B67 Sequencing of Highly Degraded, Ancient DNA From *Metasequoia glyptostroboides*

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After attending this presentation, attendees will understand the endeavors that must be considered when working with an ancient DNA sample that is in low copy number and highly degraded.

This presentation will impact the forensic community and/or humanity by expanding on low copy number applications and devising methods that can be applied to highly degraded or low copy number forensic casework samples and substrates where nuclear DNA is not able to be obtained.

Metasequoia glyptostroboides is a conifer that was prevalent in the middle-Eocene-age forests of the Napartulik, Axel Heiberg Island, within the Canadian High Arctic. These forests have been dated to be 45 million years old and today exist as mummifications. Previously, chloroplasts of *Metasequoia glyptostroboides* were found to be intact by transmission electron microscopy. Chloroplasts have their own DNA that is found in multiple copies in the cell and are more likely to be retrieved than nuclear DNA in an ancient and/or degraded sample. Additionally, plant tissues are inherently challenging as they are considerably richer in primary or secondary metabolites (than animal tissues) and these can be potential inhibitors of PCR. Due to the conservation of the arrangement of many genes in the chloroplast DNA, such as tRNA genes, numerous "universal" primers have been designed and are available for use in molecular evolutionary studies.

This work attempts to sequence a non-coding tRNA intergenic spacer region of *Metasequoia glyptostroboides*. To date, neither animal nor plant sequence data has been reported from samples older than 18 million years old. Three different extraction methods were used for DNA extraction for the ancient samples, fresh Dogwood samples (positive control), and reagent blanks (negative control) including organic, Qiagen DNeasy Plant Mini Kit, and cetyltrimethylammonium bromide (CTAB) with polyvinyl pyrolidone (PVP). Yield gels were run to gauge extraction success. All samples were amplified using the conserved primer pair *trn*L-F, which produces an expected product size of 289bp in *Metasequoia glyptostroboides*. This primer pair is between the tRNA genes *trn*L and *trn*F that code for Leucine and Phenylalanine, respectively. Product gels were run to gauge success of PCR amplification and product cleanup was performed using Rapid PCR Purification Systems. Cycle sequence reactions were performed using the ABI Big Dye Terminator v.3.1 Cycle Sequencing Kit and DTR Gel Filtration Cartridges were used for cycle sequencing cleanup. Separation and detection was performed using the ABI 3100-*Avant* Genetic Analyzer. ABI Sequencing Software v.5.1.1 and Sequencher v. 4.1.4FB19 were used for analysis. Sequencing success was compared between the three different extraction methods to determine which, if any, is most appropriate for the ancient *Metasequoia* samples. Sequencing success was determined by contiguous base pair length of the sequence obtained, if any. Extracts were then subjected to Amersham Biosciences Templiphi 100 kit for whole genome amplification (pre- tRNA amplification) to attempt to increase sequencing success.

Sequence data was obtained for positive controls extracted by the organic and CTAB methods (365 bp and 394 bp length of read, respectively). No product was observed nor was sequence data obtained from the ancient *Metasequoia* samples. However, after whole genome amplification, yield gels showed DNA smears for *Metasequoia* extracts in each of the extraction methods. Unfortunately, these samples failed to produce the expected band after tRNA amplification.

Future direction of this work includes amplifying *Metasequoia* extracts from the three extraction methods with other conserved primer pairs to explore sequence data in other regions of the chloroplast genome. Whole genome amplification will continue to be explored as a pre-amplification step in an attempt to increase sequencing success for this highly degraded ancient DNA sample.

Degraded DNA, Ancient DNA, LCN

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