Forensic Biology/DNA Laboratory
Forensic & Analytical Science Service
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NSW GOVERNMENT Health Pathology

Expert Certificate - Supplementary 3

Section 177 Evidence Act 1995

RE: Alleged Death of Samantha (David) Rose

FASS Reference Number: FS971181

Police Reference Number: E3930270

(1) I, Michele Anne FRANCO, am employed at the Forensic Biology/DNA Laboratory of the NSW Health Pathology Forensic & Analytical Science Service, Joseph Street, Lidcombe.

(2) My scientific qualifications are Bachelor of Science from the University of New South Wales, Master of Science Management from the University of Technology Sydney and I have specialised knowledge based on my training, study and experience.

(3) I acknowledge that I:

(i) have read the Expert Witness Code of Conduct in Schedule 7 of the NSW Uniform Civil Procedure Rules 2005; and

(ii) agree to be bound by the Code.

(4) Exhibit Delivery:

For previous exhibit delivery details please see previous report(s).

(5) Based on my specialised knowledge I can report as follows:

Item No	Item Description	Results
12ia	KX0001677517 Hair 'a' from area i at side of bra	DNA testing was unsuccessful.

NATA WORLD RECOGNISED ACCREDITATION

Accredited for compliance with ISO/IEC 17025 – Testing.
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The results apply to the sample(s) as received.

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14aiv a	PAX0002333697 Hair 'a' recovered from inside back left upper arm of t-shirt	The origin of the sample could not be determined due to the poor quality and quantity of mitochondrial DNA recovered.
14aiv b	QAX0002333697 Hair 'b' recovered from inside back left upper arm of t-shirt	The origin of the sample could not be determined due to the poor quality and quantity of mitochondrial DNA recovered.
14axi a	UAX0002333697 Hair 'a' recovered from outside front of t-shirt, near logo	The mitochondrial DNA profile recovered was reported as matching that of a Guinea Pig.

(6) See the attached appendix for important information.

(7) Other scientific staff have assisted with the analysis and processing of items from this case.

Reported By: Michele FRANCO

Date: 29 June 2023

APPENDIX: Overview of Mitochondrial DNA Analysis

Mitochondrial DNA

Mitochondria are found in every cell of the body (with the exception of red blood cells) and provide the energy that is required for cellular processes. The mitochondria contain their own deoxyribonucleic acid (DNA), which is different to the routinely targeted nuclear DNA (nDNA) and contains less genetic information. nDNA is located within the nucleus of cells and has two copies per cell (one from the mother and one from the father). In comparison, there are multiple mitochondria in a cell and each of these houses multiple copies of mitochondrial DNA (mtDNA), totalling 100-1000 mtDNA copies per cell. Due to the abundance of mtDNA, mtDNA analysis is very sensitive and can be used when nDNA profiling techniques are unsuccessful because nDNA is limited or degraded (e.g. compromised skeletal remains or shed hairs).

Unlike nDNA, which is inherited from both the mother and father, mtDNA is usually inherited whole from mother to child (both son and daughters) with no contribution from the father. Each sibling will have the same mtDNA profile and this will be passed on through the maternal line for many generations without significant change (except for occasional mutations). As mtDNA is maternally inherited, and all individuals who are related by a maternal link will have the same mtDNA profile, an individual's mtDNA profile is not unique. However, this feature allows any maternal relative's reference sample to be used for comparison purposes.

mtDNA Analysis

The aim of mtDNA analysis is to determine the nucleotide base sequence of the mtDNA of a sample (i.e. the order of the four nucleotide bases: A, G, C, T). Two locations in the mtDNA Control Region (a non-coding region) are usually analysed for sequence differences, commonly referred to as Hypervariable Region I (HVI) and Hypervariable Region II (HVII). These regions are known to be highly variable between individuals.

The mtDNA sequence of a casework sample is compared to a standard mtDNA sequence (referred to as the revised Cambridge Reference Sequence (rCRS)) and any nucleotide base differences are noted. This process is then repeated for any reference samples. The set of differences are then compared between a casework and reference sample.

mtDNA Profile

For reporting purposes, a mtDNA profile (or haplotype) is a list of sequence differences relative to the rCRS. When differences are observed, the nucleotide position is cited followed by the nucleotide base present at that site. Nucleotide positions that are consistent with the rCRS are not listed. A mtDNA profile will commonly include differences detected in both the HVI and HVII regions of the mtDNA Control Region. For samples which do not have full coverage of both the HVI and HVII region, a partial mtDNA profile may be reported and the significance of this comparison will be interpreted on a case by case basis.

Heteroplasmy

Heteroplasmy is the presence of more than one mtDNA type in an individual. There are two kinds of heteroplasmy; point or length heteroplasmy.

Point heteroplasmy is detected by the presence of two different nucleotide bases at a single nucleotide position.

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                                    16093
Casework Sample: ……TGTATT/CTCGTA……
Reference Sample:……TGTAT  T  TCGTA……
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For example, a casework sample may have the same sequence as the reference sample, with the exception of nucleotide position 16093. At this position, the casework sample could have both a T and C nucleotide base and the reference sample could have a T nucleotide base at this position. Due to the presence of the common (T) nucleotide base, the reference sample cannot be excluded as coming from the same source or the same maternal lineage as the casework sample.

Length heteroplasmy is detected by the presence of overlapping sequences with a variable number of repeated nucleotide bases in the HVII C-stretch region (a string of C nucleotide bases between nucleotide positions 303-315).

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Casework Sample: ……CCACCAAACCCCCCCCTCCCCCCG……      (8 C's)
Reference Sample:……CCACCAAACCCCCCC TCCCCCCG…… &   (7 C's)
                 ……CCACCAAACCCCCCCCTCCCCCCG……      (8 C's)
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For example, a casework sample may have the same sequence as the reference sample, with the exception of the number of repeated C nucleotide bases in the HVII C-stretch region. The casework sample could have eight C nucleotide bases and the reference sample could have predominantly seven C nucleotide bases (with evidence of eight C nucleotide bases as well). Due to the presence of the common (8 C's) length variant, the reference sample cannot be excluded as coming from the same source or the same maternal lineage as the casework sample.

mtDNA Profile Comparisons

The following interpretations are used for mtDNA profile comparisons between a casework and reference sample:

Exclusion (profiles are different)

In general, if the mtDNA profiles recovered from the casework and reference samples differ by two or more sequence differences, they can be excluded as coming from the same source, or the same maternal lineage.

Inconclusive (profiles have a sequence and/or length difference)

In general, if the mtDNA profiles recovered from the casework and reference samples differ by a single difference, the comparison will be reported as inconclusive.

Detection of a single difference is classified as inconclusive because it could be the result of either a true difference between two samples from unrelated individuals, or a single mutational event that has occurred between samples from two maternal relatives.

Differences can include either a sequence difference (i.e. a different single nucleotide base at a specific nucleotide position) and/or a length difference (i.e. a different number of repeated nucleotide bases in the HVII C-stretch region).

The comparison will be reported as inconclusive if samples differ:

- By a single sequence difference only;
- By not having a common length variant in the HVII C-stretch region; or,
- By a single sequence difference and by not having a common length variant in the HVII C-stretch region

Cannot Exclude (profiles are the same or consistent)

In general, if the mtDNA profiles recovered from the casework and reference samples are the same, or consistent, they cannot be excluded as coming from the same source, or the same maternal lineage.

Samples which have the same mtDNA profiles have an identical nucleotide base at every nucleotide position and an identical number of repeated nucleotide bases in the HVII C-stretch region.

Samples which have consistent mtDNA profiles have a common nucleotide base at every nucleotide position and a common number of repeated nucleotide bases in the HVII C-stretch region; thus accounting for the presence of heteroplasmy.

For conclusions regarding the origin of a sample, it is always stated that due to the maternal inheritance of mtDNA, maternal relatives are expected to share the same mtDNA profile. Therefore, mtDNA cannot be considered a unique identifier. Apparent unrelated individuals might also share this profile (inherited from some distant unknown maternal relative). The inclusion of a profile frequency estimate takes this in to consideration.

Statistical Analysis

If the casework and reference sample mtDNA profiles are the same/consistent, the profile is searched against a mtDNA population database to estimate the frequency of that mtDNA profile in the general population i.e. the number of unrelated individuals in the general population that could also share this mtDNA profile. The basis for the mtDNA profile frequency estimation is the counting method. This involves counting the number of times a particular mtDNA profile is observed in a population database. A correction factor (upper 95% confidence interval) is applied to the frequency estimate to account for database size and any sampling errors. This results in a more conservative estimate for the occurrence of a particular mtDNA profile in the general population.

mtDNA Population Database

The European DNA Profiling Group mtDNA Population Database (EMPOP) is used to estimate the expected frequency of a mtDNA profile. The EMPOP database is an online database (http://empop.online/) that is comprised of mtDNA profiles which are representative of populations from all over the world. This database is considered to be the 'gold standard' for mtDNA analysis in the forensic community, because of its size, design and quality control measures.

Quality Assurance

The Forensic Biology/DNA Laboratory has been accredited to conduct mtDNA analysis by the National Association of Testing Authorities, Australia (NATA) since January 2015. The mtDNA laboratory has an extensive quality assurance program in place that ensures the DNA testing and reporting is accurate, robust, reliable and reproducible, and to prevent, detect and minimise errors. The Reporting Biologist takes responsibility for the scientific accuracy of the analyses and opinions expressed in the report. However, under the direction of the Reporting Biologist, other suitably qualified and trained staff are involved in various processes including exhibit receipt, item examination, DNA testing and sequence analysis.

Due to the sensitivity of the mtDNA analysis procedure, contamination minimisation procedures are important and include: restricted access to the laboratory; use of personal protective equipment by staff; processing a number of control samples with case samples to monitor contamination during testing; environmental DNA monitoring of the laboratory environment and equipment; comparing all case mtDNA profiles to various elimination databases; extensive decontamination procedures; use of DNA-free equipment and consumables; strict sample handling and processing procedures; and replication of sample testing (where possible).

The mtDNA analysis procedure employed in this laboratory has been internally validated according to scientific principles and various quality assurance guidelines and standards, and each method has been demonstrated to be accurate, robust, reliable and reproducible. The validation trials and results are documented in an internal validation report. In addition, similar methods to those validated in this laboratory, have been validated extensively in other forensic DNA laboratories. Furthermore, the majority of the products (e.g. reagents, instrumentation and software) in use in this laboratory have been developmentally validated by the manufacturers, with validation trials and results being publicly documented.