dye in a fluorescence plate reader. We then turned to a real-time PCR assay using SYBR Green I. This assay has the advantage of a wider range 16ng down to 1pg, a turnaround time of ~87min (with less hands-on time than the plate reader assay) and a cost of $0.50 per sample. Both of these methods were validated by comparison with usual slot blot method on mock and real crime samples. Initial studies indicate that the methods are at least as cost effective and sensitive as the slot blot method, as well as much simpler to perform and with a greater analytical range. We have also investigated and compared the use of LUX™ primers, MGB-Eclipse™ and TaqMan™ probes for readout.

In addition to simply quantitating the human DNA in a sample, it is often of importance to determine whether and how much male DNA the sample contains. In crimes involving violence against women perpetrated by men blood may be shed by the both the victim and attacker; a method that could quickly identify those stains as male or female could allow the examiner the chance to be selective in the stains that are analyzed further by STR analysis. Another important use of sex typing would be in the STR analysis of sexual assault cases where the percentage of male DNA in differential extractions can vary widely. A method that could determine the amount of male DNA present in a sample could allow the forensic scientist to decide if a sample has ample male DNA for autosomal or Y STRs, respectively. We have tested LUX™ primers, MGB-Eclipse™ and TaqMan™ based Y satellite (Y specific) assays. Our plan is to develop a multiplex system of gender determination in conjunction with human DNA quantitation so that no additional work is required than that needed prior to STR analysis.

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