DEVELOPMENT OF A REAL-TIME QUANTITATIVE PCR ASSAY FOR DETECTING HUMAN DNA

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The process of human forensic identification involves sensitive Polymerase Chain Reaction (PCR) multiplex assays. These tests work best within a narrow range of template DNA, necessitating reliable quantification of Human DNA. Furthermore, contaminating DNA from bacteria and fungi, often associated with forensic samples, must be disregarded. With the knowledge of DNA quantity, the forensic analyst can a) amplify a pre-determined amount of target DNA, b) determine the best amplification strategy when DNA is limited and c) more effectively interpret the DNA profile obtained.

The current methodology used by the Centre of Forensic Sciences (CFS) to determine the quantity of DNA utilizes a biotiynlated oligonucleotide probe that hybridized to DNA samples immobilized on a nylon membrane. Although we have found this technique to be reliable and sensitive, it is time consuming and labour intensive. In order to meet casework demands, the CFS must dedicate one full time employee solely to the task of quantifying DNA. Efficiencies must be gained in the process of quantification if the Centre is to meet client demands for quicker analysis.

Our laboratory has recently acquired a PE Biosystems ABI PRISM® 7900HT Sequence Detector for the development of a rapid and automated Real-Time Quantitative PCR assay using TaqMan® chemistry. This technology utilizes a sequence specific probe with a fluorescent reporter dye and quencher dye that are attached to the 5' and 3' ends, respectively. When the probe is intact, the reported dye emission is quenched. During PCR, the probe anneals specifically between the forward and reverse primers. When the probe is cleaved by the 5' nuclease activity of the DNA polymerase, the reporter dye is separated from the quencher and a sequence-specific signal is generated. With each PCR cycle, additional reporter molecules are cleaved, and thus more fluorescence is detected.

The application of a simple, single tube analysis with minimal operator intervention required. With Real-Time Quantitative PCR, following pipeting of samples and reagents into reaction wells (manual loading takes approximately 40 min for 96-well plate), one has to only seal the plate, transfer it to the sequence detector and initiate the process. The results are available directly after the PCR (~100 min) with no additional processing required. The ABI PRISM® 7900HT will support automated liquid handling and bar coding. These capabilities could further reduce the labour required to generate results while improving sample tracking.

The identification of suitable primers and a sequence specific target for the assay was made possible with the use of Primer Express® Software. Two probes were selected for testing. Their sequences were derived from the reported GenBank Sequences for the loci F13B (Accession M64554) and HUMTH01 (Accession D00269) and occur downstream from the polymorphic repeat region. The probes are approximately 15bps in length with a total amplicon size of about 60bps. Quantification using SDS technology has the advantage of indicating the amount of amplifiable DNA present in a sample.

Preliminary findings with DNA extracted from 157 different individuals of unknown racial origin, indicates that the sequence's specific targets are conserved. Additional analysis with samples taken from the major racial groups are underway and results will be presented. The probes were demonstrated to be human specific with no cross-reactivity occurring when 10ng of non-primate DNA was added to the PCR reaction. Additional interference tests performed showed no change in signal obtained from 1ng of human DNA when contaminated with as much as 40ng of bacterial DNA. The system allows reliable quantification down to at least 50pg while providing four orders of linear dynamic range. Additional studies are underway to determine the viability of this assay when using forensic samples typically encountered in casework.