DEVELOPMENT OF *ALU* PCR AND *ALU* REAL-TIME PCR METHODS FOR QUANTITATION OF HUMAN DNA IN FORENSIC SAMPLES

E. Buel and J.A. Nicklas
Vermont Forensic Laboratory, Department of Public Safety, Waterbury, VT

There are a number of steps required to obtain a DNA profile from a crime scene sample. One important step in this process is the determination of the amount of human DNA contained in the sample. The forensic community relies predominately upon a technique (slot blot) to quantitate human DNA that is imprecise, time consuming and very labor intensive. We have developed new, faster and more quantitative techniques based on PCR of the primate-specific *Alu* sequence that is found in over 800,000 copies in the human genome. Primers were used to amplify a 124bp fragment of *Alu* sequence in a reaction containing SYBR Green I stain. Quantitation was performed in a fluorescent plate reader Bio-Tek FL_X800). This plate reader assay consisted of an initial zero time SYBR Green I fluorescence reading, 14 cycles of PCR and then a final fluorescence reading. Subtracting the initial time reading from the final reading removed the fluorescent contribution from the input DNA. Fluorescence background was decreased by the use of QSY7 labeled primers (method patented by Molecular Probes, Inc.). The QSY7 moiety quenches the background SYBR Green I fluorescence of the primers and of primer-dimers. We have explored a number of variables for this assay including: number of PCR cycles, multiple readings, annealing temperature, annealing time, extension time, specificity (test of animal and bacterial DNAs), Mg++ concentration and SYBR Green I concentration. Study of mock crime samples, blood spots and degraded DNA (DNAseI, sunlight or heat treated) indicated the QSY assay could correctly quantitate human DNA for amplification of STRs within the accepted range using the ABI COfiler® kit even when the DNA was highly degraded. For difficult samples such as bloodstains on denim where PCR inhibitors can be problematic, the original QSY assay could under-report the amount of human DNA in the sample. However, addition of a final concentration of 250ug/ml BSA to the QSY amplification resulted in QSY assay amplifications predictive of STR amplification results without changing the standard curve results. Comparison of the initial zero time reading with the final reading allows determination of DNA concentrations above the dynamic range of the assay (>10ng) or the presence of inhibitors or animal DNA. Our results indicate that the QSY *Alu* assay using a plate reader has a dynamic range of 10ng to 10pg in a 25ul assay and is specific for human (or other primate) DNA. We have also performed experiments using *Alu* PCR in a real-time format again using SYBR Green I stain. These results indicate that this assay is also specific for human DNA and has a range of 16ng to 3.9pg in a 10ul assay. Our results indicate that use of *Alu* PCR can be a quantitative, fast and inexpensive way to quantitate human DNA for forensic or other studies.