

| In the matter of: | Special Commission of Inquiry into LGBTIQ hate crimes – Crispin Dye                 |
|-------------------|---|
| Date:             | .15th June 2023   |
|                   |   |
| Name:             | Michele Franco  |
| Occupation:       | Group Manager, Evidence Recovery Unit at NSW Health Pathology Forensic & Analytical |
|                   | Science Service (FASS)  |
| Address:          | C/- 480 Weeroona Road, Lidcombe NSW 2141  |
| FASS Ref:         | FS232449  |

- 1 This statement made by me accurately sets out the evidence that I would be prepared, if necessary, to give in court as a witness. The statement is true to the best of my knowledge and belief, and I make it knowing that, if it is tendered in evidence, I will be liable to prosecution if I have wilfully stated in it anything that I know to be false, or do not believe to be true.
- 2 I am currently employed as the Group Manager, Evidence Recovery Unit at the NSW Health Pathology Forensic & Analytical Science Service (FASS). I have held this position since February 2018.
- 3 I have been employed as a Forensic Biologist by the NSW Department of Health, since 1985.
- 4 My scientific qualifications are a Bachelor of Science from the University of New South Wales and Master of Science Management from the University of Technology Sydney and I have specialised knowledge based on my training, study and experience.
- 5 This statement is given in response to questions raised in a letter from the Special Commission of Inquiry into LGBTIQ hate crimes dated 2<sup>nd</sup> June 2023 to Clint Cochrane, Laboratory Manager, Forensic Biology/DNA, Forensic & Analytical Science Service. The questions are restated below, followed by my responses.



# **Questions**

| ltem             | Exhibit     | Notes  |
|------------------|-------------|--|
|                  | Number      |  |
| Jeans            | X0000638075 | According to crime scene exhibit sheet, this exhibit also includes |
|                  |             | the belt.  |
| Socks            | X0000638076 |  |
| T-shirt          | X0000638077 |  |
| Shoes            | X0000638078 |  |
| Blue/denim shirt | X0000638079 |  |
| Blood sample/    | TBC         |  |
| blood swab       |             |  |
| Head hair sample | X0000638080 |  |
| Extendable baton | X0001547254 | This exhibit is linked to the event on EFIMS, however it is not    |
|                  |             | clear how or if this exhibit is linked to the matter.              |

**DNA Testing** 

1. The results of the DNA testing carried out on the exhibits listed in the table above. Where particular tests and/or analysis cannot be undertaken, or are considered to be of no utility, please refer to this in the statement.

Note: The reference head hair sample submitted from Crispin Dye was too degraded to obtain a full profile. Therefore, a sample of the apparent blood staining from the left cuff of the shirt (item 3aix) that Crispin Dye was wearing, was tested to infer his DNA profile. The DNA profile matched the partial profile recovered from the reference head hair sample but recovered more markers than the hair sample.

| Item<br>(See 'attachment 1' for<br>description of areas<br>for jeans, shirt and shoes.)                              | Police EFIMS<br>number | Results-<br>DNA testing using PowerPlex 21®   |
|--|------------------------|---|
| Jeans (with belt)<br>(item 1)<br>Tape-lifts of<br>• areas i, iii, iv<br>• areas ii, xi, xii<br>• areas v, vi, vii, x | X0000638075            | Not enough DNA recovered for DNA testing<br>Partial DNA profiles recovered consistent with<br>originating from Crispin Dye.<br>Partial profiles originating from at least two<br>contributors. Crispin Dye could not be excluded as<br>one of the contributors. <b>Unknown male 'A'</b> can't be<br>excluded as the second contributor. |
| • areas viii, ix   |                        | Partial profiles originating from at least two<br>contributors. Crispin Dye could not be excluded as<br>one of the contributors. Add contributor/s<br>weak/complex.   |



Accreditation for compliance with ISO/IEC 17025 - Testing. This document shall not be reproduced, except in full.

| Cut out of area xiii -<br>staining of the outside right<br>back pocket |             | Screening test for blood was positive<br>DNA profile appears to originate from two<br>contributors. Crispin Dye could not be excluded as<br>one of the contributors.<br>Assuming Crispin Dye is one of the contributors,<br>the profile of the second contributor, ( <b>unknown</b><br><b>male 'A'</b> ) was determined and has been entered<br>onto the DNA database. |
|--|-------------|--|
| Cut out area of area xiv –<br>inner left thigh                         |             | Screening test for blood was positive. The partial profile matched the profile of Crispin Dye.   |
| Socks (item 8)   | X0000638076 | Not examined. It is likely that the socks would have been hidden under the jeans/shoes.  |
| T-shirt (item 6)   | X0000638077 |  |
| Hairs (total 14 hairs)<br>collected from inside of<br>shirt            |             | 3 hairs - unsuitable for nuclear DNA typing as no<br>roots were present.<br>11 hairs - the hairs all appeared to be shed hair (in<br>telogen* phase) and were unsuitable for DNA<br>testing. The hairs have been stored.   |
| Hairs (total 10 hairs)<br>collected from outside of<br>shirt           |             | 2 hairs - unsuitable for nuclear DNA typing as no<br>roots were present.<br>8 hairs were in telogen* phase. DNA typing of these<br>hairs were unsuccessful as there was insufficient<br>DNA recovered.   |
| Shoes (item 7)   | X0000638078 |  |
| Tape-lift of area i  |             | Not enough DNA recovered for DNA testing.  |
| Tape-lift of area ii   |             | DNA testing unsuccessful as no profile recovered.  |
| Tape-lifts of areas iii and iv   |             | DNA testing unsuccessful as profile too weak to interpret.   |
| area v –back right heel  |             | A screening test for blood was positive. DNA testing unsuccessful as profile recovered too weak to interpret.  |
| area vi- back right heel   |             | A screening test for blood was positive.<br>This area has been stored.   |



|   |             | ]   |
|---|-------------|---|
| Blue/denim shirt<br>(item 3a)   | X0000638079 |   |
| Hair i – top left shoulder  |             | Partial profile - <b>unknown male 'B'</b> . This profile has been entered onto the DNA database.  |
| Tape-lifts of areas ii, iii<br>Inside front left pocket   |             | Partial profiles originating from at least two<br>individuals. The major component is consistent with<br>originating from Crispin Dye. Additional<br>contributor/s weak/complex   |
| iv. Hair strand- inside left<br>front pocket  |             | This hair has been stored.  |
| Tape-lifts of areas v, vi<br>Inside front right pocket  |             | Partial profiles originating from at least three<br>individuals. Crispin Dye could not be excluded as one<br>of the contributors. Additional contributors<br>weak/complex.  |
| vii. Hair strand- inside right<br>front pocket  |             | This hair has been stored.  |
| Area viii – staining on front<br>right collar   |             | Screening test for blood was positive.<br>DNA testing unsuccessful as profile too weak to<br>interpret.   |
| Area ix- staining on left<br>sleeve cuff<br><u>used as a reference</u><br><u>sample for Crispin Dye</u> |             | Screening test for blood was positive.<br>Partial profile consistent with partial profile of head<br>hair sample from Crispin Dye.  |
| <u><b>3b</b>. white card -</u> front left<br>pocket   |             | Screening test for blood was positive on the stained<br>area. The partial profile matched the profile of<br>Crispin Dye.<br>A tape-lift on an area next to the stained area was<br>unsuccessful as there was insufficient DNA<br>recovered. |
| <u><b>3c</b>. Post-it note</u> – front left<br>pocket - three hairs                                     |             | One hair unsuitable for nuclear DNA typing as no<br>roots were present.<br>Two hairs were in telogen* phase. DNA typing of<br>these hairs were unsuccessful as there was<br>insufficient DNA recovered.                                     |
| Blood sample/<br>blood swab<br>(item 4)   | ТВС         | Screening test for blood was positive.<br>The partial profile matched the profile of Crispin<br>Dye.  |



| Head hair sample (item 2)<br>from Crispin Dye   | X0000638080 | Partial profile obtained.   |
|---|-------------|---|
| Extendable baton –<br>(item 5)<br>This exhibit is linked to the<br>event on EFIMS, however it<br>is not clear how or if this<br>exhibit is linked to the<br>matter. | X0001547254 |   |
| Areas i, iii, vii - handle  |             | DNA testing unsuccessful as profile too weak to interpret                           |
| Area ii - handle  |             | Partial profiles originating from at least 2 individuals.<br>Too weak to interpret. |
| Area iv – 2 <sup>nd</sup> tier of baton   |             | Partial profile, <b>Unknown male 'C'</b>  |
| Area v - 3 <sup>rd</sup> tier of baton  |             | Mixture of at least 3 contributors. Too weak and complex for interpretation         |
| Area vi – rounded tip of<br>baton   |             | Not enough DNA recovered for DNA testing  |

\*DNA testing on a hair root in telogen phase is unlikely to produce a profile.

#### DNA testing using Y-STRs (Yfiler Plus™)

DNA using Y-STRs was conducted on areas v and xiii of the jeans (item 1), on the left cuff of the shirt (item 3a, area ix) and on the reference head hair sample from Crispin Dye. The partial Y-STR profiles recovered from the head hair sample and from the left cuff were consistent with originating from the same individual.

The Y-STR profile recovered from areas v and xiii appeared to originate from two contributors. Crispin Dye could not be excluded as one of the contributors. Assuming Crispin Dye is one of the contributors, the profile of the second contributor, (**unknown male 'Y1'**) was determined and has been entered onto the Y-STR DNA database.

### 1. (continued)

#### Please also specifically address the following:

#### a. Discovery of two items (a white card and yellow post-it note) in the pocket of Mr Dye's shirt;

A folded white card, enclosed inside a yellow folded post-it note, was located deep down inside the left front pocket of Crispin Dye's shirt (item 3a). It was on the side nearest to the left arm (away from the stained part of the pocket). The card (item 3b) was discovered when the inside of the pocket was just about to be sampled to try to recover any trace DNA from the pocket lining. Once removed from the pocket an apparent phone number was seen, hand-written on the yellow paper (item 3c). A small brown non-continuous, print-like stain was seen on the white card, which was tested and reacted positive to the screening test for blood. A partial male DNA profile was recovered from the stain which could have originated from Crispin Dye and was different to unknown males 'A', 'B' and 'C'.



Accreditation for compliance with ISO/IEC 17025 - Testing. This document shall not be reproduced, except in full.

#### b. Discovery of a DNA profile on the rear pocket of Mr Dye's jeans;

The stain on the back pocket of the jeans from Crispin Dye gave a positive reaction to the screening test for blood. A section of the stain was DNA tested which recovered a partial DNA profile. The remaining section of stain was stored in the freezer. In order to obtain the larger fragments of DNA that were missing from the partial profile, arrangements were made to send the remaining stain to ESR in New Zealand so that they could test the blood using the DNA testing system called MiniFiler<sup>™</sup>.

#### c. Discovery of hairs on Mr Dye's clothing and on the yellow post-it note; and

The loose hairs adhering to Crispin Dye's long-sleeved shirt and T-shirt were collected. There were 3 hairs adhered to the sticky part of the yellow post-it note which were also retained. All of the hairs collected were examined to determine the suitability for DNA testing (except two hairs from the shirt (item 3a), one from each pocket, which have been stored). See the results in the table above.

#### d. The reasons for requesting the assistance of ESR in conducting DNA analysis.

The partial DNA profile from male 'A' obtained from the jeans is unlikely to link with any old reference samples on the database. This is because the old reference samples collected prior to about 2010 have typically only been profiled in the Profiler Plus<sup>®</sup> system, which only dealt with 9 locations of the DNA rather than the current 20 locations. There are not enough DNA markers present in the partial profile on the jeans to link with the reference samples typed in Profiler Plus<sup>®</sup>. This limitation applies only to database searching, nominated reference samples can be compared directly to the recovered partial DNA profile.

Therefore the sample was sent for MiniFiler<sup>™</sup> testing to ESR, which is not believed to be available for forensic testing in Australia, in an attempt to recover more information for upload to assist in linking to current samples.

2. If a DNA profile other than Mr Dye's was identified in connection with any of the exhibits, whether the following persons could be excluded as contributors:

| a. | NP128           | (DOB:   |       | ) |
|----|-----------------|---------|-------|---|
| b. | Richard William | Leonard | (DOB: |   |
| с. | NP12            | 29      | (DOB: | ) |
| d. | NP127 (         | DOB:    | )     |   |

We have reference profiles from the following individuals-

- **NP128** (DOB:
- Richard William Leonard (DOB:

• NP127 with same year but <u>different date of birth</u> to DOB: . The CNI number for the sample that we have is **a second second**.

The above three individuals are excluded as the contributor of the unknown male 'A' profile from the stained area of the jeans from Crispin Dye. They are also excluded as the contributor of the profile of unknown males 'B' and 'C'.

• NP129 (DOB: Constraints)

3. Any impact the passage of time between the collection of these exhibits and the current DNA analysis, and/or the storage of the exhibits in that time, has had had on the ability to recover a DNA profile. Please specifically address any matters associated with the fragility or degradation of the exhibits. DNA degrades over time but as the samples were kept in a dry condition, they were still useable. The passage of time has enabled the advancement of technology used by the laboratory to recover DNA profiles different to the victim on the jeans and the hair recovered from the shirt.



4. If there has been any adverse impact on the DNA analysis due to the passage of time, whether these impacts would have been ameliorated if testing had been carried out: a. At the time of the original police investigation in December 1993 - August 1994;

While DNA testing could have been carried out in 1994 when PCR typing began, the system used was inferior to current DNA typing methods. For example, DNA typing at the time was affected by inhibitors that may be extracted from a sample such as that from the blue dye of the jeans. In 1994 to 1995, there also needed to be suspects available for any meaningful comparisons to be made. If the testing had commenced in 1994 the limited information gained could only be compared to a suspect directly connected and typed in the same DNA typing system of that time. DNA typing results obtained in 1994 could not be uploaded onto a database, unless re-examined in DNA typing systems used later. Also, the biological sex of the DNA contributor could not be determined in 1994.

#### b. After the first review by the Unsolved Homicide Team in May 2005;

DNA testing could commence in 2005 but the DNA typing system used at the time was greatly affected by inhibitors that could be present in the blue dye of the denim jeans. If a profile was able to be obtained, it could be entered onto the DNA database and searched for a link to a person or crime scene.

#### c. After the second review by the Unsolved Homicide Team in May 2016

In 2019, the laboratory was using the PrepFiler<sup>™</sup> extraction method which improved the DNA recovery from samples. Also the DNA typing kit, PowerPlex 21<sup>®</sup> system, was more sensitive and less affected by the presence of inhibitors in a sample. Any unknown profiles recovered could be searched on the NSW DNA database and also on the national DNA database (NCIDD). The laboratory was testing using Y-STRs but did not have a NSW Y-STR searching database until 2020.

5. To the extent not already addressed by your answer to Question 4, the forensic analysis that could have been carried out in 1994, 2005 and 2019 and any advances which are available today that were not at these times. Other comments

If testing had been carried out in 1994, it may not have been useful unless there were reference samples from suspects to compare. The discrimination capacity of the DNA typing system was much lower at this time, unlike the high discrimination capacity available today.

Please see the following table for an outline of some of the major improvements in technology implemented in the laboratory, with the associated benefits obtained, over the years specified.



| Method                      | 1993- 1994   | May 2005   | November<br>2019  | Testing in 2023   |
|-----------------------------|--|--|---|---|
| Blood<br>Grouping<br>System | Haptoglobin,<br>Phosphoglucomutase,<br>Erythrocyte Acid<br>Phosphatase,<br>Group Specific<br>Component,<br>Adenylate Kinase  | Obsolete   | Obsolete  | Obsolete  |
| Extraction of<br>DNA        | 1994-1995<br>DNA testing still in its<br>infancy<br>Only limited cases<br>tested<br>Phenol/chloroform or<br>Chelex   | <b>Chelex</b><br>Not as efficient as<br>chemistries later<br>developed, resulting in<br>lower & less purified<br>DNA yields.   | This method h   | PrepFiler™<br>as a greater<br>ecover purified                         |
| Quantification              | Quantiblot <sup>®</sup>  | Quantifiler™ Human<br>(2006 - 2017)  | Quantifiler <sup>™ ·</sup><br>(from mid- 20   |   |
| of recovered<br>DNA         | Based on visual<br>comparison of test<br>samples to<br>diluted DNA<br>standards. Less<br>precise & less<br>sensitive than modern<br>technology, which<br>may lead to less<br>optimal downstream<br>DNA processes.  | Only determines the<br>total amount of DNA -<br>does not have a marker<br>specific for male DNA<br>present. Does not have<br>a degradation index (DI)<br>that indicates the<br>quality of the DNA<br>within a<br>sample. | Gives more information about<br>the type of DNA present in a<br>sample. Has a marker specific<br>for male DNA present.<br>Has a degradation index (DI)<br>that indicates the quality of the<br>DNA within a sample (i.e. the<br>amount of large DNA fragments<br>compared to the small DNA<br>fragments). |   |
| DNA Profiling<br>Typing Kit | (1994)<br>Amplitype®PM + HLA<br>DQA1 Kit<br>Low discriminating<br>power between<br>individuals. Unable to<br>determine male or<br>female DNA (did not<br>include sex marker of<br>DNA). Needed<br>reference samples for<br>comparison &<br>interpretation. | Profiler Plus®<br>limited to 9<br>DNA markers (plus sex<br>marker).<br>Not as<br>sensitive as later DNA<br>profiling kits. Didn't deal<br>as well with inhibitors<br>or degraded<br>samples compared to<br>later kits.   | 20 markers of<br>sex marker.<br><b>Yfiler Plus™</b> (f  | This kit tests for<br>the DNA plus the<br>rom 2019)<br>STR markers on |



| Capillary<br>electrophoresis                            | No | 3100   | <b>3500xl</b><br>Improved sensitivity and<br>increased dynamic range which<br>improved analysis of strong<br>samples.   |
|---|----|--|---|
| Software to<br>improve DNA<br>mixture<br>interpretation | No | Νο   | STRmix mixture interpretation<br>software<br>Improved capabilities to<br>interpret mixture of DNA from<br>more than one person.   |
| Searching DNA<br>Database                               | No | Autosomal database<br>Person to scene and<br>scene to scene<br>matching on NSW DNA<br>database | Autosomal database<br>Person to scene and scene to<br>scene matching on NSW and<br>National DNA database (NCIDD)<br>Familial searching capabilities.<br>Y-STR database (NIFA)<br>(started 2020) |

- 6. Any other comments you wish to make regarding forensic analysis in this matter of relevance to the Inquiry.
  - The original P377 that was prepared by police for the exhibits collected in 1993 states that there ٠ is a 'pair of faeces-stained blue jeans'. The jeans had extensive brown staining around the buttock area and therefore it was difficult to differentiate any blood-like staining from the brown staining due to the age of the exhibit. Over time the blood changes and becomes brown.
  - The T-shirt was not received intact due to the middle cut down the centre, possibly by ٠ paramedics. This may affect the positioning of hairs from inside/outside surfaces.
  - The profile recovered from the jeans by ESR recovered overlapping markers with the FASS ٠ typing system. The profiles obtained at these overlapping markers are consistent with each other.

See the attached appendices for additional information.

Signature: 15.6.23

Date:



| Jeans (item 1)                    | Location of areas mentioned in the report                      |
|-----------------------------------|--|
| i                                 | Outside right back hip   |
| ii                                | Outside left back hip  |
| iii                               | Outside right front hip  |
| iv                                | Outside left front hip   |
| v                                 | Inside surface of back right pocket                            |
| vi                                | Inside surface of outer layer of back right pocket             |
| vii                               | Inside surface of back left pocket                             |
| viii                              | Inside surface of outer layer of back left pocket              |
| ix                                | Left sleeve cuff   |
| x                                 | Inside surface of outer layer of front right pocket            |
| xi                                | Inside surface of left front pocket                            |
| xii                               | Inside surface of outer layer of front left pocket             |
| xiii                              | back right pocket  |
| xiv                               | Inner left thigh   |
| Shirt (Item 3a)                   |  |
| ii                                | Inside surface of outside layer of front left pocket of shirt  |
| iii                               | Inside surface of front left pocket of shirt                   |
| v                                 | Inside surface of outside layer of front right pocket of shirt |
| vi                                | Inside surface of front right pocket of shirt                  |
| vii                               | Front right collar of shirt                                    |
| ix                                | Left sleeve cuff   |
| 1 x pair of maroon shoes (Item 7) |  |
| i                                 | Front right of right shoe as worn                              |
| ii                                | Front left of right shoe as worn                               |
| iii                               | Front right of left shoe as worn                               |
| iv                                | Front left of left shoe as worn                                |
| v                                 | Back right heel of right shoe as worn (lower)                  |
| vi                                | Back right heel of right shoe as worn (upper)                  |

# APPENDIX: Overview of Procedures and Methods used in the Forensic Biology/DNA Laboratory (FBL), NSW Forensic & Analytical Science Service (FASS)

# Contents

| 1.  | Introduction                            |
|-----|---|
| 2.  | Analyses carried out in the Laboratory2 |
| 3.  | Biological fluid testing                |
| 4.  | Differential DNA extraction             |
| 5.  | Unsuccessful results                    |
| 6.  | DNA profile interpretation              |
| 7.  | Statistical overview                    |
| 8.  | Interpretation software                 |
| 9.  | Reports                                 |
| 10. | Case review                             |
| 11. | DNA searchable databases7               |
| 12. | Further testing and/or interpretation7  |
| 13. | Transfer and persistence of DNA         |
| 14. | Specialist DNA testing                  |
| 15. | Quality assurance                       |
| 16. | Quality system documentation            |
| 17. | Secure storage                          |
| 18. | Sample retention                        |

# 1. Introduction

- 1.1 DNA (or deoxyribonucleic acid) is a molecule found in most cells of the body. Nuclear DNA is contained in the nucleus of the cells and it carries the code for the characteristics and functions of the body. DNA is inherited from the parents, half from the mother and half from the father. Barring mutation, body materials such as blood, semen, saliva, hairs, and skin cells from one person will all contain the same DNA.
- 1.2 While DNA in different individuals is largely the same, there are areas of the DNA that show considerable variability. Forensic DNA testing targets these areas so that, except for identical twins, the probability of discrimination between different people is extremely high.
- 1.3 While FASS has been using DNA testing since 1989, the processes used and the areas of DNA targeted have changed over the years. Since 1994, DNA analysis using PCR has been used in this laboratory. PCR (polymerase chain reaction) involves targeting specific areas of the DNA and copying (or amplifying) these targeted areas many millions of times. PCR allows a DNA profile to be developed from very small amounts of biological material. Since 1996, the forensic use of PCR for DNA analysis has involved determining the size variation that exists at specific DNA areas. Many scientific papers have been published demonstrating that this technology produces accurate, reliable, and robust results.
- 1.4 In the late 1990s, all Australian forensic laboratories introduced the Profiler Plus<sup>®</sup> system, which targeted nine highly variable areas (loci (singular: locus)) of the DNA and one area determining sex. In 2012, 18 loci were adopted to form the core comparison group for the National Criminal Investigation DNA Database (NCIDD). In 2013, the PowerPlex<sup>®</sup> 21 system was introduced at FASS. This system targets 20 highly variable areas and one area determining sex. Note: Profiler Plus<sup>®</sup> and/or Identifiler<sup>™</sup> results may still be reported for some historical cases.

# 2. <u>Analyses carried out in the Laboratory</u>

- 2.1 The reporting scientist takes responsibility for the scientific accuracy of the analyses and opinions expressed in the Expert Certificate. However, the receipt of exhibits, casework analyses, DNA testing, and other related activities are usually carried out by numerous trained staff within the laboratory. This is standard practice in all types of scientific laboratories.
- 2.2 All involvement of the staff in the processes and protocols is fully documented and their identities and details of their specific involvement can be provided, if required. All staff have undergone and passed relevant competency-based training and are subject to ongoing review of their performance. The qualifications of the staff are appropriate for the tasks performed. For example, all scientists must have as a minimum, a Bachelor of Science degree majoring in a relevant field.

# 3. Biological fluid testing

3.1 The laboratory may employ a number of different confirmatory tests for blood and semen, as well as chemical screening tests for blood, semen, saliva, urine, and faeces. The circumstances of the case and condition of the evidence items will determine which tests may be indicated. Appropriate wording is used in reports to reflect the specificity of the tests performed. There is no screening or confirmatory test available for skin cells.

# 4. Differential DNA extraction

4.1 A differential DNA extraction is performed on samples where both spermatozoa (sperm cells) and non-sperm cells (for example, skin cells or blood) may be present, in an attempt to separate the spermatozoa from the other cell types. Under some conditions, DNA from the non-sperm cells may appear in the profile of the sperm cell fraction and/or DNA from spermatozoa may appear in the profile of the non-sperm cell fraction, resulting in mixed DNA profiles.

# 5. <u>Unsuccessful results</u>

5.1 A DNA result reported as 'unsuccessful' could indicate one of several outcomes, such as there was no DNA detected; or the amount of DNA recovered from the sample was below the laboratory threshold for routine further DNA testing. 'DNA testing was unsuccessful' will also be reported where routine further DNA testing has been carried out but no DNA profile was recovered; or a very limited amount of DNA profile information was recovered, and as such, the result is not suitable for meaningful comparison.

# 6. DNA profile interpretation

6.1 At the completion of testing, the raw DNA data are analysed independently by two scientists (or a scientist and an expert reading system) in the DNA laboratory. The analysed DNA profiles are then released to the Case Management Unit for interpretation by the reporting scientist. There is at least one technical review check by an appropriately trained scientist prior to the results being reported.

# 7. <u>Statistical overview</u>

7.1 If there are differences in the DNA profiles generated from good quality, high yield DNA samples, then these samples could not be from the same person. Where there are no differences between the DNA profiles of two samples, then these samples could be from the same person. A statistical calculation can be carried out to estimate the weight of this evidence. There may be occasions where the

complexity and/or low yield of DNA prevents any interpretation from being conducted.

- 7.2 The standard statistic used by the FBL to evaluate the weight-of-evidence is the likelihood ratio. This approach is widely regarded by scientists and statisticians as being the most appropriate method to assess the value of evidence and provides a balanced, logical, transparent, and robust approach. The likelihood ratio considers the probability of the findings under two competing scenarios. These scenarios are typically chosen to reflect the positions of the prosecution and defence regarding the findings. For example, suppose a DNA profile has been recovered from a bloodstain left at the scene of a crime. The recovered DNA profile matches the DNA profile of the defendant. The prosecution may suggest that the DNA originates from the defendant. The defence may instead propose that the DNA originates from an unknown individual who is unrelated to the defendant. The likelihood ratio approach evaluates the probability of the findings under each of these scenarios.
- 7.3 The ratio of these probabilities is then calculated (hence, likelihood ratio). A likelihood ratio greater than 1 supports the proposition on the numerator (typically the prosecution proposition) while a likelihood ratio less than 1 supports the proposition on the denominator (typically the defence proposition). The greater the magnitude of the likelihood ratio, the greater the relative degree of support provided by the findings. A likelihood ratio of 1 provides equal support to both propositions and can be considered 'neutral' or 'inconclusive'.
- 7.4 Alternative statistical scenarios can be considered, if required. If it is proposed that the DNA originates from a close biological relative of the individual, it is preferable that a reference sample from the relative is collected and made available for analysis. If a reference sample is unavailable, a likelihood ratio can be calculated using the defence proposition that the DNA originates from a specified relative (for example, from a sibling of the person in question), rather than from an unrelated individual. It is advisable to contact the laboratory well in advance of any court proceedings if alternative scenarios need to be considered so that the necessary statistical calculations can be carried out.
- 7.5 In order to assess the probability of the findings, information regarding the relative proportion of each DNA type in the relevant population is required. Such information is contained in a population database of person samples in which the relative proportions of the different DNA types are listed.
- 7.6 There is much evidence to show there is little difference between the match statistics generated from different databases of similar racially comprised populations used in Australia and in other countries. All statistics are calculated using national Australian frequency databases specific for the Caucasian, Aboriginal, and Asian populations. Collectively, these three ethnicities account for the majority of people living in Australia.
- 7.7 In most cases, the race of the person who left the DNA is unknown and cannot be assumed. The likelihood ratio evaluates the probability of obtaining the DNA profile

if the DNA originates from a specified individual rather than if the DNA originates from an unknown, unrelated individual in the Australian population. The most appropriate databases to use in this calculation are the national Australian frequency databases introduced above. However, calculations can be made using specific ethnic databases if the ethnicity of the offender (as opposed to the defendant) is known.

- 7.8 Adjustments are made to the calculation to account for sampling uncertainty, that is, the fact that the relative proportions of the different DNA types have been determined using a sample taken from the larger population. A co-ancestry correction (Fst, also referred to as a theta correction) is also applied to account for the possibility that the defendant and the source of the DNA (if it did not originate from the defendant) may share common ancestry. The inclusion of these correction factors results in a more conservative likelihood ratio match statistic.
- 7.9 The laboratory incorporates a conservative cut-off of 100 billion when reporting the final likelihood ratio match statistic (where a billion is defined as 1000 million). The calculated likelihood ratio is often many times larger than this. The selection of 100 billion as the cut-off value was an arbitrary decision and was based on the perceived difficulties in comprehension of very large numbers by a non-scientific audience. A likelihood ratio of this magnitude provides extremely strong support for one proposition versus the alternative.

# 8. <u>Interpretation software</u>

- 8.1 In 2013, the FBL introduced STRmix<sup>™</sup>, a specialist probabilistic genotyping software that can aid in the interpretation of DNA profiles. STRmix<sup>™</sup> is particularly useful in the interpretation of weak or complex DNA profiles. STRmix<sup>™</sup> uses extensively validated methods to interpret forensic DNA profiles. The methods used by STRmix<sup>™</sup> make better use of the quantitative data contained in the DNA profile, allowing for improved discrimination between true donors and non-contributors.
- 8.2 STRmix<sup>™</sup> uses a statistical process called stratification to combine match statistics from the Caucasian, Asian, and Aboriginal populations and produce a single figure that is representative of the Australian population as a whole. The stratified likelihood ratio is essentially a weighted average that considers the proportion of each of these sub-populations within the wider Australian population.
- 8.3 In addition to accounting for sampling variation and co-ancestry, STRmix<sup>™</sup> incorporates a number of additional factors that ensure the final figure reported is highly conservative. This includes consideration of the effect that run-to-run variation associated with the STRmix<sup>™</sup> software would have on the likelihood ratio. Due to these factors, the reported figure can be considered to be close to the lower limit of the range of possible likelihood ratio values.
- 8.4 STRmix<sup>™</sup> currently requires the user to make a determination regarding the number of contributors to the DNA sample and carry out the interpretation in

STRmix<sup>™</sup> under a specific assumption. For weak or complex DNA results, the number of contributors may not be able to be assigned with a high degree of confidence. Additional analyses may be carried out in STRmix<sup>™</sup> to examine the effect on the statistical weight of evidence if the number of assumed contributors is varied. In many cases, varying the number of assumed contributors to a mixture has no effect on the reported figure. In those instances, the findings of these additional analyses may not be included in the report in order to aid comprehension but are retained in the case file and available upon request.

- 8.5 Sometimes, multiple typed individuals may be found to be possible contributors to a mixed DNA profile.
- 8.6 For example, a mixed DNA profile may be recovered that originates from at least two contributors. Reference samples have been submitted from two known individuals and both could be possible contributors to the DNA recovered. In this example, one could consider the following pairs of propositions (Note: other propositions may also be considered depending on case circumstances):

A. The DNA originates from Individual 1 and an unknown, unrelated individual versus the DNA originates from two unknown, unrelated individuals.

B. The DNA originates from Individual 2 and an unknown, unrelated individual versus the DNA originates from two unknown, unrelated individuals.

C. The DNA originates from Individual 1 and Individual 2 versus the DNA originates from two unknown, unrelated individuals.

- 8.7 Commonly, the laboratory will evaluate match statistics for sets A, B, and C above. These sets of propositions evaluate the weight of evidence against each individual separately as well as together to confirm that both individuals could be cocontributors to the recovered DNA. The match statistics for set C, or for all three sets, may be included in the report.
- 8.8 Other statistical scenarios may also be appropriate and may be carried out and included in the report. Any assumptions made for the statistical calculations are stated in the report. Further statistical calculations may also be considered upon request. It is advisable to contact the laboratory well in advance of any court proceedings if alternative scenarios need to be considered so that the necessary statistical calculations can be carried out.

# 9. <u>Reports</u>

9.1 Reports are prepared in accordance with the National Association of Testing Authorities (NATA) requirements. They contain all the relevant information considered pertinent to the case. While they are, of necessity, a summary of the total analysis, no important findings are intentionally omitted. Further details of the analyses performed are contained in the case file and in other records stored in the laboratory.

- 9.2 Reports also contain opinions of the reporting scientist. These opinions are based on the experience of the scientist, communication with peers, courses, scientific papers, attendance at conferences, and studies carried out within the FBL and by other laboratories.
- 9.3 The reporting of forensic biology/DNA results covers many varied fields including biochemistry, immunology, molecular biology, statistics, and genetics. Due to the varied nature of the fields and the diverse range of materials that the reporting scientist may use to support their opinions, it is difficult to provide a comprehensive list of reference materials. As an example, reporting a DNA statistic includes literature and other materials related to population genetics, genetics, Bayesian theory, probability, sampling variation, etc. However, if required, the laboratory can provide a list of selected texts and other materials that are routinely used in the laboratory. It is not complete and does vary but it does give a reasonable reference list on which the opinions reported by scientists are based.

# 10. Case review

- 10.1 All results undergo a technical review prior to release by a suitably qualified forensic biologist to check for scientific and technical correctness. Additionally, all case files are subject to an administrative review prior to completion. This is designed to check for consistency with laboratory policy and ensure the completeness and correctness of any reports issued.
- 10.2 All results presented in an Expert Certificate (or other form of expert statement) are independently reviewed by two senior scientists.

# 11. DNA searchable databases

11.1 DNA searchable databases are used for linking DNA profiles, either within NSW or, where permitted, between NSW and another State or Territory of Australia. All suitable DNA profiles from crime scene samples are uploaded to the database unless the sample profile matches, or is strongly presumed to match, a volunteer or victim. Uploaded crime scene profiles are matched against DNA profiles from persons and other crime scene profiles contained on the DNA database as permitted under the matching tables found within the <u>NSW Crimes (Forensic Procedures) Act 2000</u>.

# 12. Further testing and/or interpretation

12.1 In situations where limited amounts of DNA are present, where the DNA is degraded, or where there is DNA from multiple individuals (DNA mixtures), weak and/or complex DNA profiles may be recovered. These profiles can be particularly

challenging and may require further testing before an interpretation can be progressed.

- 12.2 Where limited information regarding the case circumstances and the significance of the submitted items has been provided, the laboratory may not carry out statistical interpretation of these difficult profiles in favour of interpreting other results. This approach is taken to streamline the reporting process and is generally applied where it is considered that the lengthy interpretation of multiple complex results will not provide additional information.
- 12.3 If the exhibit is of particular importance to the case, the laboratory can be contacted with a request to carry out further testing and/or interpretation. It should be noted that some results may be considered unsuitable for further testing and/or interpretation. If further testing is carried out, some results may remain unsuitable for interpretation. It is advisable to contact the laboratory well in advance of any court proceedings if further testing and/or interpretation is required.

# 13. Transfer and persistence of DNA

- 13.1 The transfer and persistence of DNA is affected by a number of factors and there are several mechanisms by which DNA may be transferred.
- 13.2 Direct transfer (or 'primary transfer') involves the transfer of DNA on to an object, surface, or person directly from the source of that DNA. This may be via physical touch or contactless transfer of body fluids (for example, blood transferred following injury or droplets of saliva projected during conversation).
- 13.3 Indirect transfer (for example 'secondary transfer') involves the transfer of DNA indirectly via an intermediary. In this manner, it is possible for a person's DNA to be deposited on to an object, surface, or person that they have not had direct contact with.
- 13.4 The direct transfer of DNA is affected by many factors including, but not limited to: the amount of DNA available for transfer, the length and nature of the contact, and the nature of the surfaces and/or objects involved.
- 13.5 The indirect transfer of DNA is affected by many factors including, but not limited to: the number of transfer steps between the source of the DNA and the evidence item in question, and the time, and events, which may have occurred in between transfer steps.
- 13.6 In many situations, particularly when dealing with low-level DNA and where the DNA cannot be scientifically attributed to a particular body fluid, it may not be possible to provide an opinion regarding the probability of obtaining the observed results given a particular transfer scenario.

Authorised by: C. FIELD

- 13.7 Methods to 'age' DNA are not in widespread use within forensic laboratories. If exposed to adverse environmental conditions such as UV light, heat or humidity, DNA will degrade to such an extent that it may fail to be detected. Conversely, DNA may be successfully typed months or even years later if stored undisturbed in favourable conditions.
- 13.8 If 'how' or 'when' the DNA was transferred is at issue, it is advisable to contact the laboratory well in advance of any court proceedings to determine whether an opinion can be provided.

# 14. Specialist DNA testing

- 14.1 Y-STR testing targets areas on the Y chromosome only and is therefore male specific. All males along the same paternal line (for example, father, brother, son) are expected to have the same Y-STR profile, barring mutations.
- 14.2 Mitochondrial DNA testing targets DNA recovered from the mitochondria in a cell and is passed on the maternal line.
- 14.3 Separate appendices are available in relation to Y-STR and mitochondrial DNA testing.

# 15. Quality assurance

- 15.1 The FBL has been accredited by NATA (National Association of Testing Authorities, Australia) for Forensic Biology/DNA testing since 1999. The FBL is accredited to ISO 17025 standards.
- 15.2 The FBL has an extensive quality assurance programme in place to ensure uniform and reliable testing and reporting and to detect and prevent errors. This is achieved in a variety of ways including the control of all documents and forms, the review of all methods and documents, full traceability of all exhibits, and formal and ongoing staff training programmes and competency assessments.
- 15.3 The laboratory participates in external and internal forensic proficiency testing programmes. NATA monitors the performance of accredited forensic science laboratories in external proficiency testing programmes.
- 15.4 There are many quality system checks within the FBL including DNA sample transfer system checks, contamination minimisation protocols, and the use of positive and negative controls where appropriate.

### 16. Quality system documentation

16.1 Detailed policies, procedures and methods are held in the Laboratory and are available upon request.

### 17. <u>Secure storage</u>

17.1 The entire FBL is a secure area under restricted and controlled access. An extensive alarm system is in operation after hours. From the time of receipt until dispatch all items of evidence are stored within this secure facility.

### 18. <u>Sample retention</u>

18.1 Permitted person samples and DNA extracts from crime scene evidence, wherever possible, are retained indefinitely. Items from crime scenes are returned to the Police as soon as practicable.

ID:FRM-FBL-36 Y-STR Appendix

### **APPENDIX: Overview of Y-STR testing**

Y-STR testing employs the same technology as conventional DNA typing (using the PowerPlex 21<sup>TM</sup> System). The difference is that the gender-determining chromosome of the male (the 'Y' chromosome) is targeted in the Y-STR test. This can be particularly useful in a case where the DNA recovered from an item is a mixture of both male and female DNA. As females do not possess a Y chromosome (only X chromosomes) this difference is exploited in order to target only the male DNA in a male/female DNA mixture.

The mode of inheritance of DNA markers typed in conventional versus Y-STR testing differs and hence, a different method is needed to interpret the statistical weight of a match. DNA markers, identified using the PowerPlex 21 <sup>TM</sup> System, are passed down to the child from both the mother and father, and the inheritance of each individual type is independent of each other. With Y-STRs however, the DNA is passed down from the father to the son as a whole unit or 'haplotype', virtually unchanged (except for occasional mutations) from one generation to the next. Therefore the haplotype of a man should be the same as his biological brothers and sons (and all other males along the paternal lineage).

In order to assign a statistical weight to a haplotype that matches a person, a counting method can be used as to the occurrence of this profile in population databases. A correction factor which takes into account the size of the sampled population is normally incorporated into this frequency estimate. At present the Forensic & Analytical Science Service has a local population database which includes samples of Aboriginal, Asian, Middle Eastern and European individuals. A larger database –YHRD (Y Chromosome Haplotype Reference Database) is also available online at www.yhrd.org.