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Sequencing the Mammoth Cytochrome b gene: A Genetic Look Into the History of Prehistoric Columbian Mammoths

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During the excavation process associated with the reconstruction of Cleveland reservoir dam near the Huntington Canyon area of UT, the massive skeleton of a Columbian mammoth was unearthed. Excavation of the remains ensued, and bone samples from this prehistoric mammal were sent to the Molecular Archaeogenetic Research facility at Brigham Young University. These samples were studied in hopes of unlocking many of the mysteries associated with this ancient animal.

Utilizing modern extraction, amplification, and sequencing techniques, this project set out to sequence the mammoth cytochrome b gene. Such sequences, if attained, would allow for evolutionary comparisons between prehistoric mammoths, and modern relatives.

This study proceeded on bone samples that were excavated from the Huntington Canyon site as stated previously. Investigation involved a four-step process: DNA extraction from the mammoth bone, amplification of the extracted DNA, sequencing of the samples, and analysis and comparison of the ancient samples to samples of modern relatives.

As stated, the first step in the process is to extract the DNA from the mammoth bone fragments. This was accomplished by drilling through the outer sheath of bone (periosteum), and pulverizing the tissue next to the interior surface of the sheath. The powdered bone was then collected and DNA was extracted using glass beads or ion exchange chromatography extraction protocols.

After extraction, the material was initially amplified using a technique termed primer extension preamplification (PEP). This technique utilizes primers that bind non-specifically across the entire genome, amplifying all available DNA, and repairing any damage that may have occurred after the death of the animal. Following this PEP reaction, specific segments of interest, in this case the cytochrome b gene, were amplified utilizing polymerase chain reaction (PCR). This method consists of creating an environment in which specific sequences of DNA can be made millions of times in a short time period. Mitochondrial DNA (such as that studied here) is especially conducive to amplification in ancient samples, because numerous copies of this distinctive DNA exist within a single cell.

The final steps attempted were to sequence the DNA fragments and compare this data to that of modern sequences of living animals. Such comparisons we hoped would elucidate evolutionary relationships between mammoths and their modern relatives.

Unfortunately there were some difficulties associated with amplifying the ancient DNA. Many of the chemicals used to extract ancient DNA inhibit amplification processes. Careful balances between chemicals must be met to ensure that the DNA is extracted, and yet a significantly small amount of these chemicals must be used to ensure that amplification can take place. Thus this

project met with mixed results. While much of the cytochrome b gene has been sequenced at this point, several key areas remain under investigation. Doubtless new procedures that allow for more accurate means of amplification would help much in this endeavour. As things now stand the project relies much on repetition. In other words, a number of attempts improve your chances of isolating DNA, but do not guarantee such success.

In closing, while not every single aim I had in mind was met while working on this project, I still think that I accomplished much in terms of scientific based research. While I became familiar with several frustrations associated with this kind of work, I also became well aware of the joys that even small clinical successes can produce.