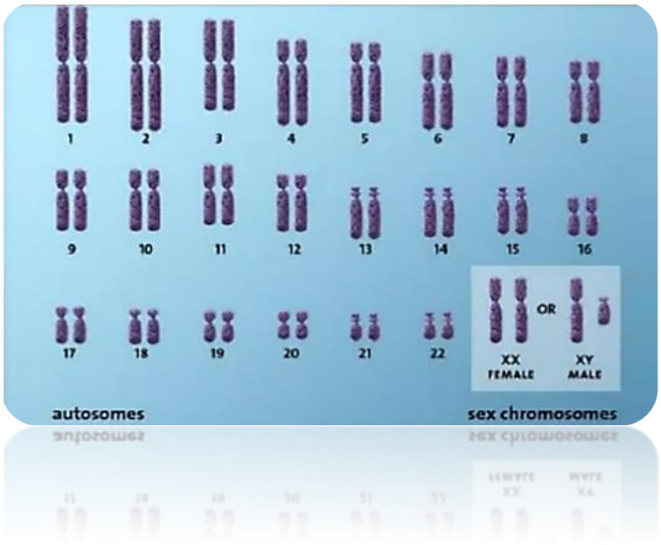
HSA Health Sciences Authority

HSA Forensic Laboratories have been accredited since 1996 and are currently accredited under the ANSI National Accreditation Board (ANAB) Forensic Testing Laboratory Programme.

All procedures and methods performed in the laboratories (as set out in this Primer) are validated to conform to international best practices and standards.

A Guide to Forensic Analysis

DNA Database Laboratory (DDL) and DNA Profiling Laboratory (DNAPL)



Contents

Deoxyribonucleic acid (DNA) is the hereditary material that contains genetic information. It is present in all cells in our body, except red blood cells, organised into structures called chromosomes, of which a human typically has 23 pairs – 22 pairs of non-sex chromosomes ('autosomes') and 1 pair of sex chromosomes ('X' and 'Y' chromosomes). Hence, DNA can be extracted from various biological materials such as blood (white blood cells are the main source of DNA in blood), semen, saliva, muscle tissue, bone marrow and skin cells.

DNA Profiling is a scientific technique to identify the specific DNA pattern of an individual through the examination of the individual's biological specimen. This pattern is called a DNA profile.

Forensic DNA Profiling is the application of this technique to criminal investigations. It involves comparing DNA profiles obtained from crime exhibits to those of persons of interest, allowing for the assessment of their potential involvement in the crime under investigation.

Standard (conventional) DNA analysis focuses on autosomal DNA, which yields a unique profile for each individual, barring identical twins. The power of discrimination of standard autosomal DNA analysis is, therefore, high.

1.1 Generation of Reference DNA Profiles…..……..

A reference DNA profile is obtained from biological material, typically blood or buccal (cheek) cells, collected from a known individual such as a person of interest. This reference profile is then compared against the DNA profiles obtained from the crime exhibits.

Reference samples are processed by the DNA Database Laboratory (DDL).

1.2 Generation of DNA Profiles from Crime Exhibits……..

Crime exhibits are processed by the DNA Profiling Laboratory (DNAPL).

The generation of a DNA profile begins with extraction of DNA from the biological material (such as blood, semen, saliva, muscle tissue, bone marrow and skin cells) on the exhibit, followed by quantitation of the extracted DNA, amplification at specific DNA loci, and detection of the amplified DNA. The DNA profile is then interpreted and compared against reference DNA profiles.

When an individual's DNA profile cannot be matched to the DNA profile obtained from the exhibit, it could be due to:

- No contact and therefore no DNA was deposited onto the exhibit.
- Contact was made but was not detected due to insufficient DNA present.

1.2.1 Deposition of DNA ……

This refers to the deposition of biological material onto an exhibit via either direct (primary) or indirect (secondary) transfer.

- Direct transfer includes physical contact, as well as activities such as speaking or coughing, which directly deposits an individual's DNA on the exhibit.
- Indirect transfer is when DNA from an individual is transferred to an exhibit via another individual/object.

Deposition of DNA depends on factors such as:

- Duration of contact, e.g. longer contact time may result in more DNA deposited.
- Nature of contact, e.g. more vigorous contact or more friction during contact may result in more DNA deposited.
- Activity prior to contact, e.g. sweating may result in more DNA deposited; washing of hands or wearing of gloves may reduce DNA deposited.
- An individual's propensity to shed skin cells (commonly referred to as 'shedder status'). Shedder status of individuals can range from good to poor. A good shedder typically deposits more DNA. For the same individual, shedder status may also vary at different times.

1.2.2 Persistence of DNA

This refers to the duration that biological material can remain (persist) on the exhibit that it was deposited onto. Even if sufficient biological material was initially deposited, DNA can be lost or degraded by other factors, thereby leading to a partial or even no interpretable DNA profile.

Persistence of DNA depends on factors such as:

- Removal – DNA present on the object could be removed by cleaning or subsequent handling.
- Environment – Degradation will occur due to environmental factors such as sunlight (UV radiation), temperature, moisture, and microbial activity. Although the integrity of the biological material deteriorates over time, it is possible to remain detectable for over a year in indoor, room temperature environment. If stored frozen, biological material can remain stable for many decades.
- Substrate – Different substrates vary in their ability to retain biological material. In general, porous materials such as cotton fabrics have higher retention ability.

1.2.3 Inhibition effects on DNA processing

This refers to substances that can interfere with the DNA processing. Even when sufficient DNA is present and has persisted on an exhibit, the presence of inhibitors can prevent successful DNA profiling. Examples of inhibitors include soil, denim dyes, glue, and certain types of fingerprint powder.

1.2.4 Limitations of Forensic DNA Profiling

Given the current technology, DNA profiling does not allow for:

- Determining ‘when’ the biological material was deposited.
- Determining ‘how’ the biological material was deposited, whether by direct or indirect transfer.
- Determining the biological material source of the DNA in a mixture – standard DNA analysis cannot distinguish whether detected DNA originated from blood cells, sperm cells, epithelial cells or other cell types.

Commonly used terminology……

Reference sample: A biological sample obtained from a known individual, e.g. Blood, Buccal.

Chromosome: A thread-like structure made up of DNA and proteins. It carries genetic information arranged in a linear sequence.

Sex chromosomes: The pair of chromosomes that determines the gender of an individual with female being X, X and male being X, Y.

Autosome: A chromosome that is not a sex chromosome.

Gene: Unit of heredity, transferred from parent to child. Consists of a stretch of nucleotides (basic building blocks of DNA) forming part of a chromosome. The order (sequence) of the nucleotides determines the different types of genes.

Locus (pl. loci): Specific position of a gene on a chromosome. Also referred to as genetic marker(s).

Allele: Alternative versions of a gene at a particular locus.

Short Tandem Repeat (STR) loci: The loci used to discriminate between individuals in forensic DNA profiling. Common sets of loci have been adopted by the international forensic DNA community based on guidance by the US FBI and the European Network of Forensic Science Institutes and made available in commercial amplification kits. An allele at a locus consists of a short stretch of nucleotides which is repeated a variable number of times in tandem. The number of repeats is used to name an allele.

Example: Locus CSF1PO (Alleles 6, 7)
... TAGA TAGA TAGA TAGA TAGA TAGA ... - 6 repeats of the "TAGA"
... TAGA TAGA TAGA TAGA TAGA TAGA TAGA ... - 7 repeats of the "TAGA"

Workflow in the DDL

The reference samples from known individuals are collected by the law enforcement agencies. Blood or buccal cells are deposited onto a sample collection card that is sealed and labelled with a unique reference number label. This number is the identifier provided by the law enforcement agency and the identity of the individual is anonymous to the laboratory.

Upon receipt in the DDL, each sample is assigned a unique laboratory number.

Phase 1: DNA Processing of Reference Samples

DNA processing of reference samples consists of 2 steps: Amplification and Detection of Amplified DNA.

DNA Amplification .....

A punch-out of the sample card is taken for amplification.

The Polymerase Chain Reaction (PCR) is an amplification process which replicates a single copy of targeted DNA into multiple copies. This allows for the production of sufficient amounts of DNA to be detected.

Detection of Amplified DNA .....

The amplified DNA is detected through fluorescence using a genetic analyser.

Phase 2: Report Generation of Reference Samples

When all the necessary tests are performed and the results obtained, the Analyst reviews and interprets all examination records and results and prepares a report. These are reviewed by another Analyst before the report is released.

Autosomal DNA Profile .....

Locus	Female Sample	Male Sample
D3S1358	14, 15	15, 16
vWA	17, 18	14, 16
D16S539	11, 12	9, 10
CSF1PO	10, 12	11, 12
TPOX	8, 8	8, 8
Yindel	N.D.	2
Amelogenin	X, X	X, Y
D8S1179	13, 13	12, 13
D21S11	30, 30	28, 31
D18S51	15, 19	12, 15
DYS391	N.D.	11
D2S441	10, 14	14, 15
D19S433	14, 15	14, 15
TH01	8, 9.3	7, 9.3
FGA	23, 24	24, 26
D22S1045	11, 14	11, 16
D5S818	11, 11	11, 11
D13S317	11, 11	11, 11
D7S820	10, 11	7, 12
SE33	19, 29.2	17, 25.2
D10S1248	13, 15	12, 15
D1S1656	18.3, 18.3	13, 16
D12S391	18, 20	18, 19
D2S1338	19, 23	20, 23

N.D.: Not Detected

Each reference DNA profile has 24 loci comprising of 21 autosomal and 3 gender-specific loci. There is a maximum of two alleles for an autosomal locus as a pair is inherited, one allele from each parent.

Amelogenin is generally detected as X,X in females and X,Y in males.

Yindel and DYS391 will only be detected in males with one allele each as it is located on the Y-chromosome.

The DNA profile of the known individual is used as a reference for which to compare against unknown profiles obtained from crime exhibits.

Workflow in the DNAPL

The crime exhibits are submitted to DNAPL for analysis. Each exhibit is labelled with a unique marking assigned by the law enforcement agency and properly sealed. Upon receipt, each exhibit is assigned a unique laboratory number.

Phase 1: Examination for Biological Materials on Crime Exhibits

In the first phase, exhibits are examined for the presence of biological material based on the nature of the case and/or instructions from the Investigation Officer (IO).

The most commonly encountered examination request is for Touch DNA. Touch DNA usually refers to skin cells/DNA deposited on a surface. There is no test to determine where touch DNA might be found on an exhibit. To collect touch DNA, the area on an exhibit (where it is suspected that the person of interest might have deposited his/her DNA) can be swabbed. These swabs are then subjected to DNA processing.

For the examination of biological fluids, a **presumptive test** is first performed to preliminarily identify the presence of a specific biological fluid of interest. These tests typically detect a common component present in the fluid in question. If this is positive and if there is sufficient material, **confirmatory tests**, where available, may be subsequently performed to definitively establish the identity of the biological fluid. It is noteworthy that presumptive and confirmatory tests often target different components of the fluid, thereby enhancing the robustness of the identification process. A sample for DNA processing is then obtained by swabbing or cutting out the area on the exhibit that tested positive for the biological fluid.

After DNA processing, the residues, which are remains of the swabs/cuttings, are generally returned alongside with the corresponding exhibits.


Swab


Cutting


Residue

Examination for Blood ……..

Presumptive test for blood

Kastle-Meyer (KM) test: Based on the detection of haem (iron component found within haemoglobin in red blood cells).

A positive result may also be obtained from animal blood and vegetable matter, such as potato and horseradish.

Confirmatory test for human blood

OBTI test: Antibody-based detection of human haemoglobin found in red blood cells.

A positive result may also be obtained from animal blood, such as primate, horse and Mustelidae (e.g. badger and weasel).

Examination for Semen ……..

Presumptive test for semen

Acid Phosphatase (AP) test: Based on detection of acid phosphatase, a protein produced by the prostate gland.

A positive result may also be obtained from materials that contain AP, e.g. vaginal fluid, cauliflower, sweet potato and shiitake mushroom.

Confirmatory test for human semen

SERATEC® PSA Semiquant: Antibody-based detection of Prostate-Specific Antigen (PSA), a protein produced by the prostate gland.

RSID™-Semen: Antibody-based detection of Semenogelin, a protein produced by the seminal vesicles.

PSA can be found at low levels in other body fluids such as breast milk and urine, while Semenogelin has been found in some cancer cell lines. Thus, the Laboratory uses the concordant detection of PSA and Semenogelin as indicative of the presence of semen.

Fluorescence-based Microscopy: The detection of spermatozoa using a microscope is conclusive of the presence of semen.

Examination for Saliva .....

Presumptive test for saliva

RSID™-Saliva: Antibody-based detection of human salivary α-amylase (specifically AMY1 isoform), a protein produced by the parotid gland.

A positive result may also be obtained from perspiration, breast milk, and tears.

There is, to date, no well-accepted confirmatory test for saliva.

Examination for Urine .....

Presumptive test for urine

RSID™-Urine: Antibody-based detection of Tamm-Horsfall protein (THP) produced by the loop of Henle within the kidney.

A positive result may also be obtained from gorilla, canine, equine and rat urine.

There is, to date, no well-accepted confirmatory test for human urine.

Phase 2: DNA Processing of Crime Exhibits

DNA processing consists of 4 steps: DNA Extraction, Quantitation, Amplification and Detection of Amplified DNA.

DNA Extraction.....

DNA must first be separated from other cellular material before it can be analysed. This is done by lysing (breaking open) the cells to release the DNA for downstream analysis.

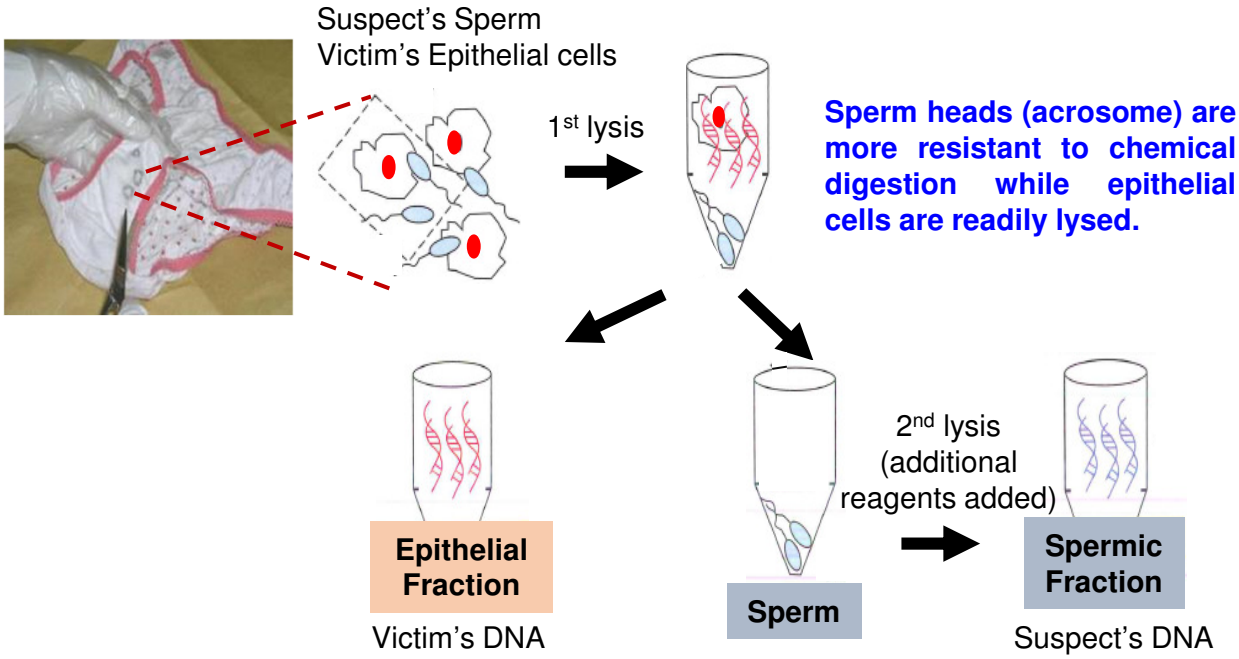
This can be achieved using 2 types of extraction methods: Standard DNA extraction and Differential DNA extraction.

(1) Standard DNA Extraction

Standard DNA extraction involves lysing all types of cells to release the DNA.

(2) Differential DNA Extraction

Differential DNA extraction attempts to separate sperm, if present, from non-sperm cells via a two-step process. In the first step, non-sperm cells are lysed to release their DNA into the **Epithelial Fraction**, while leaving the sperm intact. In the second step, the sperm are lysed to release their DNA into the **Spermic Fraction**. This extraction method is used in sexual assault cases to separate the DNA from the suspect's sperm and victim's epithelial cells.



DNA Quantitation .....

Human-specific DNA quantitation is performed to determine the optimum volume of DNA extract to use in the amplification step.

DNA Amplification .....

The Polymerase Chain Reaction (PCR) is an amplification process which replicates a single copy of targeted DNA into multiple copies. This allows for the production of sufficient amounts of DNA to be detected.

Detection of Amplified DNA .....

The amplified DNA is detected through fluorescence using a genetic analyser.

Phase 3: Interpretation, Matching of DNA Profiles and Report Generation of Crime Exhibits

When all the necessary tests are performed and the results obtained, the Analyst reviews and interprets all examination records and results and prepares a report. These are reviewed by another Analyst before the report is released.

Autosomal DNA Profile ……..

The autosomal DNA profile obtained after detection in the genetic analyser can be broadly classified as either “Not interpretable" or "Interpretable”.

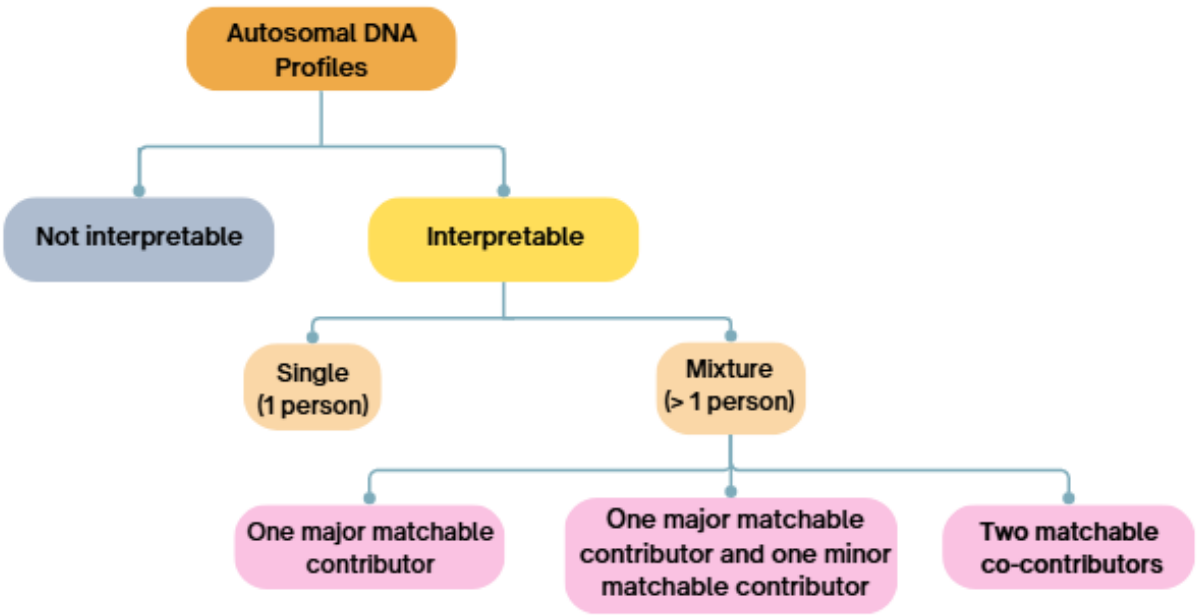
An interpretable DNA profile is either a single source DNA profile or a mixed DNA profile (mixture). A single source DNA profile has a maximum of two alleles at each locus and originated from a single contributor. A mixture has more than two alleles at multiple loci and it could include:

1) one major matchable contributor,
2) one major matchable contributor and one minor matchable contributor, where the two contributors are present in different quantities/proportion and therefore distinguishable, or
3) two matchable co-contributors, present in roughly similar quantities/ proportion and therefore not distinguishable.

The mixture could additionally have a component that cannot be interpreted which may result from insufficient DNA and/or DNA from multiple persons with no distinct contributor(s) observed. Matching cannot be performed for this component.

After interpretation, each matchable contributor is designated, e.g. “UNKNOWN MALE 1”, and compared to reference DNA profile(s), if provided. If there is no reference sample or there is no match to given reference DNA profile(s), the unknown remains as is.

If there is a match to a given reference DNA profile, the reference is stated as a contributor. The Table of alleles for the DNA profile(s) matched to reference(s) and the Table of Match Statistics can be found on Appendices I and II within the DNA report.



(1) Not interpretable

S/N	Exhibit Information	Area(s) examined	Examination for Biological Fluid	Match Analyses
1	**1.001** **"X1"** Two swabs	(A) Swabs	Not applicable	No interpretable DNA profile

The DNA profile obtained could not be interpreted due to insufficient DNA and/or DNA from multiple persons with no distinct contributor(s) observed.

(2) Interpretable DNA Profile – Single

S/N	Exhibit Information	Area(s) examined	Examination for Biological Fluid	Match Analyses
2	**1.002** **"X2"** One swab	(A) Swab	Not applicable	Contributor: UNKNOWN MALE 1
3	**1.003** **"X3"** One swab	(A) Swab	KM (blood): Pos OBTI (human blood): Pos	Contributor: S123456
4	**1.004** **"X4"** One swab	(A) Swab	Semenogelin (semen protein): Pos PSA (semen protein): Pos	Epithelial fraction Contributor: A112233 Spermic fraction Contributor: S123456 A112233 can be reasonably expected as a contributor.

In this example:

i. The DNA profile obtained from “X2” is designated as UNKNOWN MALE 1. This is where no reference sample is provided or when there is no match to the reference DNA profile(s). An exclusion statement would be stated if it does not match to the reference DNA profile(s).

ii. The DNA profile obtained from “X3" is found to match the reference DNA profile of S123456. A Random Match Probability (RMP) statistical calculation is provided to assess the significance of the DNA match.

The RMP provides the probability of observing the DNA profile in question in a population of randomly selected and unrelated individuals.

iii. Differential DNA extraction was performed for “X4”, thus two fractions (Epithelial fraction and Spermic fraction) were obtained.
The DNA profile obtained from the epithelial fraction is found to match the reference DNA profile of A112233. As the exhibit had originated from A112233 (based on information on exhibit origin provided by the submitting agency), it is reasonably expected for A112233’s DNA profile to be found on the exhibit. Table of alleles and match statistics will not be provided.
The DNA profile obtained from the spermic fraction is found to match the reference DNA profile of S123456. A RMP statistical calculation will be performed for this contributor.

(3) Interpretable DNA Profile – Mixture : One matchable contributor

S/N	Exhibit Information	Area(s) examined	Examination for Biological Fluid	Match Analyses
5	**1.005** **"X5"** One T-shirt	(A) Exterior	Not applicable	Contributor: S123456*

The DNA profile obtained from "X5" has a major matchable contributor and it is found to match the reference DNA profile of S123456. A RMP statistical calculation will be performed for this major component.
The * denotes that there is a component that is not interpretable in addition to the matchable contributor(s). Please refer to Page 12.

(4) Interpretable DNA Profile – Mixture : One major matchable contributor and one minor matchable contributor

S/N	Exhibit Information	Area(s) examined	Examination for Biological Fluid	Match Analyses
6	**1.006** **"X6"** One swab	(A) Swab	Not applicable	Major contributor: A112233 Minor contributor: S123456

The DNA profile obtained from "X6" has a major matchable contributor and it is found to match the reference DNA profile of A112233. There is also a minor matchable contributor and it is found to match the reference DNA profile of S123456. Separate RMP statistical calculations will be performed for each matchable contributor.

(5) Interpretable DNA Profile – Mixture : Two matchable co-contributors

S/N	Exhibit Information	Area(s) examined	Examination for Biological Fluid	Match Analyses
7	**1.007** **"X7"** Two swabs	(A) Swabs	Not applicable	Co-contributors: A112233 + S123456
8	**1.008** **"X8"** One dress	(A) Interior	Not applicable	Co-contributors: A112233 + S123456 A112233 can be reasonably expected as a contributor.

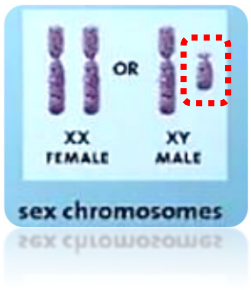
i. The DNA profile obtained from "X7" is a mixture of two matchable contributors. Reference DNA profiles of A112233 and S123456 can be included as co-contributors.
A different statistical calculation, referred to as the Combined Probability of Inclusion (CPI), is used to assess the significance of the DNA match.

The CPI provides the probability of an individual, in a population of randomly selected and unrelated individuals, being included as a possible contributor to the DNA mixture.

ii. The DNA profile obtained from "X8" is a mixture of two matchable contributors. Reference DNA profiles of A112233 and S123456 can be included as co-contributors.
As the exhibit had originated from A112233 (based on information on exhibit origin provided by the submitting agency), it is reasonably expected for A112233's DNA profile to be on the exhibit.
The other contributor is found to match the reference DNA profile of S123456. A RMP statistical calculation will be performed for this contributor.

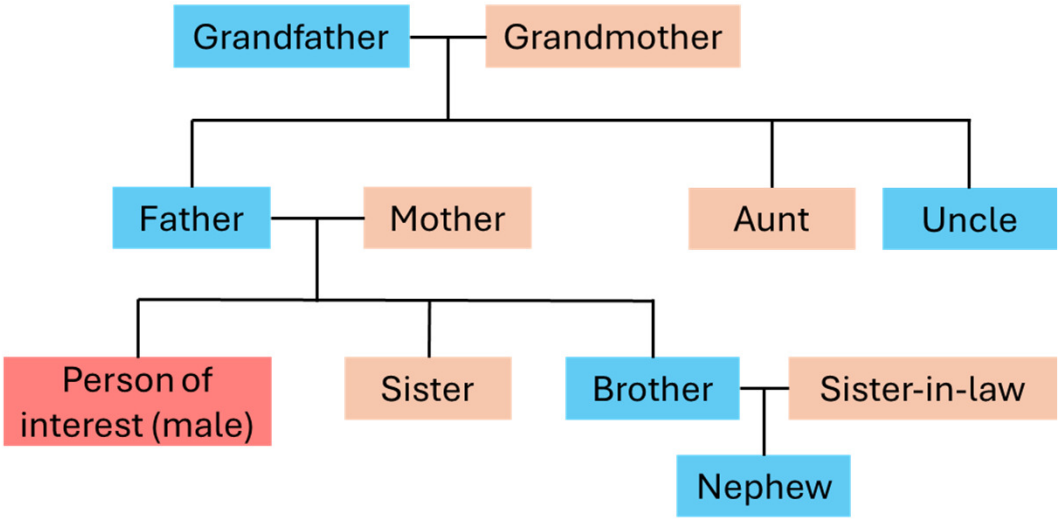
Y-chromosome DNA Analysis……..……..

Another type of DNA testing is called Y-chromosome DNA analysis. The Y-chromosome DNA profile is obtained by an additional amplification after standard autosomal DNA analysis, focusing on loci on the Y-chromosome.



As the Y-chromosome is unique to males, this type of analysis can be very useful in cases where the male suspect's DNA may be masked by the excessive female victim's DNA when using standard autosomal DNA analysis, e.g. sexual assault cases.

The Y-chromosome is inherited in a patrilineal manner i.e. passed down unchanged from father to son. Looking at the pedigree tree (below), this would mean that all the paternal male relatives (in Blue), barring mutations, will have the same Y-chromosome DNA profile as the Person of interest (in red). The Y-chromosome DNA profile is thus not unique to an individual, unlike an autosomal DNA profile, and does not differentiate between males in the same family tree.



The power of discrimination of Y-chromosome DNA analysis is, therefore, considerably less than that of standard autosomal DNA analysis.

Y-chromosome DNA Profile of Reference Samples

Locus	Sample
DYS576	19
DYS389I	13
DYS635	24
DYS389II	29
DYS627	21
DYS460	11
DYS458	17
DYS19	15
YGATAH4	13
DYS448	19
DYS391	11
DYS456	15
DYS390	24
DYS438	12
DYS392	13
DYS518	37
DYS570	17
DYS437	15
DYS385	11, 14
DYS449	30
DYS393	13
DYS439	12
DYS481	22
DYF387S1	35, 37
DYS533	13

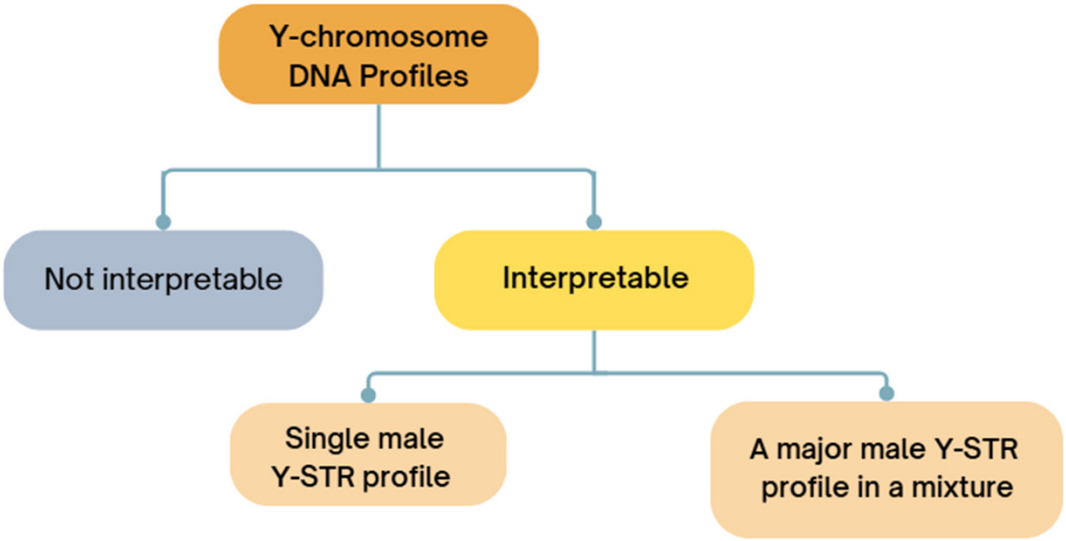
The reference Y-chromosome DNA profile has 25 loci. There is generally only one allele at each locus, with the exception of DYS385 and DYF387S1.

The Y-chromosome DNA profile of the known individual is used as a reference for which to compare against unknown Y-chromosome DNA profiles obtained from crime exhibits.

Y-chromosome DNA Profile of Crime Exhibits…….

The Y-chromosome DNA profile obtained after detection in the genetic analyser can be broadly classified as either “Not Interpretable" or "Interpretable”.

The Y-chromosome DNA profile is interpretable only if there is a single male Y-STR profile or a major male Y-STR profile in a mixture.



(1) Not interpretable

S/N	Exhibit Information	Area(s) examined	Y-STR Match Analyses
1	**1.001** **"Y1"** One swab	(A) Swab	No interpretable DNA profile

The Y-STR DNA profile could not be interpreted due insufficient male DNA and/or DNA from multiple males with no distinct male contributor(s) observed.

(2) Interpretable DNA Profile – Single

S/N	Exhibit Information	Area(s) examined	Y-STR Match Analyses
2	**1.002** **"Y2"** One swab	(A) Swab	Contributor(s): Y-STR Profile 1
3	**1.003** **"Y3"** One swab	(A) Swab	Contributor(s): Y-STR Profile 2 S987654 and/or any of his paternal male relatives can be included.

i. The Y-chromosome DNA profile obtained from “Y2“ is designated as “Y-STR Profile 1”. This is where no reference sample is provided or when there is no match to the reference DNA profile(s). An exclusion statement would be stated if it does not match to the reference sample(s).

ii. The Y-chromosome DNA profile obtained from “Y3“ is designated as “Y-STR Profile 2”. This profile obtained is found to match the male reference DNA profile of S987654. A RMP statistical calculation will be performed. A RMP statistical calculation is provided to assess the significance of the DNA match.
When read with the autosomal DNA report where no male autosomal profile is detected, paternal male relatives of S987654 who share the same Y-chromosome DNA profile (barring mutations) can also be included since the Y-chromosome is paternally inherited.

The RMP provides the probability of observing the Y-chromosome DNA profile in question in a population of randomly selected and unrelated male individuals.

(3) Interpretable DNA Profile - Mixture : One matchable contributor

S/N	Exhibit Information	Area(s) examined	Y-STR Match Analyses
4	**1.004** **"Y4"** One swab	(A) Swab	Contributor(s): Y-STR Profile 3*

The Y-chromosome DNA profile obtained from “Y4" has a major matchable component and is designated as “Y-STR Profile 3”.

The * denotes that there is a component that is not interpretable in addition to the matchable contributor(s).