A91 A Study of the Environmental Effects on DNA Extracted from Degraded Tissue Samples

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After attending this presentation, attendees will see the correlation between degradation of DNA, inhibition of the amplification process, the way these manifest themselves and the method of analysis that provides the most information for sample identification.

This presentation will impact the forensic community by examining the rate of natural degradation of DNA recovered from bodies at crime scenes. This should provide an estimate of time since death for such samples. In addition, by developing a correlation between the quality of real time results and the STR profile, we hope to provide laboratories with the information needed to determine which method of analysis: SNPs, miniSTRs, multiplex STRs etc. will produce the best estimates for sample identification.

The goal of this presentation is to examine the effect of environmental degradation on tissue and bone samples on DNA typed using multiplex PCR analysis. In this project special attention is paid to the connection between measurements of degradation using real-time PCR quantification and the quality of the recovered profiles for these samples using commercial STR kits.

Using real-time PCR we can generate a virtual yield gel, providing information on the relative amounts of intact and degraded DNA in a sample. This permits the analyst to estimate the potential of generating a full or partial profile using either standard sized or mini STR kits. Ultimately, this should create a more streamlined system in which the relative age of a sample can be determined and the optimal method of analysis can be utilized.

This study involves determination of natural rates of nuclear DNA degradation, under a variety of conditions, to develop a series of timelines for decay. These timelines will then be correlated to the quality of nuclear DNA through the use of real-time PCR utilizing a series of primers varying in amplicon length. These results will then be correlated to the probability of generating a full STR profile, a partial STR profile, or no profile at all. Tissue and blood samples obtained from bodies placed at the University of Tennessee Anthropological Center. Four types of burials were compared: above ground, buried under debris, below ground, and submerged in water.

A series of tissue samples were obtained over weekly intervals and 25 mg of each sample was extracted using the QIAGEN blood and tissue kits and quantified using a series of multilocus Alu based primers with amplicon sizes of 82, 189, and 234 bp respectively. For all three sets of primers, samples 0 to 1 week old yielded DNA recoveries concentrations between 9ng/uL and 12ng/uL. For samples 2 to 4 weeks old, the small and medium primer sets yielded concentrations that would be available for STR analysis while the larger primer set did not. For samples 8 weeks old or older, all three primer sets show concentrations less than 0.05ng/uL, with the majority of samples yielding undetectable amounts of DNA. Samples that could be quantified using real-time PCR were then amplified using PowerPlex® 16 and analyzed on an ABI PRISM® 310 Genetic Analyzer. Certain samples were also examined using melt curve analysis and other techniques to determine the presence of inhibition. For many of the buried and brush covered samples, inhibition of the amplification process seems to be occurring as seen in melt curve analysis.

The results of this study indicate that samples can be successfully analyzed using real-time PCR to establish the quality of the sample and that these analyses correspond to a particular success rate in the generation of full profiles.

In addition, the data provides information on the relative rates of loss of nuclear DNA in tissue from recovered bodies.

DNA, Degraded, Environmental

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