

SER019-17 Final Report

File #: SER019-17

Date: 28 March 2017

Report of Expert

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

I, the undersigned, as requested by Chase Kloetzke, The Field Reports & Technology Group, 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 Chase Kloetzke, The Field Reports & Technology Group. This sample was designated the following case and sample number by the Paleo-DNA Laboratory (PDL):

PDL Case Designation	PDL Sample Designation	Sample Type	Comments
SER019-17	1	Tooth	1 part Maxilla with an exposed tooth

Table1. Samples submitted to the Paleo-DNA Laboratory.

EXAMINATION REQUESTED: Ancient DNA Analysis: extraction of DNA, mitochondrial DNA profiling and nuclear DNA feasibility test.

REQUIREMENTS REQUESTED: Determine if any genetic information could be extracted from 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, 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.

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EXAMINATION METHODOLOGY:

All aDNA 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.

Sample Preparation

The tooth is surface cleaned with sterile water, 70% ethanol and another washing of sterile water. The tooth is then milled into a fine powder using a mixer mill.

DNA Extraction

Total Demineralization [Loreille et al, 2007]:

Approximately 0.57g of sample powder is mixed with 9.0mL 0.5M EDTA, 150uL 20% Lauryl Sarcosinate, and 50uL Proteinase K (20mg/mL) in a sterile 15.0mL tube. This reaction is incubated overnight at 56°C with gentle agitation. The resulting supernatant is transferred to next step.

Silica Bead Purification [modified Boom et al, 1990]:

The supernatant is mixed with 18mL 4M Guanidinium Thiocyanate and 15uL silica. This is allowed to sit for 4 hours at 4°C [to allow DNA to bind to silica] after which the supernatant is removed and the remaining silica washed with Working Wash Buffer (10mM Tris-HCl, 50mM NaCl, 1mM EDTA, anhydrous ethanol) and 100% ethanol, then allowed to dry. The silica is resuspended in 55uL sterile water and incubated for 1 hour at 56°C to allow DNA to unbind from silica and dissolve in the water. The resulting supernatant is transferred to the next step.

Size Exclusion Column Purification [Matheson et al, 2009]:

The purified DNA extract is further filtered using Biorad Micro Bio-Spin P30 Chromatography Columns as per manufacturer's instructions.

****It is important to note that an extraction control (negative) is carried through this entire process as a quality control measure.****

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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, 3uL 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 sizes are approximately 250bp in length and overlap in order to span the human mitochondrial HV1 and HV2 region. Primers used amplify regions mt15971-16258, mt16191-16420, mt1-280, mt155-389.

Primer Information:

15971F	5'-TTA ACT CCA CCA TTA GCA CC-3'	AFDIL Primer
16258R	5'-TGG CTT TGG AGT TGC AGT TG-3'	AFDIL Primer
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
1F	5'-GAT CAC AGG TCT ATC ACC C-3'	PDL
280R	5'-GAT GTC TGT GTG GAA AGT GG-3'	PDL
155F	5'-TAT TTA TCG CAC CTA CGT TC-3'	AFDIL Primer
389R	5'-CTG GTT AGG CTG GTG TTA GG-3'	AFDIL Primer
429R	5'-CTG TTA AAA GTG CAT ACC GCC-3'	PDL

AFDIL (Armed Forces DNA Identification Laboratory) developed primer.
PDL (Paleo-DNA Laboratory) developed primer.

Amplicons and Length for aDNA analysis.

15971F – 16258R = 287bp
16191F – 16420R = 229bp
1F – 280R = 279bp
155F – 389R = 234bp

Each PCR reaction batch includes a positive and negative PCR control as well as the negative extraction control. Each amplicon is amplified at least twice for replication.

Quantification

Nuclear DNA is targeted using Life Technologies Quantifiler™ Human DNA Quantification kit as per manufacturer's instructions run on the Cepheid Smart Cycler® II.

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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 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 37°C for 15 minutes, then the enzymes are deactivated at 80°C 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.

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							
	16223	16234	16298	16325	16327	72	73	194	249	263	290	291	315.1
RCRS	C	C	T	T	C	T	A	C	A	A	A	A	-
Sample 1	T	T	C	C	T	C	G	T	del	G	del	del	C

Table 2. Final mitochondrial DNA results obtained. Regions analyzed 16024-16365bp, 73-340bp. 'RCRS' is the Revised Cambridge Reference Sequence. '-' is an undefined base position. 'del' is a deleted base position.

The above profiles in Table 2 are indicative of mitochondrial Haplogroup 'C1'.

Mitochondrial DNA haplogroup C (Chochmingwu) likely arose on the high plains of Central Asia between the Caspian Sea and Lake Baikal around 50,000 years ago. C is considered to be a characteristic Siberian lineage, and currently accounts for over 20 percent of the entire mitochondrial gene pool in the area. In addition to Siberia and Asia, haplogroup C is also found in the Americas and is one of five mtDNA haplogroups found in the indigenous peoples of the Americas, the others being A, B, D, and X.

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Region	Haplogroup C (%)
Middle East	1
East Europe	2
Caucasus	4
Central Asia	12
East Asia	5
Siberia	30
North America	18
South and Central America	21

Table 3. Estimated Worldwide Haplotype frequencies of 'C' in various regions. Data not available for regions not shown. Excerpt from Mitomap.org.


 The results for the nuclear DNA feasibility test were positive. There is sufficient nuclear DNA detected for future testing opportunities.

The combination of replication, fragment sizes obtained, 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 a previous handler.

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NOTES:

Controls were run at every step of the analysis and gave expected results. 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 SER019-17. Your feedback is important to us! Please fill out our customer survey at <http://lucas.lakeheadu.ca/customer-survey>.

Technical Manager: 
Stephen Fratpietro

Date: 31 March 2017