LAKEHEAD UNIVERSITY
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SER036-11 Final Report

File #: SER036-11

Date: 08 September 2011

Report of Expert

Expert's Name: Stephen Fratpietro
Title: Technical Manager, Paleo-DNA Laboratory

I, the undersigned, as requested by Carrie Sulosky, submit my professional opinion in reference to the following matter: This examination of exhibits is connected to a genetic heritage inquiry to determine ethnicity & sex.

ITEM EXAMINED:

The following items (see Table 1) were submitted for genetic analysis by Carrie Sulosky. These samples were designated the following case and sample number by the Paleo-DNA Laboratory (PDL):

PDL Case Designation	PDL Sample Designation	Sample Type
SER036-11	1	Tooth

Table1. Samples submitted to the Paleo-DNA Laboratory.

EXAMINATION REQUESTED: Genetic Analysis using mitochondrial DNA (mtDNA) to determine ethnic background and nuclear DNA (nDNA) to determine the sex of the individual.

REQUIREMENTS REQUESTED: Determine if any genetic information could be extracted from sample using mtDNA and nDNA. Unless otherwise discussed, the industry standard extraction, purification and mitochondrial & sexing 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

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purpose, or any other warranty, 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:

The type of genetic analysis agreed to for the sample is as follows:

1) Extraction and purification of DNA.
2) PCR analysis using mitochondrial DNA primers and amelogenin sexing primers.
3) Visualization of PCR product using gel electrophoresis.
4) Direct sequencing PCR analysis of amplified product.
5) Separation of sequence product using capillary electrophoresis.
6) Analysis of data, if any.

Detailed Methodology

Sample Preparation: The tooth was surface sterilized with 10% bleach, rinsed with sterile water, and dried with 70% ethanol. Next, the tooth was milled into a fine powder and divided into aliquots.

Extraction and Purification: A total demineralization extraction, utilizing EDTA and Proteinase K was performed on the sample followed by a silica bead purification and additional size exclusion column purification.

Polymerase Chain Reaction: Human nuclear DNA was quantified using the Applied Biosystems Quantifiler® kit. A standard Platinum Taq DNA Polymerase PCR reaction was performed using primers specific for the human mtDNA hypervariable regions (HVI base pairs 16024-16365, HV2 base pairs 73-340) and the amelogenin region for sexing. Amplicon targets were 100 - 300 bp sizes.

Gel Electrophoresis: All PCR reactions were run on a 6% Polyacrylamide Gel stained with ethidium bromide for visualization of PCR product.

Sequencing: Any PCR product obtained was purified with Applied Biosystems recommended purification protocols, direct sequenced with Applied Biosystems Big Dye Terminator Chemistry and run on the ABI 3100 Genetic Analyzer.

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RESULTS: The results below relate only to the items tested. A mitochondrial DNA profile spanning hypervariable region 1 (HV1) and hypervariable region 2 (HV2) was obtained for each sample submitted. The results from replicating the data is identical, therefore the resulting calculated uncertainty is zero for these test samples. Mitochondrial DNA (mtDNA) is passed down through the maternal line. Only females pass on their mtDNA profile to their offspring. A mtDNA profile is reported in Table 2 as differences from a universal mtDNA reference sequence called the Revised Cambridge Reference Sequence (RCRS). The base position and base call of the differences from the RCRS is stated below.

Sample	mtDNA nucleotide base positions			
	HV1	HV2		
	16129	73	263	315.1
RCRS	G	A	A	-
Sample 1	A	G	G	C

Table 2. Final mitochondrial DNA results obtained in 100% of sequences analyzed. Regions analyzed 15971-16420bp, 1-389bp. 'RCRS' is the Revised Cambridge Reference Sequence . ' – ' is an undefined base position.

The above profile in Table 2 is indicative of Haplogroup 'H'.

Sample	mtDNA nucleotide base positions								
	HV1				HV2				
	16129	16223	16294	16311	73	199	250	263	315.1
RCRS	G	C	T	T	A	T	T	A	-
Sample 1	A	T	Y	Y	G	Y	Y	G	C

Table 3. Final mitochondrial DNA results obtained in 50% - 100% of the sequences analyzed. Regions analyzed 15971-16420bp, 1-389bp. 'RCRS' is the Revised Cambridge Reference Sequence . ' – ' is an undefined base position. 'Y' is an undetermined pyrimidine (C or T).

The above profile in Table 3 is indicative of Haplogroup 'M'.

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Table 4 represents polymorphisms only, separated into levels of certainty; polymorphisms found in 100% of the generated sequences, 50%-100% of the generated sequences, and polymorphisms found in less than 50% of the generated sequences, which are most probably artifacts of damaged DNA or contamination. Common Polymorphisms found in 100% of the data generated for each sample were deduced to be the profile unique to the individual sample and placed in Table 2, however, polymorphisms represented in Table 3 cannot be discarded and must be considered . Polymorphisms of Probable Damage/ Contamination were not deemed true polymorphisms of the sample due to their random occurrence and inability for replication but are noted to show the complete analysis.

Sample	Common Polymorphisms (100%)	Possible Polymorphisms (>50%)	Probable Damage/Contamination (<50%)
Sample 1	16129A, 73G, 263G, 315.1C	16223T, 16294Y, 16311Y,199Y, 250Y	16223C, 195C, 198T, 309.1C

Table 4. These profiles are represented by the nucleotide position and the nucleotide polymorphism eg. 16223T. 'Y' is an undetermined pyrimidine (T or C).

No nuclear DNA was detecting using the Quantifiler® Human DNA Quantification Kit. An attempt was done on the sample to determine sex. No amelogenin sexing results could be obtained.

The combination of replication, fragment sizes obtained, difficult to obtain results, no nuclear DNA present, procedures in place for laboratory sterilization and elimination of Paleo-DNA Laboratory DNA profiles suggest the results are authentic and not contamination. However, no modern comparison samples were submitted with this batch from the archaeologists or any other individual who may have handled the sample and potentially contaminated it. Therefore, we cannot guarantee that these profiles are authentic and not from a previous handler.

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NOTES:

The above profiles do not match any staff member or laboratory user at the Paleo-DNA Laboratory, past or present. 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 SER036-11. Feel free to fill out our customer survey at: http://lucas.lakeheadu.ca/customer-survey.

Technical Manager: _____ Date: 08 Sept 2011

Stephen Fratpietro

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