B30 Real-Time PCR Methods for Analysis of Forensic Samples

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After attending this presentation, attendees will understand rapid quantitative and inexpensive tests for determination of sample DNA quantitation and gender determination

This presentation will demonstrate that new methods will be a valuable addition to DNA analysis by saving time and money and improving the quality of human DNA quantitation.

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 handson time than the plate reader assay) and a cost of $0.50 per sample. Both of these methods were optimized by adjusting primer concentration, annealing and extension time and temperature, SYBR Green concentration and polymerase concentration. The assay was validated by comparison with usual slot blot method on mock and real crime samples and by STR analysis based on assay quantitation results. The studies indicate that the SYBR Green Alu method is 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.

PCR quantitation methods that use probes (oligonucleotides with fluorescent dyes attached) have the advantage that plateau effects are lessened and a greater dynamic range can be achieved. We have also investigated and compared the use of LUX primers (Invitrogen), MGBEclipse (Epoch Biosciences) and TaqMan (ABI) probes for readout. The LUX system has a low fluorescence output and appears not to be sufficiently reproducible. The Eclipse system has been optimized and validated with case work samples and can detect from 256ng down to 1pg of DNA at about the same cost as the SYBR™ Green assay. The TaqMan assay can also detect from 256ng down to 1pg DNA; optimizations and validations are underway.

In addition to simply quantitating the total 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 which stains are 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. These assays are able to discriminate male and female DNA over 100,000 fold and accurately quantitate male DNA. Experiments are underway to optimize these assays and pick the best assay from among these methods. The plan is then to develop a multiplex system of gender determination in conjunction with human Alu DNA quantitation. Such an assay will greatly reduce the work and expense in analysis of crime scene samples by quickly determining which samples are important and which contain sufficient DNA of the correct gender to yield valuable and conclusive STR results.

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