B6 Determining the Quantity and Quality of DNA Using Real Time PCR

Sarah E. Hughes, BSc, and Bruce R. McCord, PhD, Florida International University, 11200 SW 8th Street, Miami, FL 33199*

By attending this presentation, attendees will learn the basics of real time PCR, how real time PCR can be used as a more sensitive method of DNA quantitation than the current slot blot method, and how to determine the level of degradation in DNA by amplifying different length fragments.

This presentation will impact the forensic community and/or humanity by offering a more sensitive method of DNA quantitation which also provides useful information regarding the quality of the DNA present in the sample, thus allowing the analyst to select an appropriate DNA typing method.

DNA extracted from forensic samples such as bone, hair, or fingernail scrapings may not be in optimal condition. Instead, the DNA may be badly degraded or may be present in low copy number. It is important to quantify genomic DNA prior to amplification using human specific probes; however, techniques such as slot blot quantitation can have difficulty in detecting DNA present in degraded and low copy number samples. Real Time PCR offers an alternative method for quantifying such samples. Real Time PCR is a sensitive method of DNA quantitation with a dynamic range of 1 picogram to 16 nanograms of DNA (1). As this technique can detect picogram amounts of DNA, it is perfectly suited to the detection of DNA in low copy number and degraded samples. However, in its present config- uration, real time PCR cannot provide information on the quality of a DNA sample.

The goal of this research project was to develop a technique to use real time PCR as a method to probe the quality of the DNA extracted from forensic samples. Previous work from this laboratory has demonstrated that the PCR reaction is sensitive to the size of the DNA template (2). By using several primer sets, each amplifying a different length fragment, the level of DNA degradation can be determined. Since degraded DNA is frag- mented into shorter templates, it is expected the primers which amplify the shorter amplicons will detect larger quantities of DNA. The amount of DNA detected using each primer set can be compared to determine the extent of the DNA degradation. Preliminary results have shown that the quantity of DNA detected differs depending on the length of the amplified fragment, with more DNA being detected with the shorter primer set.

In this research project, DNA was extracted from various simulated forensic samples using a phenol chloroform extraction and centrifugal fil- tration. The level of DNA degradation was assessed using a series of PCR primers which amplify different length amplicons. The DNA was quanti- tated with real time PCR and three different primer sets were used in the amplification. The amount of DNA degradation was then determined by comparing the quantitation results obtained for each of the different primer sets. The DNA was also typed using both a commercially available STR kit and miniSTR's. The DNA typing results show that by knowing the level of degradation, the success or failure of the DNA typing method can be deter- mined prior to analysis. This allows the analyst to choose an appropriate method – STRs, miniSTRs, mtDNA, or SNPs to analyze the results. References:

Nicklas JA, Buel E. Development of an Alu-based, real-time PCR method for quantitation of human DNA in forensic samples. J Forensic Sci 2003; 48(5):936-944.

Chung DT, Drabek J, Opel KL, Butler JM, McCord BR. A study on the effects of degradation and template concentration on the amplification efficiency of the STR Miniplex primer sets. J Forensic Sci 2004; 49(4): 733-740.

Real Time PCR, DNA Quantitation, Degraded DNA

 ** Presenting Author*