Lakehead UNIVERSITY | Paleo-DNA Laboratory
Centre for Analytical Services

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SER012-18 Supplemental Report

File #: SER012-18 Supplemental

Date: 04 May 2018

Report of Expert

Expert's Name: Stephen Fratpietro, M.Sc., B.Ed.
Title: Technical Manager, Paleo-DNA Laboratory

I, the undersigned, as requested by David Noble, Kettle Creek Battlefield Association, submit my professional opinion in reference to the following matter: This examination of exhibits is connected to an ancient DNA analysis.

ITEMS EXAMINED:
The following items (see Table 1) were submitted for genetic analysis by David Noble, Kettle Creek Battlefield Association. These samples were designated the following case and sample number by the Paleo-DNA Laboratory (PDL):

PDL Case Designation	PDL Sample Designation	Sample Type	Comments
SER012-18	4	Soil	Target I
SER012-18	5	Soil	Target II
SER012-18	6	Soil	Target III
SER012-18	7	Soil	Target IV
SER012-18	8	Soil	Target V
SER012-18	9	Soil	Target VI

Table1. Samples submitted to the Paleo-DNA Laboratory.

EXAMINATION REQUESTED: Ancient DNA Analysis: extraction of DNA and human mitochondrial DNA feasibility test.

REQUIREMENTS REQUESTED: Determine if any genetic information could be extracted from the sample. Unless otherwise discussed, the industry standard extraction, purification and amplification protocols were to be used and attempted in this case.

The Paleo-DNA Laboratory agreed to work on the project in accordance with high scientific and professional standards, but as we had not been involved with the collection and storage of the sample, nor have we inspected the sample, nor have we assessed the condition of the sample, the Paleo-DNA Laboratory did not promise success in achieving any desired result. The Paleo-DNA Laboratory undertook this project giving no warranty of fitness for a particular purpose, or any other warranty,

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expressed or implied, on the results of your project or the tests carried out pursuant to your project. This includes no guarantee or warranty that the recommended protocol will achieve your desired results.

EXAMINATION METHODOLOGY:

All soil samples are prepared pre-amplification in a room dedicated specifically to limited quantity DNA samples. This environment is monitored quarterly for the presence of DNA. This lab has restricted access and requires protective gear to be worn at all times: tyvek suit covering head and feet, gloves, hairnet, facemask. All persons entering this lab have their DNA profiled and kept for future comparison.

DNA Extraction

DNA extraction was performed on ~500mg of soil sample using the FastDNA™ Spin Kit for Soil (MP Biomedicals) as per manufacturer's instructions. This extraction methodology was performed in duplicate.

PCR Amplification

DNA is amplified in 25uL reactions using Quanta Biosciences™ AccuStart™ II PCR Supermix (2X) with 12.5uL of AccuStart II PCR Supermix (2X), 0.25uL of 10uM each primer, 3-12uL template. Cycling parameters: hot start of 94° for 2 min, and 50 cycles of 94°C for 30s, 60°C for 1 min., 72°C for 2 min.

The amplicon size is approximately 229bp in length and spans half of the human mitochondrial HV1 region. Primers used amplify the region mt16191-16420.

Primer Information:

16191F	5'-CCC ATG CTT ACA AGC AAG TA-3'	Kolman etal. 2000. Am. J. Phys. Anthropol. 111(1): 5-23
16420R	5'-TGA TTT CAC GGA GGA TGG TG-3'	Vigilant etal. 1989. PNAS. 86: 9350-9354

Each PCR reaction batch includes a positive and negative PCR control.

GEL Electrophoresis

PCR products are mixed with a dye and loaded onto a 6% Polyacrylamide Gel (PAGE) that uses electricity to separate any DNA products produced by the PCR reaction. The gel is stained with ethidium bromide that binds to the DNA in the gel and fluoresces under ultra violet light. A picture is taken for visual verification of amplification products

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present within the PCR reaction. Each primer region will produce a DNA band of a specific size if DNA is present.

Successful PCR products are purified by mixing 20uL of PCR product with 2uL *Exo I* nuclease [Lucigen] and 4uL of Shrimp Alkaline Phosphatase (SAP) [Thermo Fisher]. The mixture is incubated at 37ºC for 15 minutes, then the enzymes are deactivated at 80ºC for 15 minutes.

Sequencing

Purified PCR products are direct sequenced with the Life Technologies Big Dye Terminator Ready Reaction Kit v3.1 in both the forward and reverse direction. 0.5uL Big Dye Terminator Ready Reaction Mix v3.1, 0.25uL 10uM primer, 2uL 5x Big Dye Terminator Sequencing Buffer, 4.2uL of sterile water, and 3uL purified PCR Product. Cycling parameters: Hot Start of 96°C for 60s; 15 cycles of 96°C for 10s, 50°C for 5s, 60°C for 75s; 5 cycles of 96°C for 10s, 50°C for 5s, 60°C for 90s; and 5 cycles of 96°C for 10s, 50°C for 5s, 60°C for 2 min. Sequencing products are purified with a sodium acetate/ethanol precipitation as per Applied Biosystems Automated DNA Sequencing Chemistry Guide. Sequencing products are resuspended in 15uL Hi-Di Formamide and run on the ABI 3130xl for sequencing analysis.

Mitochondrial sequencing data is edited and aligned to the Revised Cambridge Reference Sequence using Gene Codes Sequencher™ Software v4.10.1

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RESULTS: The results below relate only to the items tested.

Below are the findings for two independent DNA extractions performed on each soil sample.

Sample	Extraction 1	Extraction 2
Target I	No DNA Detected	No DNA Detected
Target II	Human mtDNA Detected	Human mtDNA Detected
Target III	Human mtDNA Detected	No DNA Detected
Target IV	Human mtDNA Detected	No DNA Detected
Target V	Human mtDNA Detected	No DNA Detected
Target VI	No DNA Detected	Human mtDNA Detected

Human mitochondrial DNA analysis is a very sensitive procedure where extremely small amounts of human DNA can be detected. In this batch of samples, human mtDNA was detected in at least one or both DNA extractions for Target II, Target III, Target IV, Target V, and Target VI. The variation in results could be due to variations in human DNA concentration within the soil composition.

It is important to note that it cannot be discerned from this analysis whether the DNA detected in these soil samples is from remains previously buried in it or from modern contamination.

NOTES:
Controls were run at every step of the analysis and gave expected results. This analysis complies with the requirements requested by the client. Details of the experimental procedures and analysis of this case are found in the case file of the Paleo-DNA laboratory, case number SER012-18. Your feedback is important to us! Please fill out our customer survey at http://lucas.lakeheadu.ca/customer-survey.

Technical Manager: Stephen Fratpietro Date: 07 May 2018

Stephen Fratpietro