



B196 An Analysis of Highly Degraded DNA From Bone Samples Using Probe Capture Enrichment of the Entire Mitochondrial Genome and Next Generation Sequencing

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After attending this presentation, attendees will better understand the efficacy of using probe capture enrichment of the entire mitochondrial genome coupled with next generation sequencing to analyze highly degraded DNA from bone samples.

This presentation will impact the forensic science community by evaluating a method that can be used to successfully analyze highly degraded DNA samples from crime scenes and to identify victims of mass disaster situations.

Forensic DNA samples are often highly degraded, making them unsuitable for traditional methods of DNA analysis such as Short Tandem Repeat (STR) analysis because target sequences and primer binding sites are not intact. Mitochondrial DNA (mtDNA) is useful for analyzing degraded DNA because of its high copy number. Traditional methods of mtDNA analysis, including Sanger sequencing, are commonly used to only target only the HVI/HVII regions of the mtDNA genome, limiting the discriminatory power of mtDNA analysis. Probe capture is a novel technique that uses mtDNA-specific probes to enrich and capture the entire mitochondrial genome. Analyzing the entire mitochondrial genome would allow detection of discriminating information outside of the HVI/HVII regions and increase the discriminatory power of mtDNA. Next generation sequencing, a massively parallel, clonal, and high-throughput technique, is an excellent tool for analyzing the entire mitochondrial genome of degraded DNA and allows for mixture detection and resolution. The purpose of this project was to determine whether probe capture and next generation sequencing on the Illumina® platform could be used to successfully analyze highly degraded mtDNA from bone samples dating to approximately 100, 2,000, and 4,000 years ago.

This optimized probe capture method for enrichment of the entire mtDNA genome was tested on forensically relevant bone samples dating to 19-20 BC, 10-8 BC, and 4000-1000 BC. Seven bone samples recovered from a comingled tomb in Rijeka, Croatia, and dating to approximately 100 years ago were successfully sequenced with coverage of the mitochondrial genome ranging from 52%-100%. The average read length for these samples ranged from 73bp-79bp, demonstrating that the DNA is highly degraded. Six prehistoric bone samples dating to approximately 2,000 years ago were successfully sequenced with coverage of the mitochondrial genome ranging from 26%-100%. The last set of samples was recovered from a necropolis on the island of Korčula, Croatia, and dates to approximately 4,000 years ago. These six samples were successfully sequenced with coverage of the mitochondrial genome ranging from 75%-100%. In this presentation, the results of this study and the historical information gathered from these results will be discussed in further detail.

Degraded DNA, Mitochondrial DNA, Probe Capture