B183 DNA-Protein Crosslink Reversal and Mitochondrial DNA Amplification From Formaldehyde-Treated Unknowns From the Korean War

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After attending this presentation, attendees will understand the concept of formaldehyde-induced DNA-protein crosslinks and their effect on Polymerase Chain Reaction (PCR). Attendees will further learn how to use heat denaturation to reverse DNA-protein crosslinks for successful DNA amplification. The downstream ramifications of heat denaturation will be explained, and protocol modifications to accommodate single-stranded DNA (ssDNA) will be discussed.

This presentation will impact the forensic science community by offering guidance on how to successfully reverse formaldehyde-induced DNA-protein crosslinks from embalmed human remains.

Of the more than 800 Korean War Unknowns that were embalmed in formaldehyde and buried in the National Memorial Cemetery of the Pacific in Hawaii, only a few dozen have been identified to date. The formaldehyde treatment of the remains inflicted DNA-Protein Crosslinks (DPCs) that inhibit standard PCR amplification. Since the 1990s, attempts to conduct DNA testing of these Unknowns have been largely unsuccessful until Next Generation Sequencing (NGS) technologies became available. In 2015, the Armed Forces DNA Identification Laboratory (AFDIL) validated a hybridization capture and Illumina® sequencing protocol to obtain mitochondrial genome data from these and other degraded DNA samples. While this method was shown to be nearly 100% successful when tested with traditional degraded samples, it proved to be half as robust for the formaldehyde-treated Korean War Unknowns (with a success rate of 47%). Moreover, the DNA that could be sequenced from these Unknowns was shown to be very short in length, approximately 65 base pairs (bp) on average. The observations made over the course of the NGS validation indicated that most of the endogenous DNA was unamplifiable due to the presence of inhibitory DPCs.

To bolster the success rate of DNA testing for this set of Korean War Unknowns, a novel DPC reversal and DNA extraction procedure was recently developed at the AFDIL. The procedure involves standard bone powder demineralization, DPC isolation and denaturation, followed by proteinase K digestion, and DNA extraction and purification. For this, 29 samples and 6 reagent blanks were extracted with varying DPC denaturation temperatures (65°C, 72°C, and 95°C) and DNA purification methods (QIAGEN® EZ1® DNA Investigator, Microcon®, and Amicon® Ultra-4). After DPC reversal extraction, DNA was quantified using the Qubit® fluorometric assay as well as the Plexor® HY System. Amplification was attempted using a modified AmpFℓSTR® Y Filer® technique as well as mitochondrial control region primers. The results indicate that higher temperature (95°C) improves DPC denaturation and enables amplification of 100bp-250bp mitochondrial DNA fragments. Furthermore, this study demonstrated that DPC reversal at high temperature renders DNA single stranded, which has downstream

ramifications. Specifically, ssDNA can lead to DNA loss when silica-based and filter-based purification methods are employed. In addition, the validated NGS library preparation protocol at the AFDIL requires double-stranded DNA substrate. Consequently, DPC reversal requires DNA extraction techniques that are amenable to ssDNA, such as phenol chloroform extraction, as well as a complementary-strand DNA synthesis step prior to NGS library preparation. The results of the DPC reversal extraction and DNA amplification will be presented and future goals for protocol development will be discussed.

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Crosslink, DNA Extraction, Heat Denaturation