LAKEHEAD UNIVERSITY
Paleo·dna
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ELECTRONIC REPORT

File #: SER044-09

Date: 19 June 2009

Report of Expert

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

I, the undersigned, as requested by Dr Leila Hadj-Chikh, submit my professional opinion in reference to the following matter: This examination of exhibits is connected to a universal genetic identification.

ITEM EXAMINED:

The following item (see Table 1) was submitted for genetic analysis by Dr. Leila Hadj-Chikh. These samples were designated the following case and sample number by the Paleo-DNA Laboratory (PDL):

Company Designation	PDL Case Designation	PDL Sample Designation	Sample Type	Description
Erickson 2009-06-01	SER044-09	1	blood	Blood stain on paper plate

Table1. Samples submitted to the Paleo-DNA Laboratory.

EXAMINATION REQUESTED: Genetic Analysis using universal detection to determine species of the blood.

REQUIREMENTS REQUESTED: Determine if any genetic information could be extracted from sample using universal detection primers of mitochondrial DNA. 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:

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

1) Extraction and purification of DNA.
2) Quantification of Human/Primate DNA.
3) PCR analysis using universal detection primers.
4) Visualization of PCR product using gel electrophoresis.
5 Sequencing PCR analysis of amplified product.
6) Separation of sequence product using capillary electrophoresis.
7) Analysis of data, if any.

Detailed Methodology

Sample Preparation: The blood stain was removed with two sterile cotton swabs and moistened with sterile water.

Extraction and Purification: An industry standard extraction utilizing Proteinase K was performed on each swab including reagent control. Each sample was purified using a silica bead purification.

Quantification: Applied Biosystem's (AB) Quantifiler™ kit was used to detect the presence of nuclear DNA (nDNA) using real-time PCR detection. This kit can detect DNA from human , higher ape DNA samples (chimpanzee, gorilla, and orangutan), and to a lesser extent macaque monkeys.

Polymerase Chain Reaction: A standard Platinum Taq DNA Polymerase PCR reaction was performed using universal primers specific for the 12S and Cyt B region of mitochondrial DNA. 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 AB recommended purification protocols, direct sequenced with AB Big Dye Chemistry and run on the ABI 3100 Genetic Analyzer.

RESULTS: The results below relate only to the items tested. DNA was extracted from the blood on the swabs. The data obtained from the nDNA quantification was positive in terms of detecting an amount of nDNA present

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within the blood sample. This indicates the sample contains either human or higher ape nDNA.

The DNA extracted from the swabs was subjected to a universal DNA detection test which produced a readable DNA sequence below (in duplicate) for the 12S region of the mitochondrial DNA.

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GCGTAAGAGTGTTTTAGATCACCCCCTCCCCAATAAAGCTAAAACTCACCTGAGTTGTAA
AAAACTCCAGTTGACACAAAATAGACTACGAAAGTGGCTTTAACATATCTGAACACACAA
TAGCTAAGACCCAAACTGGGATTAGATACCCCACTATGCTTAGCCCTAAACCTCAACAGT
TAAATCAACAAAACTGCTCGCCAGAACACTACGAGCCACAGCTTAAAACTCAAAGGACCT
GGCGGTGCTTCATATCCCTCTAGAGGAACCTGTCCTATAATCGACA
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GTT-T-GATGGCCCTGTCCTATAATCGCTCAAACTCACCTGAGTTGTAAAAAACTCCAGT
TGACACAAAATAGACTACGAAAGTGGCTTTAACATATCTGAACACACAATAGCTAAGACC
CAAACTGGGATTAGATACCCCACTATGCTTAGCCCTAAACCTCAACAGTTAAATCAACAA
AACTGCTCGCCAGAACACTACGAGCCACAGCTTAAAACTCAAAGGACCTGGCGGTGCTTC
ATATCCCTCTAGAGGAACCTGTCCTATAATCGACA-T
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When the DNA sequence was compared to a genetic database (NCBI BLAST, Basic Local Alignment Search Tool, nucleotide database), it identified the DNA as belonging to *Homo sapiens.* An independent alignment of these sequences to the universal human mtDNA reference sequence called the Revised Cambridge Reference Sequence (RCRS) was done. The alignment indicated a 100% match to *Homo sapiens* mitochondrial DNA. Multiple mutations are present in other higher primates within this region.

The DNA extracted from the swabs was also subjected to a universal DNA detection test for the Cyt B region of the mitochondrial DNA. No results were obtained. The primers used for the Cyt B region will detect birds, mammals, reptiles, and fish but are not human-specific.

Based on the above findings, it is my opinion that the blood stain is of human origin.

RECOMMENDATIONS:

Analysis of the human mtDNA hypervariable region may help confirm the maternal origin of the blood stain whether human or something that hasn't been seen yet.

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NOTES:

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 SER044-09. Feel free to fill out our customer survey at: http://lucas.lakeheadu.ca/customersurvey.

Technical Manager: _____ Date: 19 June 2009

Stephen Fratpietro