

GENETECH

54 Kitulwatte Road, Colombo 08, Sri Lanka. CO 00800

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PRELIMINARY REPORT Ancient DNA

October 16th 2017

Our Ref No: GC 4516/23/09/2017

Preliminary report 1 on DNA analysis

A sealed cardboard box containing five plastic vials was received by us on 23rd September 2017. Seals of the cardboard box was found to be intact at the time of receipt.

Plastic vials with the following items (Table: 01) were submitted for genetic analysis by Vitaly Safarov for genetic analysis.

Five samples were designated the following code numbers prior testing by Genetech.

Table: 01

Genetech code number	Label on the container	comments
GC 4516/S1/TB	Sample 1 MARIA Tail Bone	A brownish colour material
GC 4516/S2/MF	Sample 2 MARIA FOOT	Light brownish colour particles
GC 4516/S2-B/HT	Sample 2-A MARIA Hand tissue	brownish colour particles
GC 4516/T8/SB	Tube 8 Baby SPINE BONE	A small fragment of a hard material
GC 4516/T9/H	Tube 7 Baby HAIR	15 small hair shafts

Background to the analysis

Chromosomal DNA (nuclear DNA) testing: amplification of DNA loci on human chromosomal DNA which exhibit a high degree of variation between individuals. These loci contain Short Tandem Repeat (STR) units of DNA and the number of repeats varies between individuals. The variable numbers of repeating units in the population observed for a given locus are referred to as "alleles", and the alleles are named after the number of repeating units that they contain. Each individual inherits two alleles (or two copies of one allele) from his/her biological mother and father. Following PCR, the products are genotyped and alleles are visualized as DNA profiles. The allelic profile with respect to each STR locus is referred to as the DNA profile of an individual. Each person's DNA profile is different from that of every other individual. The only exception is identical twins, who share 100 percent identical DNA.

All samples are prepared and extracted in a room dedicated to biological samples that are having limited quantity of DNA. High scientific and professional standards were strictly adhered when handling the samples in order to ensure the quality of the test results.

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- **DNA extraction** : Column based DNA purification method of DNA extraction. QIAGEN, Germany.
- **PCR amplification**: AmpF/STR Identifiler PCR Amplification Kit (Applied Biosystems, USA)
The DNA locations in AmpF/STR Identifiler kit are **specific to primates**.
- **Genotyping**: 3500xL Automated Genetic Analyzer. (Applied Biosystems, USA)
- **Data analysis**: GeneMapper ID-X Software. (Applied Biosystems, USA)

Results:

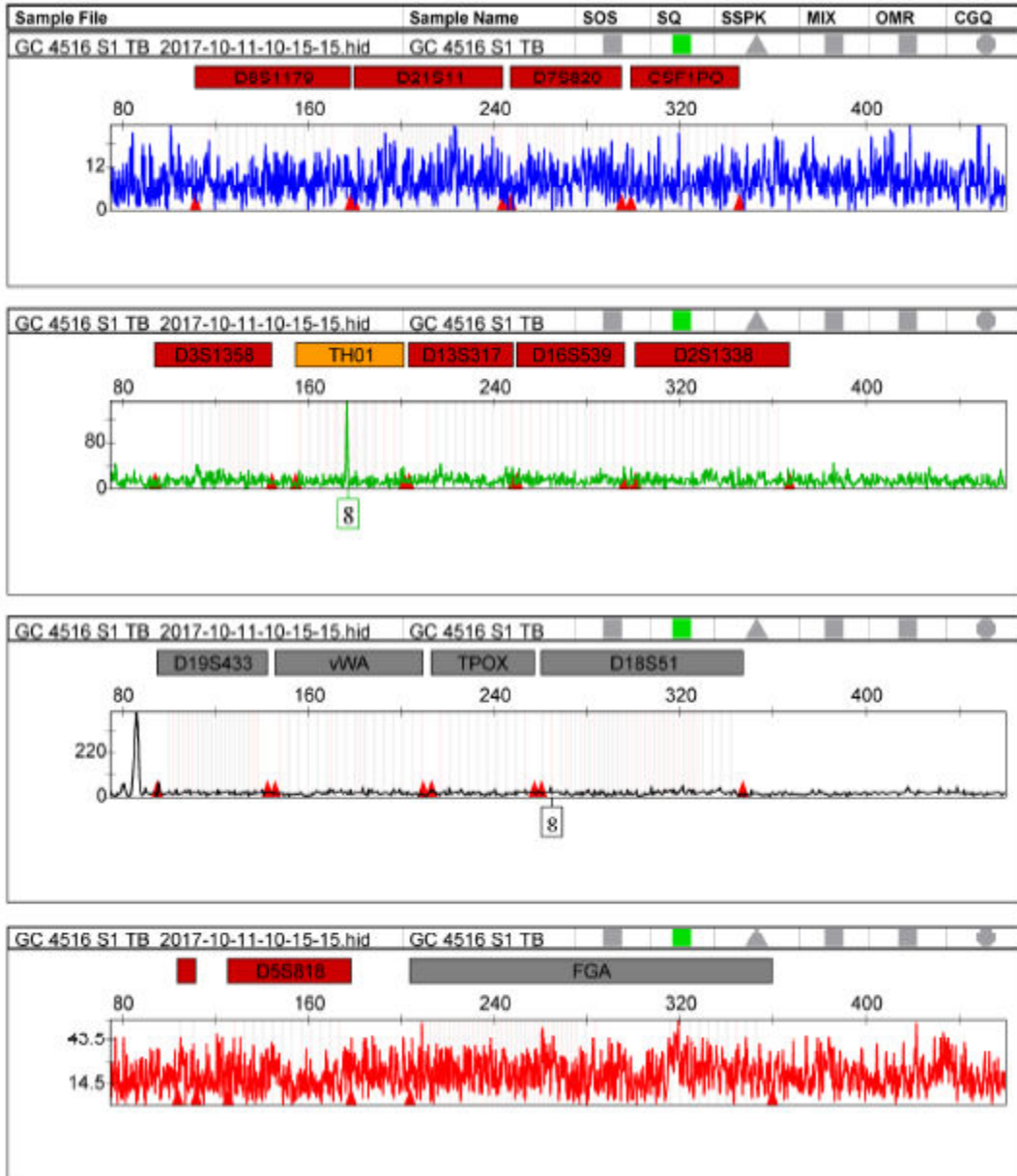
Sample label: **MARIA Tail Bone**

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GeneMapper® ID-X 1.5



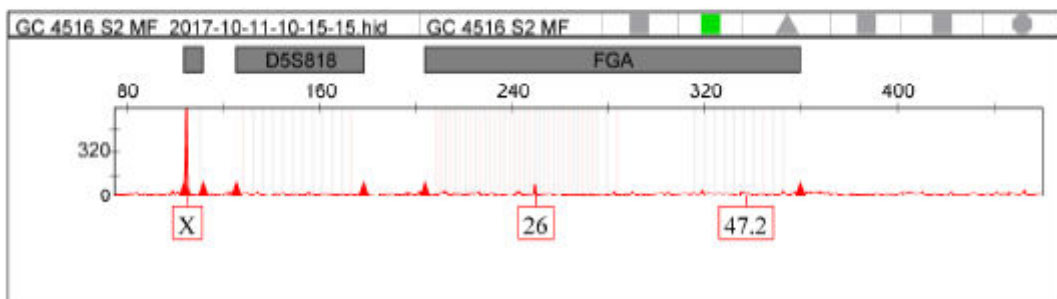
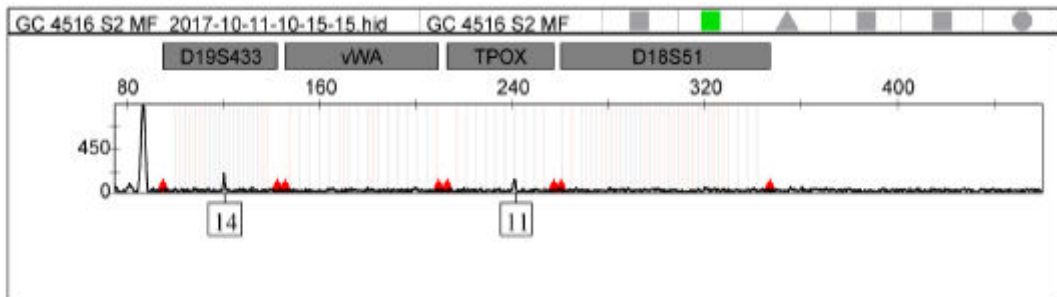
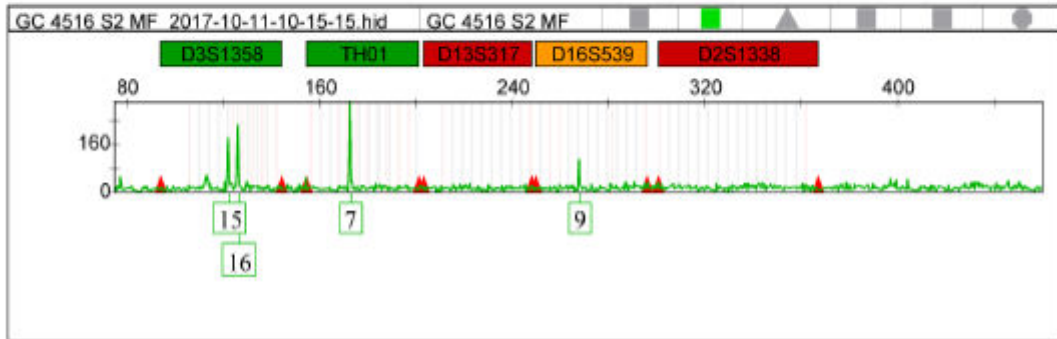
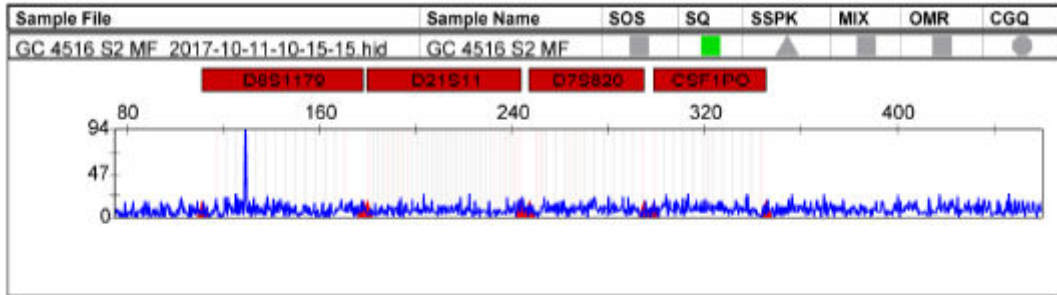
Sample label: **MARIA FOOT**

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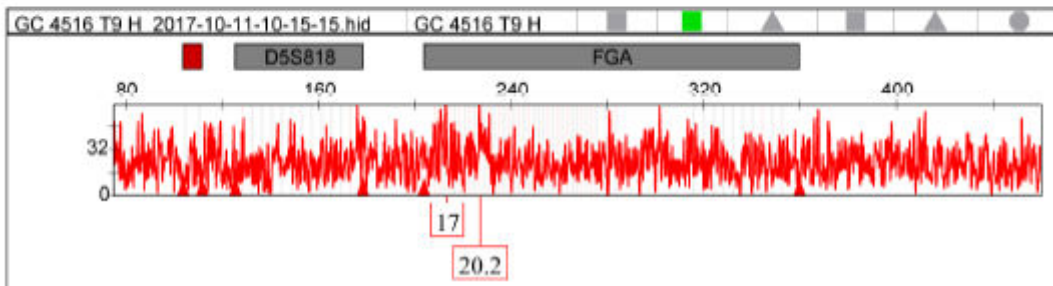
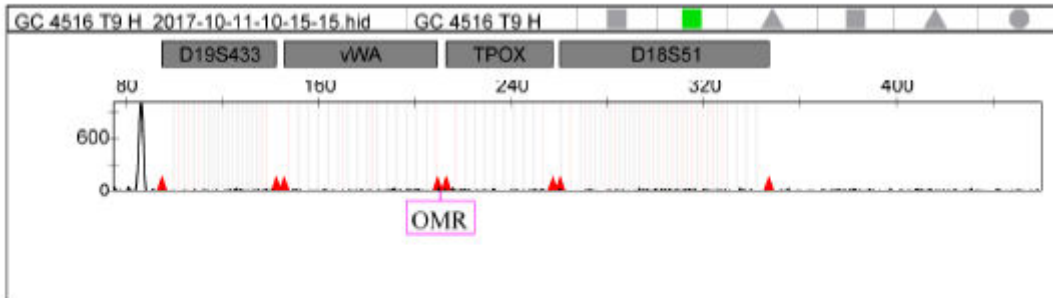
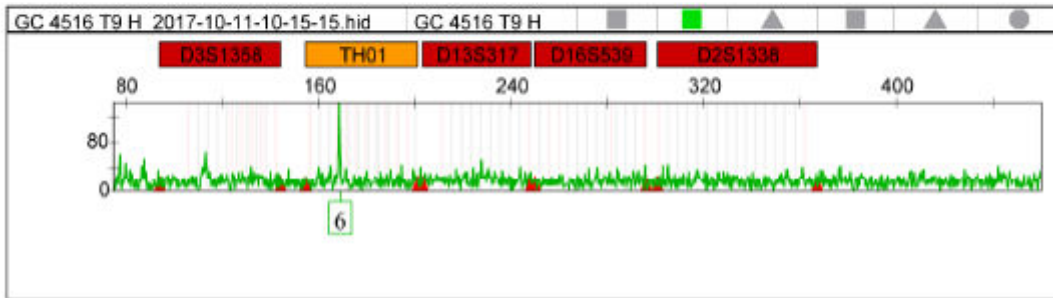
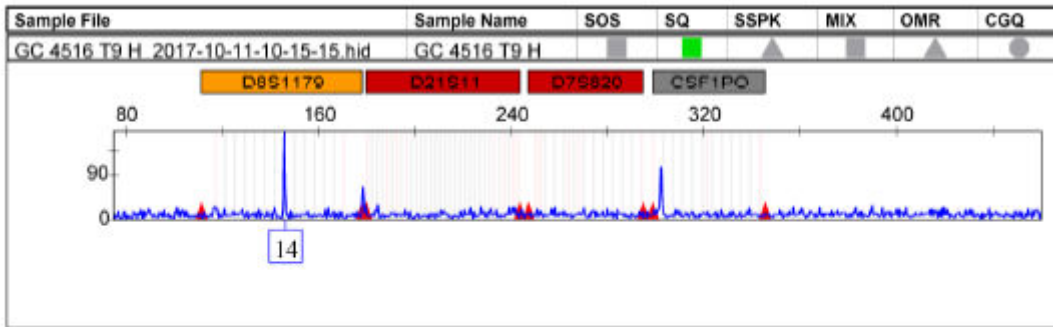
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Sample label: **Baby HAIR**

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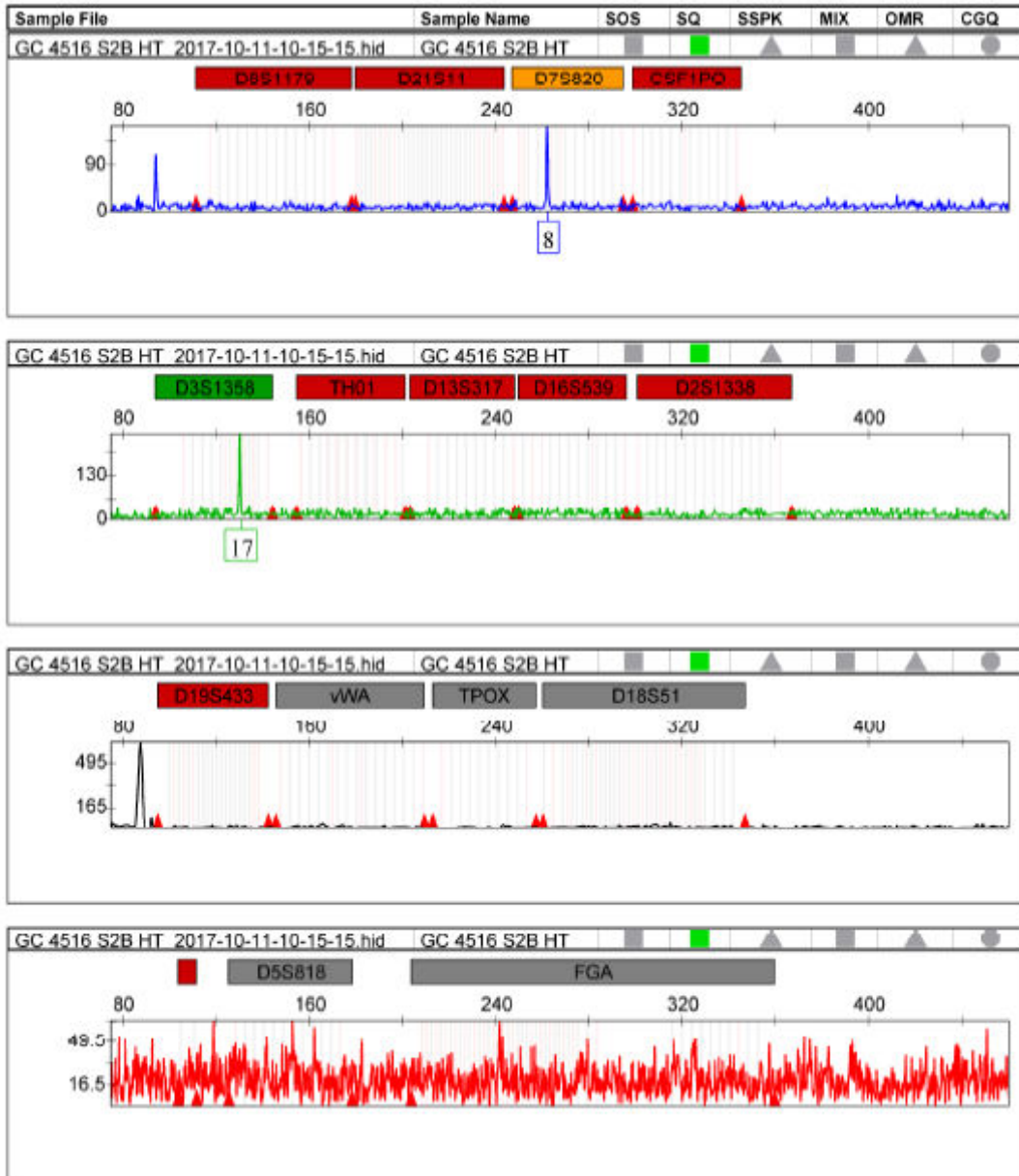
Sample label: **MARIA Hand tissue**

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Baby SPINE BONE: Quality of nuclear DNA was found to be not satisfactory to perform testing. Sample is being re-analyzing.

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Interpretation:

1. MARIA FOOT

MARIA FOOT generated a partial DNA profile having allelic data from only five (05) out of 15 Non-sexchromosomal DNA locations tested.

This result indicates that the sample MARIA FOOT contain DNA. However DNA contained in MARIA FOOT may have been partially destroyed.

The reason for the above maybe due to prolong exposure to environmental conditions such as high humidity and high temperature.

The amelogenin test for sex determination (testing of the sex chromosomes) found that sample MARIA FOOT belongs to a **FEMALE individual**.

Overall results suggests that **MARIA FOOT belongs to a primate**.

2. MARIA Tail Bone, Baby HAIR and MARIA Hand tissue

Nuclear DNA or chromosomal DNA (DNA inside the nucleus of the cells) in the samples; MARIA Tail Bone, Baby HAIR and MARIA Hand tissue was found to be significantly destroyed.

The reason for the above maybe due to MARIA Tail Bone, Baby HAIR and MARIA Hand tissue may have been subjected to degradation due to prolonged exposure to environmental conditions such as high humidity and high temperature and also the amount of biological material present may have been insufficient to be typed.

Every reasonable endeavour was made to obtain a successfully amplified PCR product. The extractions were subjected to dilution, concentration and purification techniques in order to make them typable. However the failure to amplify the DNA by PCR, despite the use of these techniques, indicates that nuclear /chromosomal DNA contained in the above samples may have been degraded beyond the point of being typable.

3. **Baby SPINE BONE:** Results of nuclear DNA (chromosomal DNA) was found to be not satisfactory for analysis. Sample is being re-analyzing.

DNA tests targeting several locations which resist against DNA degradation on the mitochondrial genome (DNA found outside the nucleus of the cells) are being conducted for all samples.

Procedures in place for laboratory sterilization and elimination and the use of controls suggest that the above results contained authentic DNA allelic data and not contamination. All the data were screened against an elimination database to identify any contamination from lab staff of Genetech. However the possibility of contamination by a previous handler prior submitting the samples to Genetech cannot be excluded.

Investigators:

Dr. Ruwan Illeperuma

Mr. Manju Fernando