H30 Using Real-Time PCR Quantification of Nuclear and Mitochondrial DNA to Develop Degradation Profiles for Various Tissues

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The goals of this study are to develop nuclear and mitochondrial DNA postmortem degradation profiles for various soft and osseous tissues using real-time quantitative PCR; to compare the effectiveness of several DNA extraction and purification methods; and to mitigate the effects of co-extracted PCR inhibitors.

This presentation will impact the forensic community and/or humanity by allowing investigators to optimize compromised tissue sampling, extraction, and purification protocols in order to facilitate the decedent identification process.

DNA based decedent identification methods are dependant on the successful extraction, purification and amplification of template molecules. Oftentimes, samples encountered in forensic contexts are compromised due to postmortem degradation and environmental exposure. As a result, investigators must have a clear understanding of DNA amplification success potential for various tissues prior to sampling. Successful molecular analysis is also dependent on a comparative awareness of extraction and purification method effectiveness. This study explores DNA degradation as well as the isolation and purification capabilities of various extraction methods and inhibitor troubleshooting strategies.

DNA was extracted from lung, liver, spleen, psoas muscle, parietal bones, ribs, vertebral bodies, and femoral shafts obtained over a two year period from 28 pigs: 14 placed in a pond shore environment and 14 deposited on land beneath a broadleaf forest canopy. Tissue samples were extracted using the Armed Forces Institute of Pathology (AFIP) organic extraction protocol along with the Qiagen QIAamp® silica adsorption protocol. A 250 bp nuclear locus and a 226 bp region of the mtDNA D-loop were then amplified using the ABI® Model 7000 Sequence Detector. A sequence specific fluorescent probe was used to estimate initial template copy number in each sample. During amplification the probe hybridizes to the target DNA sequence. TAQ DNA Polymerase subsequently cleaves a fluorescent reporter dye from the hybridized probe. Upon cleavage the reporter dye fluoresces. As additional target amplicons are produced during each PCR cycle, the fluorescent signal increases. Samples containing a greater initial template copy number will produce a detectable fluorescent signal at an earlier amplification cycle (i.e. detection threshold cycle). The relationship between initial template copy number and detection threshold cycle is used to develop a DNA degradation profile for each tissue sampled over the two year postmortem interval. The degradation profiles provide insight as to the suitability of each sample for nDNA or mtDNA analysis given a particular deposition environment and postmortem interval. The amplification efficiency of each successful reaction is also measured to assess the need for further template purification. Complete amplification failure is further evaluated using quality control PCR (qcPCR) to differentiate between amplification inhibition and DNA degradation beyond the limits of analytical sensitivity. Template samples determined to have co-extracted inhibitors are processed further to facilitate amplification. The effects of adding bovine serum albumin, sodium hydroxide, and larger quantities of Taq Polymerase on amplification efficiency are evaluated.

Based on the aforementioned information, investigators will be able to optimize compromised tissue sampling, extraction, and purification protocols in order to facilitate the decedent identification process.

DNA Degradation, Real-Time PCR, PCR Inhibition

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