



Foundations in Forensic DNA Testing for Sexual Assault Kits

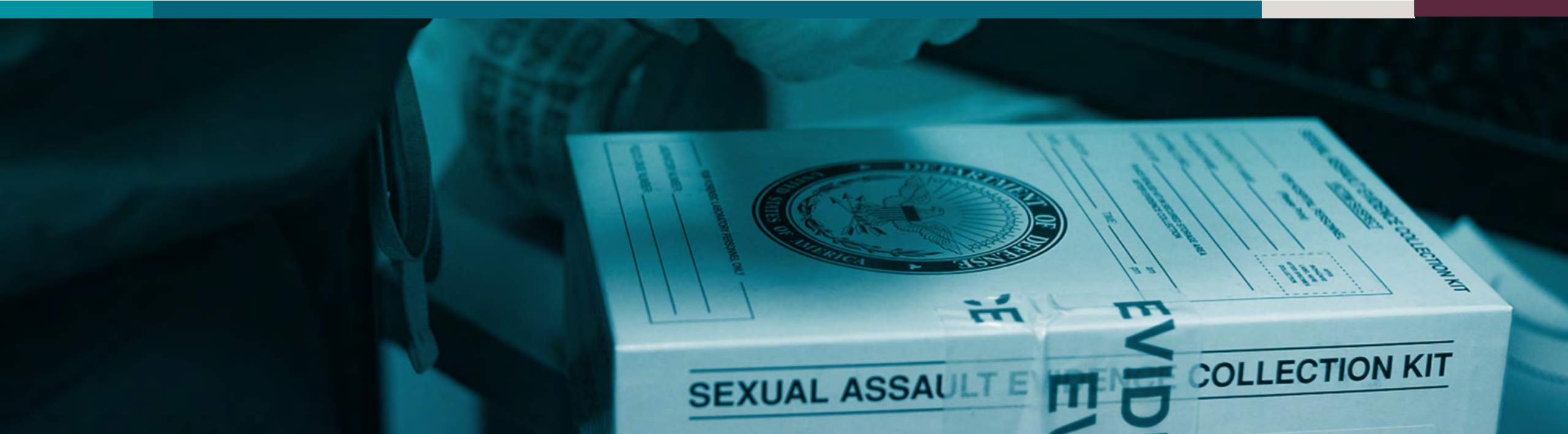
Amy Jeanguenat, MFS
amy@mindgenllc.com



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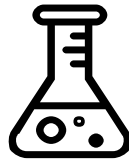
OBJECTIVES



DNA Typing



Case Study



Laboratory
Workflow

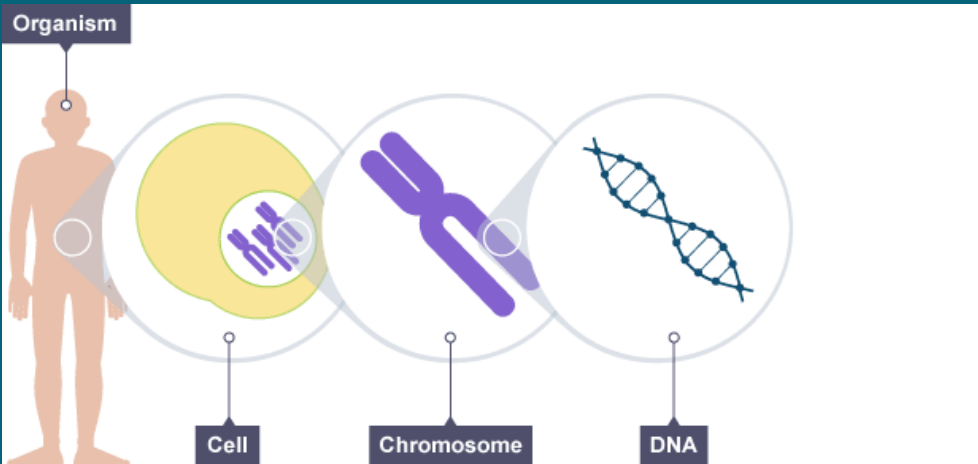


Reporting &
Conclusions



Timelines

Foundations in

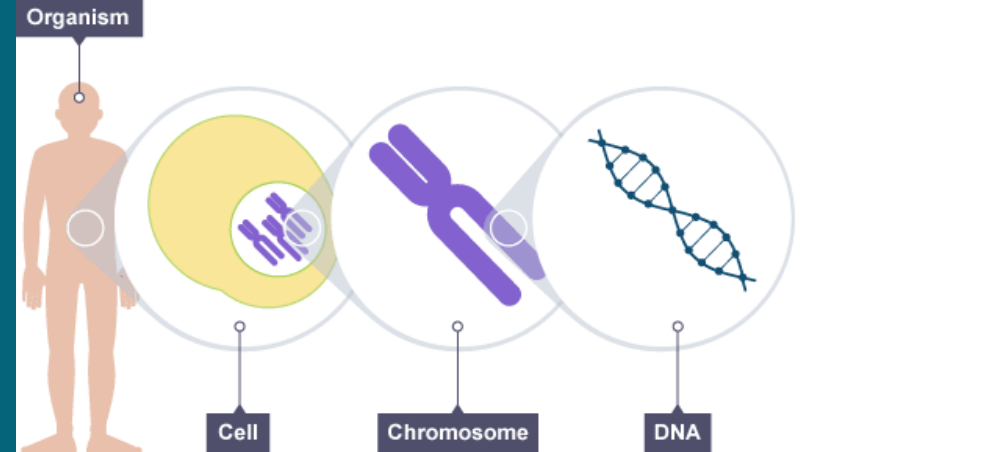


DNA Typing

- DNA= Deoxyribonucleic acid
- Genetic blueprint
- Nuclear DNA = 50% DNA from Mom/
50% DNA from Dad

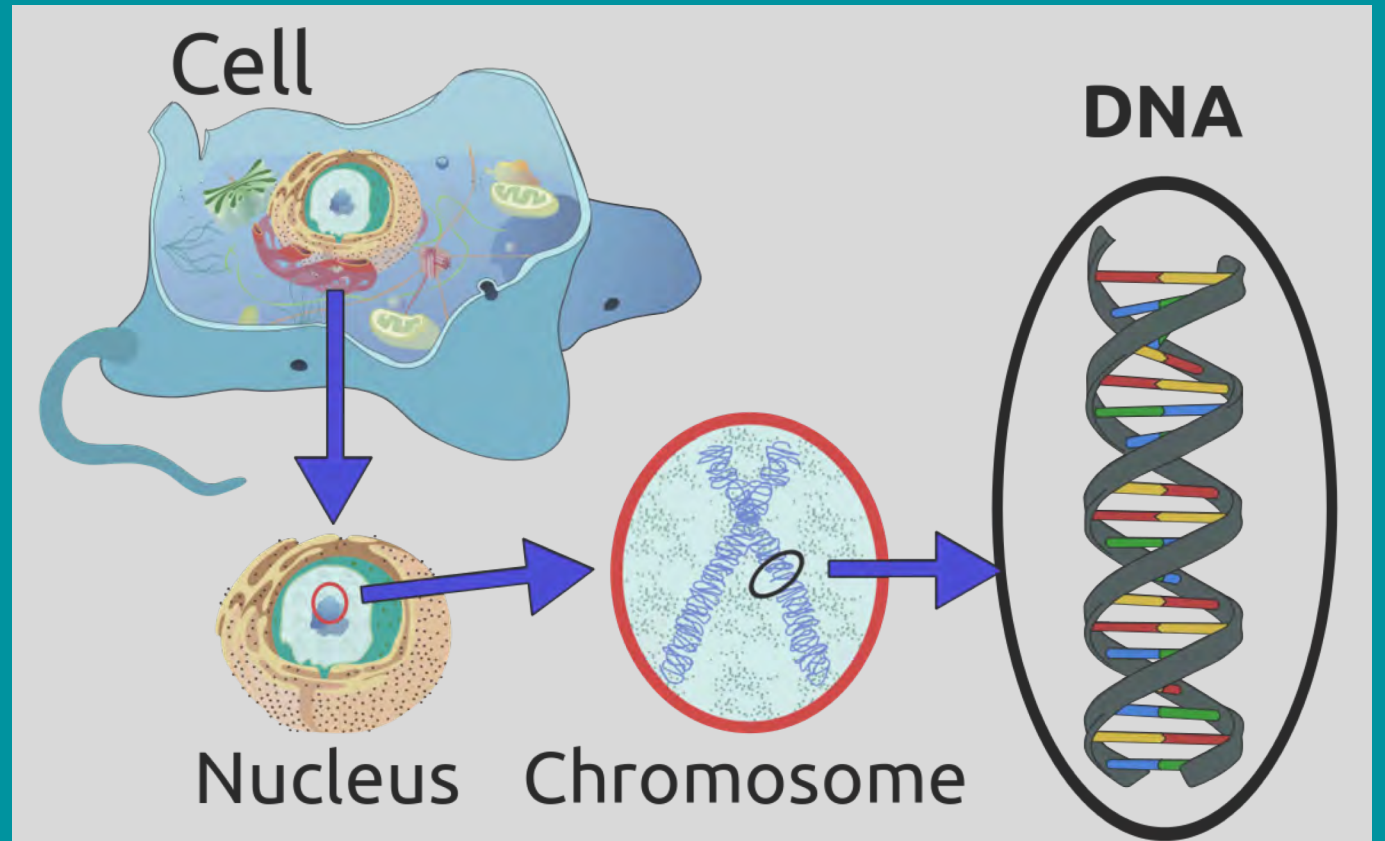


Foundations in

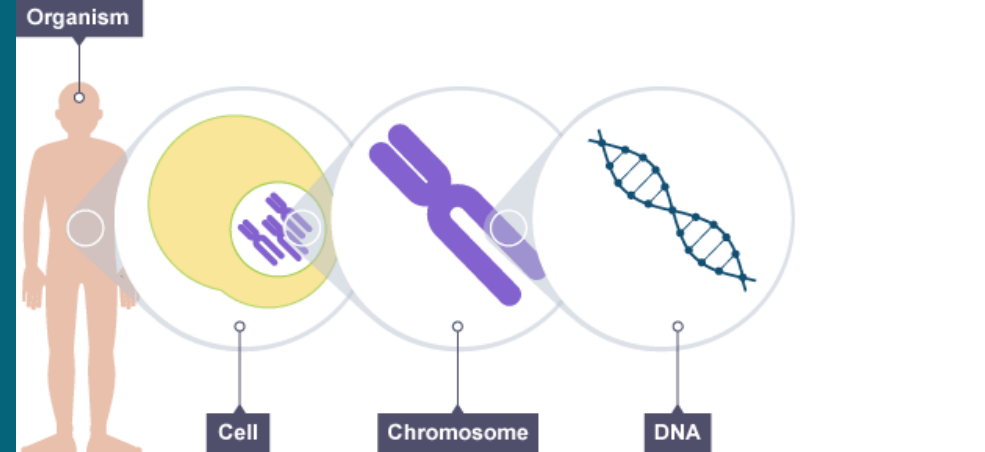


DNA Typing

- DNA is located within a chromosome structure that is housed in the nucleus of a cell



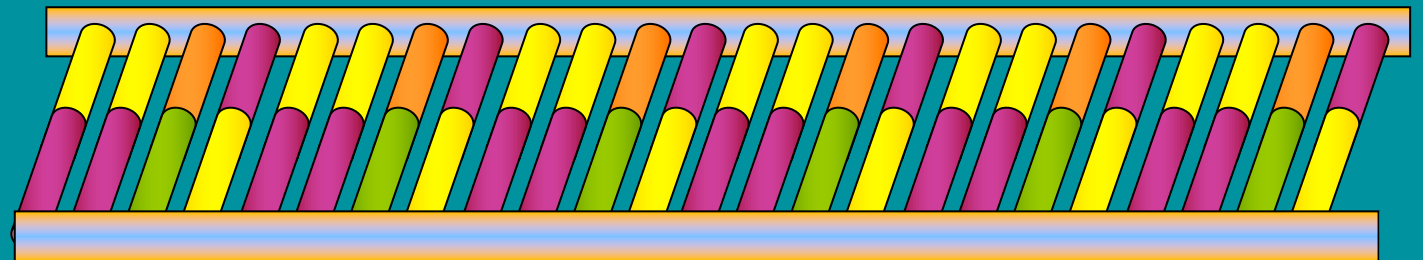
Foundations in



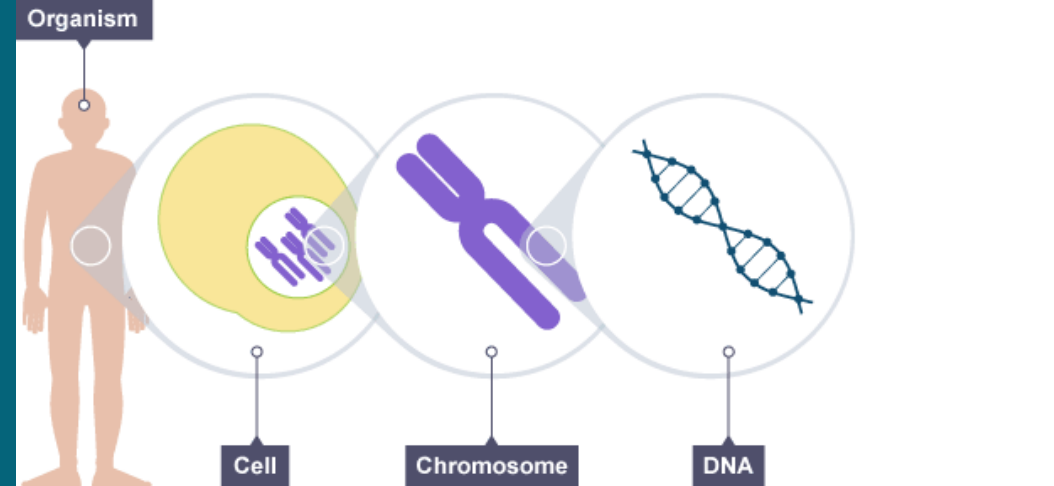
DNA Typing

- DNA consists of four bases that are the genetic 'alphabet': A, T, C, G
- Sex determining chromosomes can differentiate female (X,X) and male (X,Y)

A A G T A A G T A A G T A A G T A A G T A A G T



Foundations in

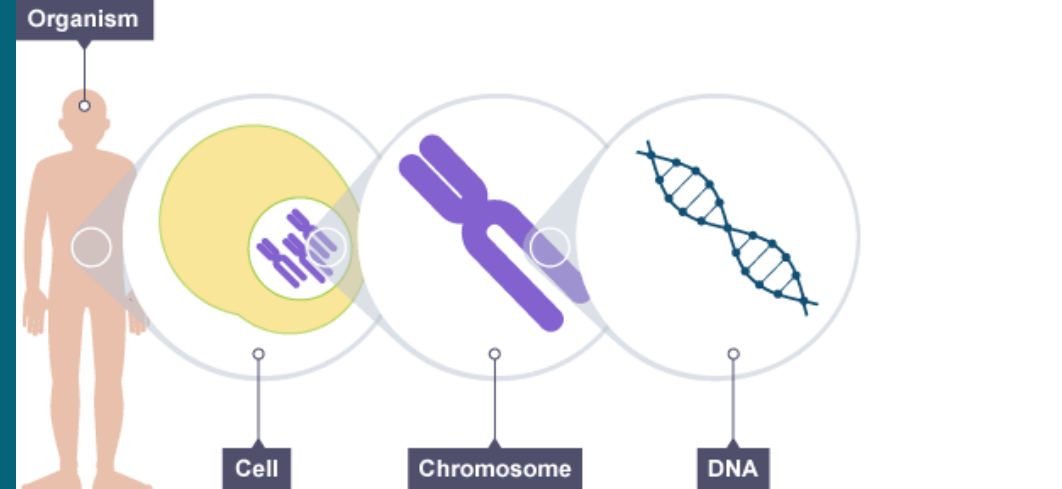


DNA Typing

- Barring rare exceptions- DNA within all cells is the same



Foundations in



DNA Typing

- DNA is 99% the same between all humans
- Forensic DNA testing targets areas in the 1% of human DNA that makes us different



STR

Foundational principle:
Found throughout the human genome, STR regions used in forensic testing are highly variable



Short segments of DNA (4-5 bases)

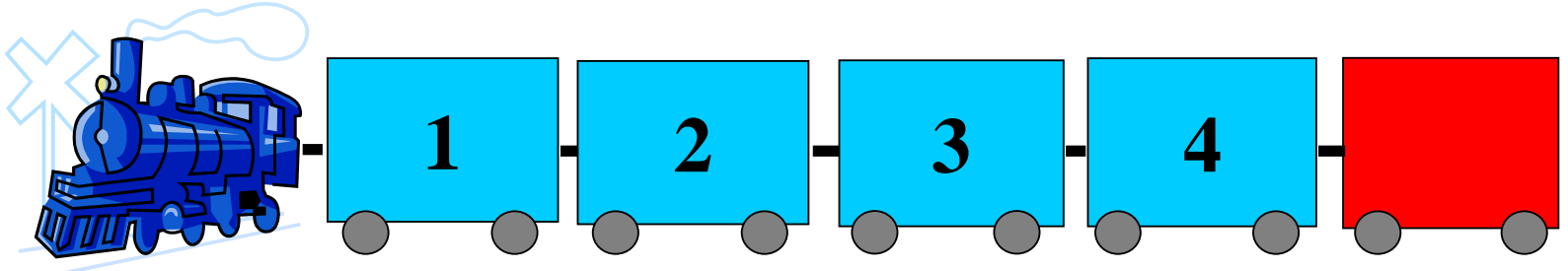
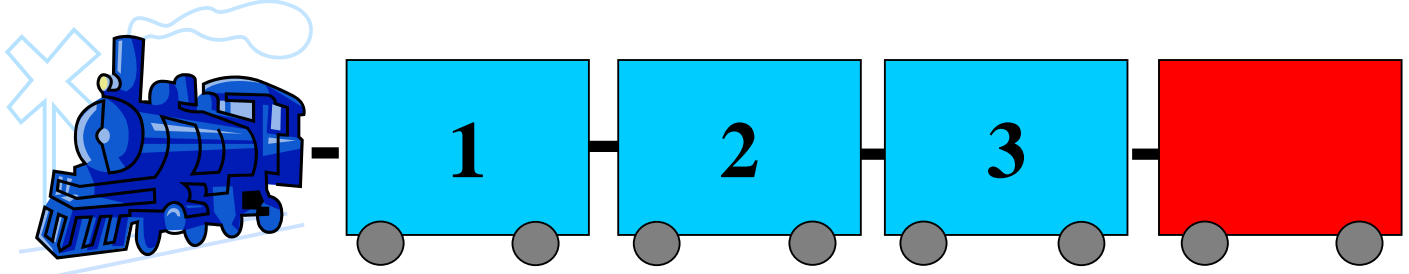
Tandem (next to each other)

Repeat over and over again (same bases recur again)

Allele: difference in the number of repeats

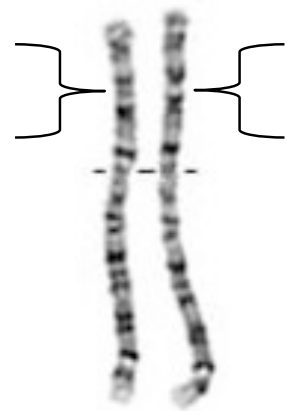
DNA Profile: Summary of differences at each location tested

Length Polymorphisms / STRs



Mom's Allele

**3 Repeats
(Box cars)
"3"**



Dad's Allele

**4 Repeats
(Box cars)
"4"**

At each location tested, an individual will have up to two different number of repeats

Y-STR

Foundational principle:
Targets STR regions only on the Y-chromosome
found in males

High levels of female DNA

Resolve male: male mixtures

Provide clarity for inconclusive STR results

Extended time intervals between incident & collection

Digital penetration



CODIS 20 LOCI

Laboratories in the U.S. that participate in NDIS are required to perform forensic DNA testing with a minimum 20 core loci

- No predictive value for disease
- Allow sharing of profiles domestically and internationally
- Aid in power of discrimination
- Reduce false matches

Locus
CSF1PO
D3S1358
D5S818
D7S820
D8S1179
D13S317
D16S539
D18S51
D21S11
FGA
TH01
TPOX
vWA
D1S1656
D2S441
D2S1338
D10S1248
D12S391
D19S433
D22S1045

Original 13

Required as
of 2017

Databases used in forensic typing

Elimination Contamination Prevention

- Collection of DNA profiles held in a searchable format from individuals whose access/role/activities are deemed to be a potential DNA contamination risk. The profiles are used to identify instances of inadvertent contamination.

Population Allelic frequency

- Aid in determining the proportion of a particular allele among the chromosomes carried by individuals in a population; how often an allele is found within a particular group of individuals aids in determining the rarity of a DNA profile.

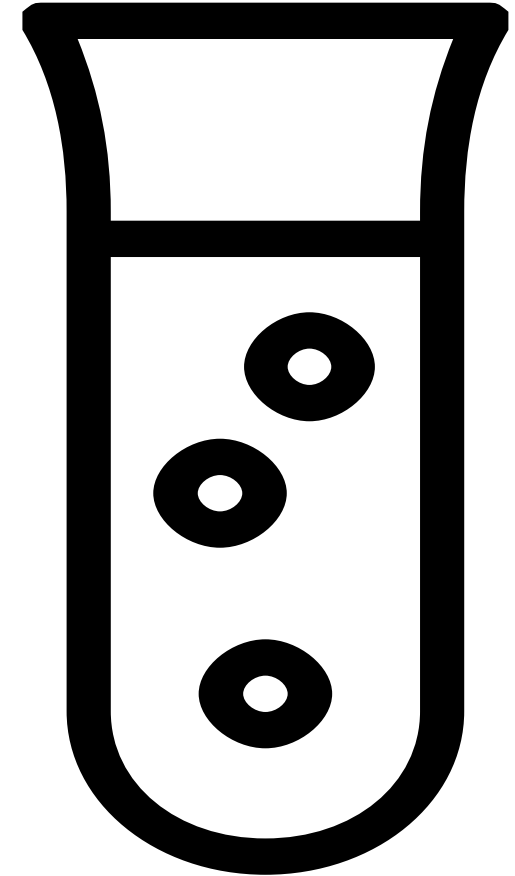
Searchable Compares known and evidence profiles

- Databases that store DNA profiles obtained from crime scene evidence and known offenders/arrestees. Profiles can be searched to link crimes together and individuals to crimes.

CONTAMINATION PREVENTION & MONITORING

Control samples: Used throughout testing to monitor contamination and procedure functionality

- Blank/Negative control: consists of reagents used in testing without the introduction of samples
- Positive control: consists of reagents used in testing and known template DNA

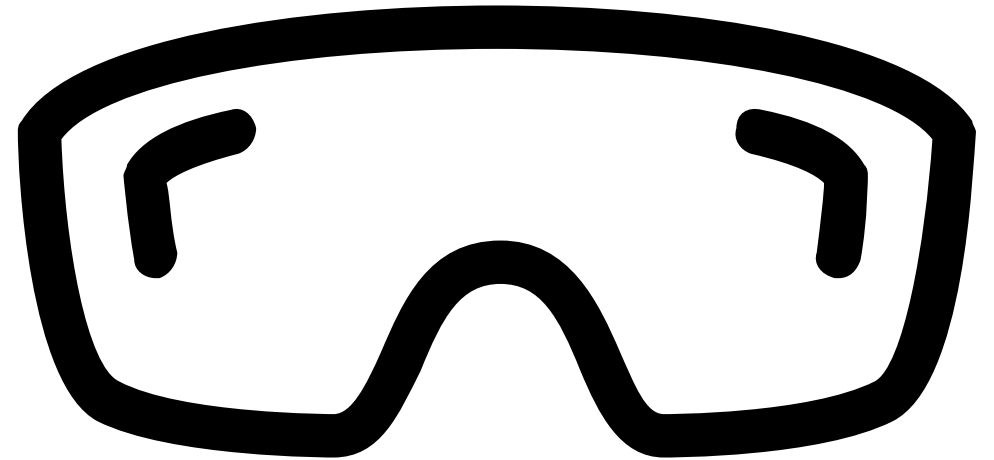


CONTAMINATION PREVENTION & MONITORING

Personal protective equipment: biohazard protection and protects against human DNA transfer

Examples:

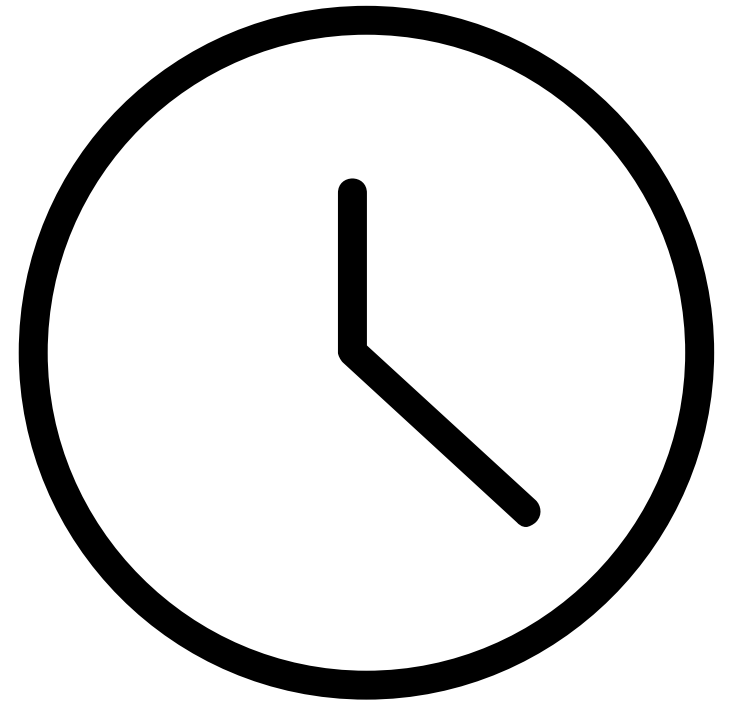
- Goggles
- Laboratory Coats
- Face Mask
- Gloves



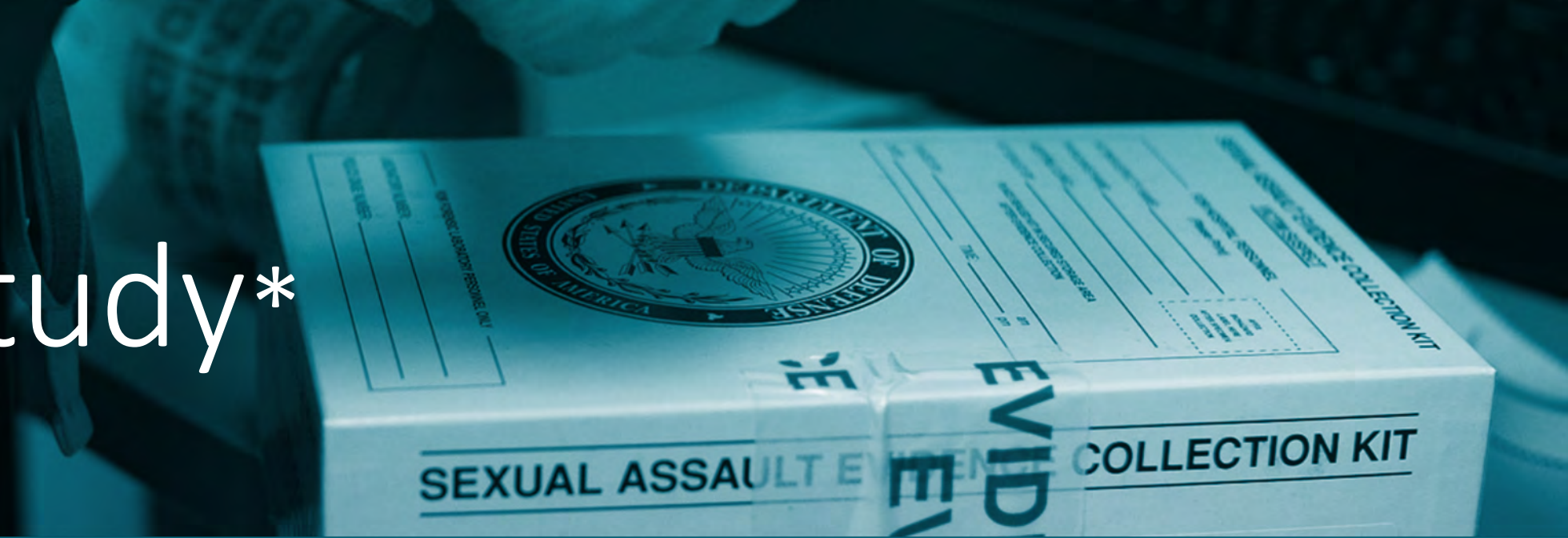
CONTAMINATION PREVENTION & MONITORING

Time & space workflow considerations

- Each item handled independently
- Evidence processed separately from known samples
- Pre and post amplification separation
- Dedicated equipment & tools



Case Study*



Katie Wells was driving home from work in the city around 3 AM. She was stopped at a red light when her car was approached by two men carrying baseball bats. Her passenger window was busted open. One man dragged her into the backseat and sexually assaulted her as the other pulled her car into an alley. The men ran away as sirens approached.

*scenario and results are fabricated but resemble actual cases



The sirens did not come for Katie. She called a friend when she felt it was safe. The friend encouraged her to call the police and go to the hospital.

The medical report documents:

Victim was fondled on the chest, while kissed on the mouth and neck, and vaginally penetrated. Victim is unsure if ejaculation occurred.



The following items were collected:

- Internal vaginal swabs (x2)
- External vaginal swabs (x2)
- Neck swabs (x2)
- Lip swabs (x2)
- Breast swabs (x2)
- Underwear
- Fingernail clippings
- Blouse and jeans
- Known sample- Katie Wells





Kit submitted to the laboratory:

Agency case #: 12-00104

Victim: Katie Wells

Suspect: Unknown





Chain of Custody Generated

Date Received: February 2, 2018

Laboratory case number: 2018-02100



GENERATE A TESTING PLAN

High throughput decision making with forensic setting: examiner

- Screen most probative samples (3+)
- Based on medical report, submitted paperwork
- Based on body location (intimate v non-intimate)
- Sample retention as appropriate

Goal:

**Generate
CODIS
eligible
Profile**

GENERATE A TESTING PLAN

Consider the results

- Input from law enforcement and legal system
- Test more items if necessary
- Determine possible suitability for Y-STR testing
- Determine need for biological fluid screening (if not performed initially)

Goal:

Obtain additional information needed for investigation & prosecution



Laboratory analyst selects three samples for testing based on the case submission and medical exam paperwork:

- Internal vaginal swabs
- Breast swabs
- Neck swabs
- Known sample from Katie Wells



SAMPLING

Remove possible biological material (substrate) from an evidence item.

Foundational techniques:

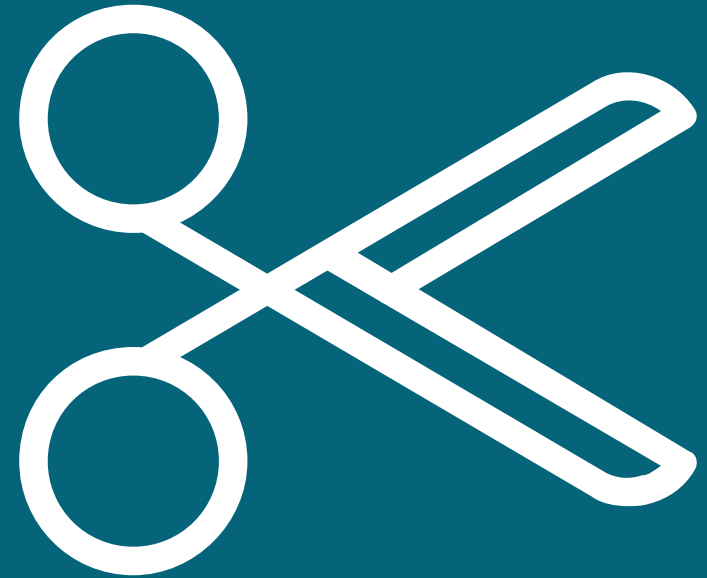
- Swab
- Cut
- Tape Lift
- Scrape





SAMPLING

- Breast swab: entire swabs consumed
- Neck swab: $\frac{1}{2}$ of both swabs consumed
- Internal vaginal swab: $\frac{1}{2}$ of both swabs consumed
- Known swab: $\frac{1}{8}$ of one swab

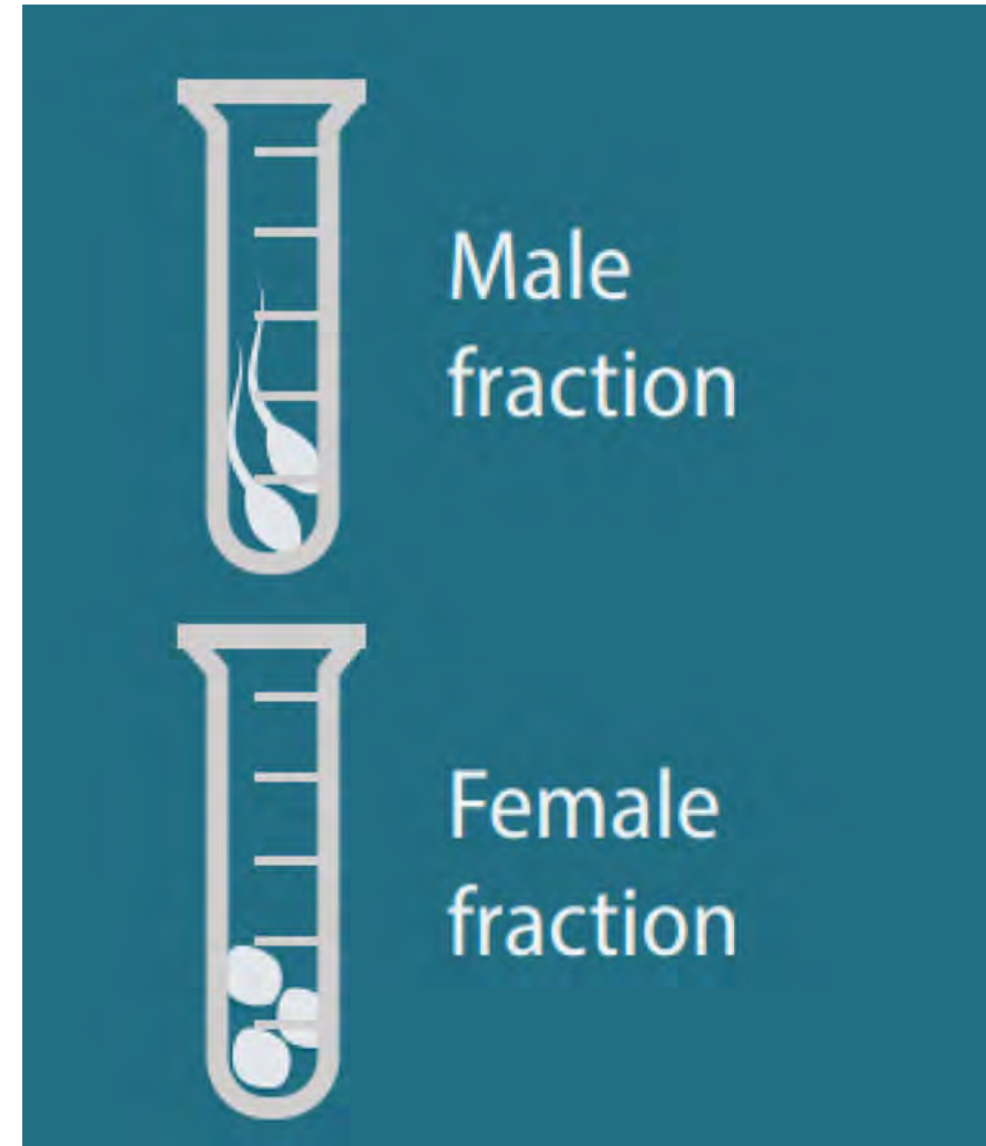


EXTRACTION

Remove cellular material from the substrate and purify DNA

Batch together similar type evidence

- Blood/Saliva
- Semen
- Touch
- Knowns

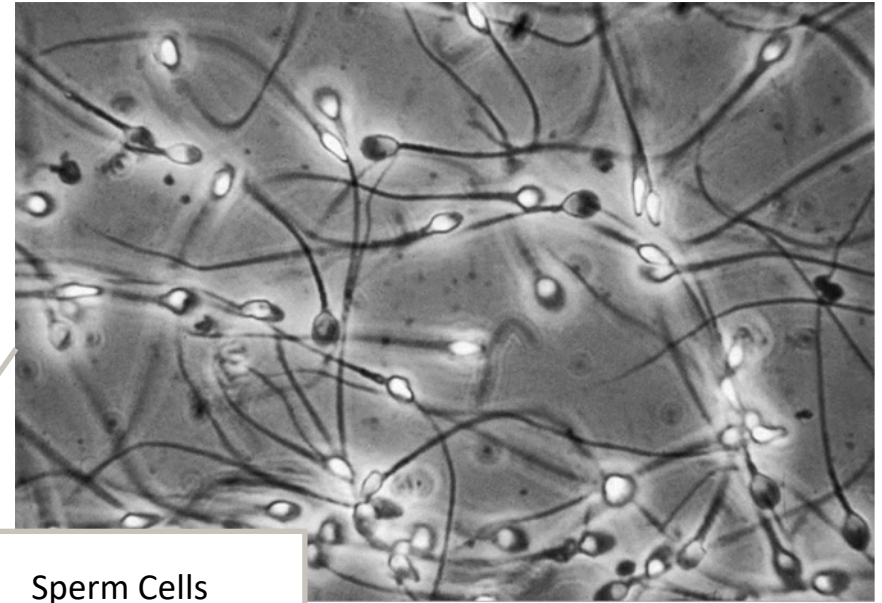


SEMEN EXTRACTION

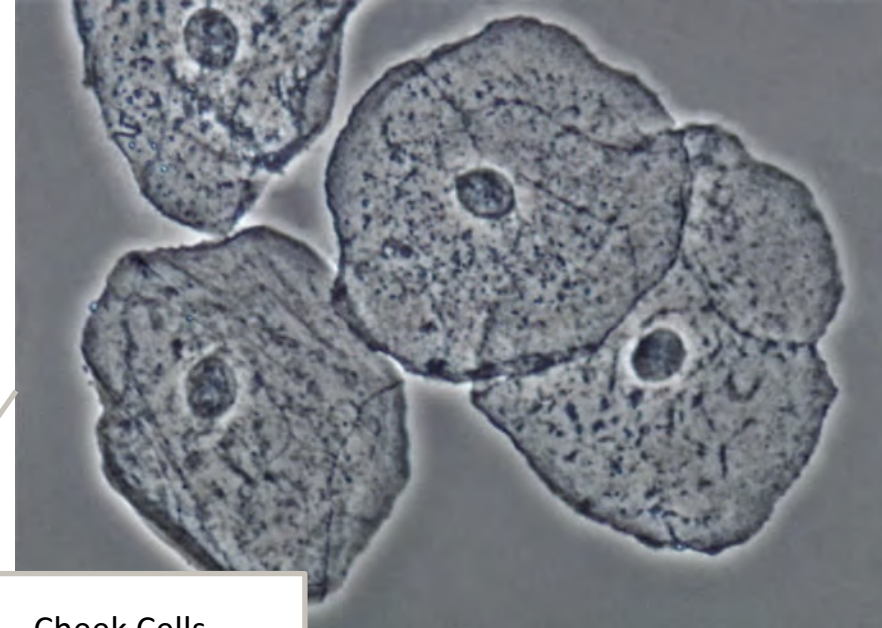
Foundational principle:

Sperm cells have a different composition than other types of cells (i.e. epithelial cells)

- Need harsher chemicals to break open and release DNA



Sperm Cells

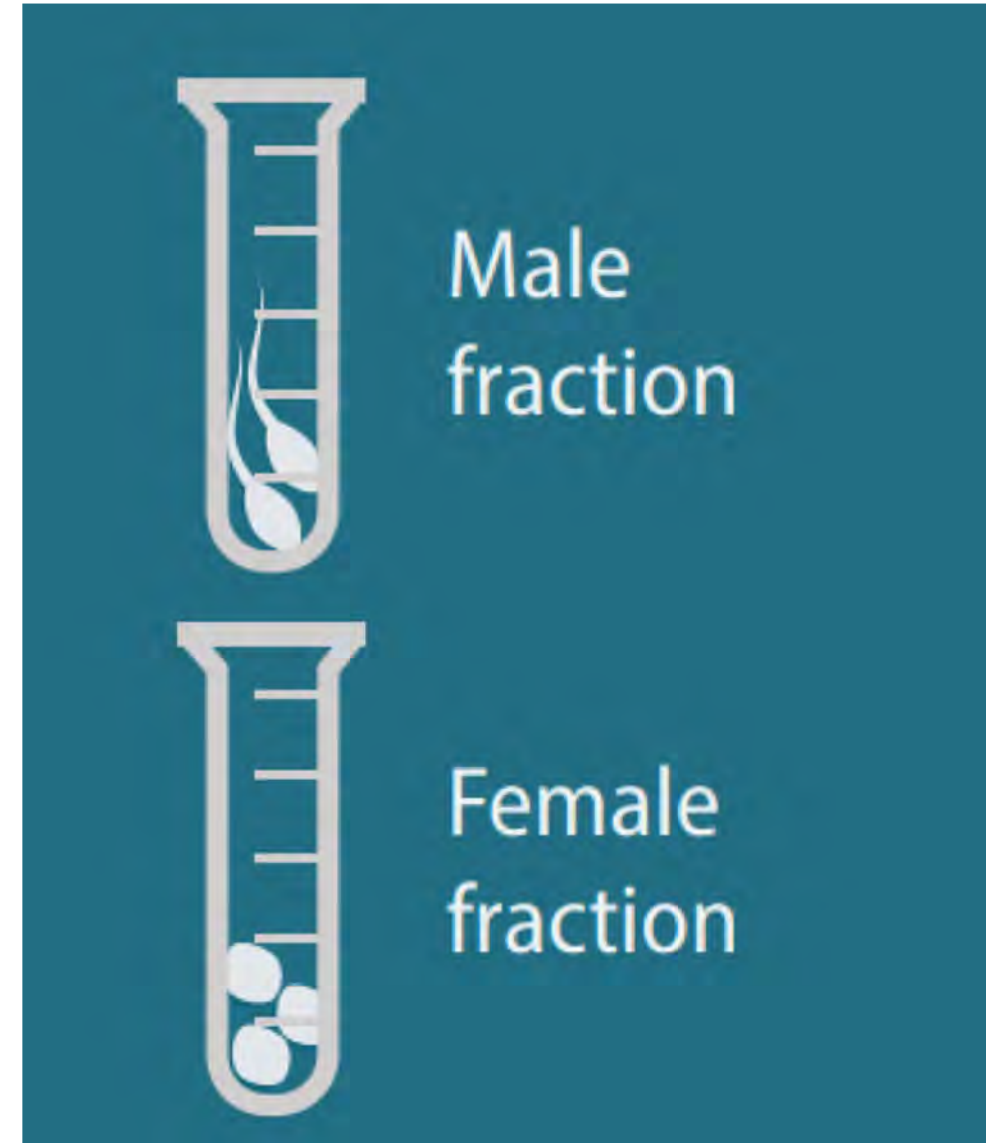


Cheek Cells

DIFFERENTIAL EXTRACTION

Foundational principle:

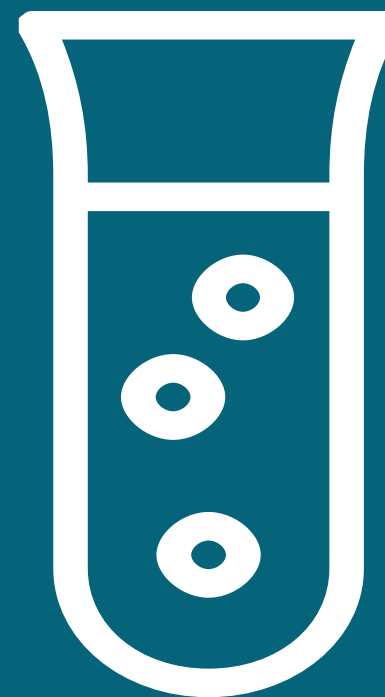
- Mild chemicals are added to disrupt non-sperm cells
- Non-sperm cellular DNA is removed (AKA- female fraction, epithelial fraction)
- Sperm cells are pelleted and washed
- Harsher chemical added to disrupt sperm cells & release sperm cellular DNA





EXTRACTION

1. Touch batch: breast (2018-02100-01)
+RB1
2. Saliva batch: neck (2018-02100-02)
+RB2
3. Differential batch: internal vaginal
(2018-02100-03)+RB3
4. Known batch: Katie Wells (2018-
02100-04 +RB4

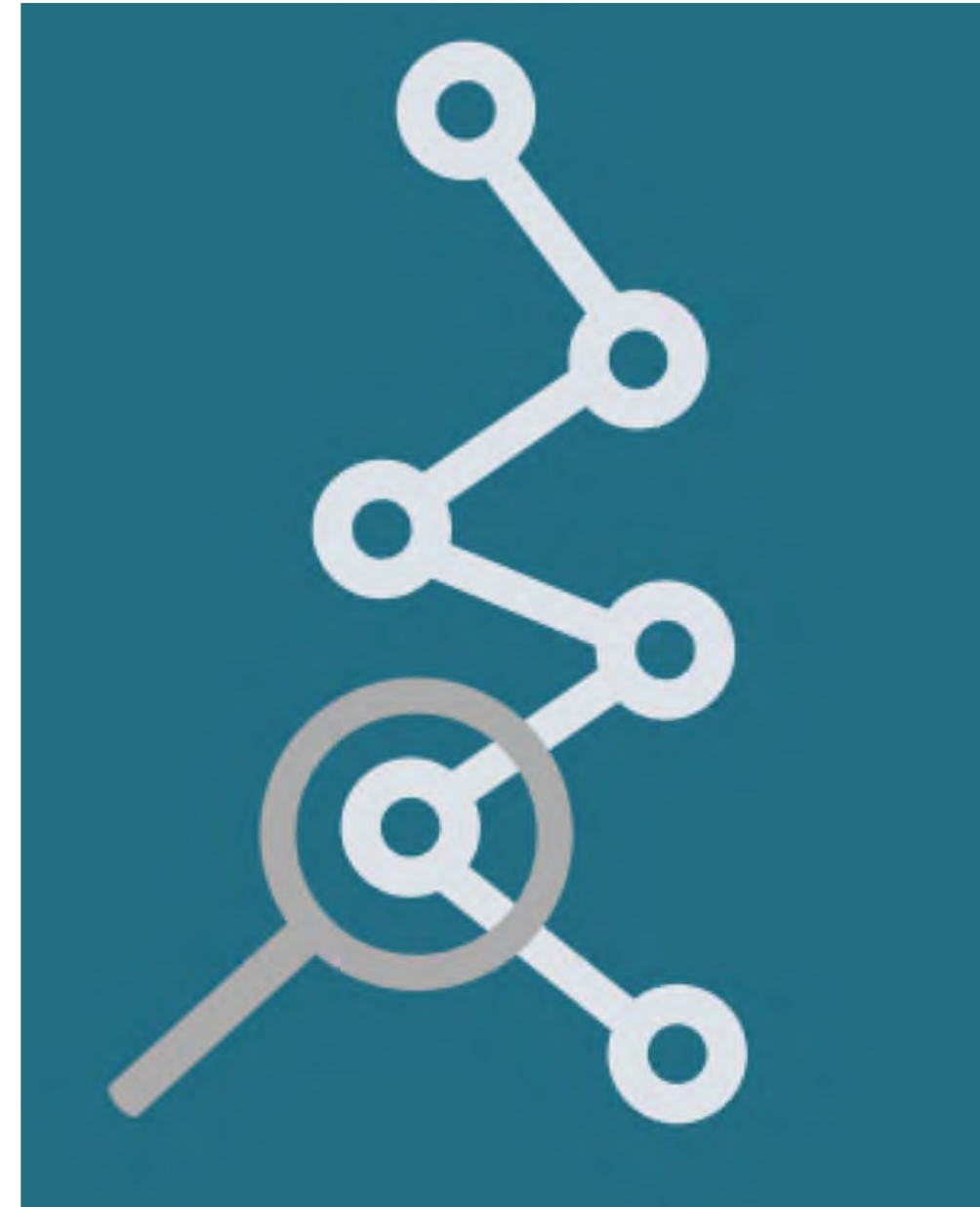


QUANTIFICATION

Estimates the amount of human and male DNA present in each sample/fraction.

Decision making:

- Target amounts for amplification
- Not proceed with DNA testing
- Proceed with STR and/or Y-STR testing

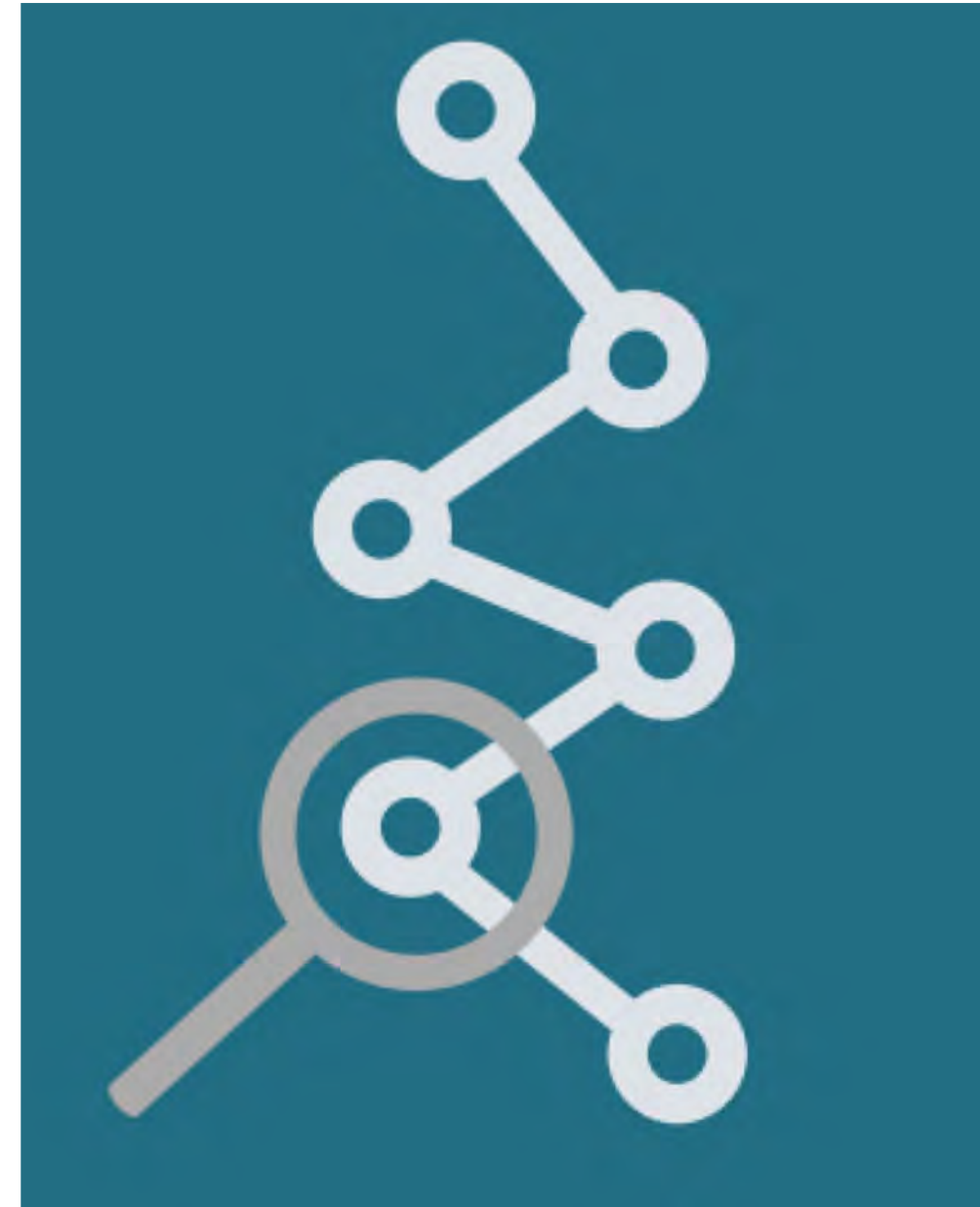


DIRECT TO DNA

DNA analysis is performed before serology to maximize the chances of obtaining CODIS-eligible profiles

DNA driven process:

- Occurs during quantification
- Determines best sample(s) to DNA test
- Better at determining possible samples that might produce a CODIS eligible profile



How much DNA do you need?



- Testing is optimized for 1 nanogram (one billionth of a gram)
- It can be performed on 0.1 ng with reasonable results



QUANTITATION/MALE DNA SCREENING

- Breast: (2018-02100-01)
 - 0.0012 ng human
 - 0.000 ng male
- Neck (2018-02100-02)
 - 0.008 ng human
 - 0.006 ng male
- Internal vaginal (2018-02100-03-SF)
 - 3.4 ng human
 - 3.0 ng male
- Internal vaginal (2018-02100-03-EF)
 - 6.0 ng human
 - 0.8 ng male



QUANTITATION/MALE DNA SCREENING

- Internal vaginal (2018-02100-03-SF)
 - 3.4 ng human
 - 3.0 ng male
- Internal vaginal (2018-02100-03-EF)
 - 6.0 ng human
 - 0.8 ng male
- Known sample (2018-02100-04)
 - 38 ng human
 - 0.0000 ng male

AMPLIFICATION

Polymerase Chain Reaction- targets specific areas in the DNA (STR regions) and amplifies them by making many copies.

Amplification allows small sample sizes to be tested and visualized by the use of fluorescent tags





AMPLIFICATION

- Neck: 2018-02100-02.1
- RB2.1
- Internal vaginal: 2018-02100-03-SF.1
- RB3-SF.1
- Internal vaginal: 2018-02100-03-EF.1
- RB3-EF.1
- POS1
- NEG1
- Known: 2018-02100-04.1
- RB4.1
- POS2
- NEG2

FRAGMENT ANALYSIS

By applying a voltage to each sample, negatively charged DNA is separated by size

A laser beam excites the fluorescent tag attached to each fragment so that the STR fragment can be visualized





FRAGMENT ANALYSIS

- Neck: 2018-02100-02.1
- RB2.1
- Internal vaginal: 2018-02100-03-SF.1
- RB3-SF.1
- Internal vaginal: 2018-02100-03-EF.1
- RB3-EF.1
- POS1
- NEG1
- LADDER A
- LADDER B
- Known: 2018-02100-04.1
- RB4.1
- POS2
- NEG2
- LADDER C
- LADDER D

STR ANALYSIS

STR regions are sized and assigned a number (i.e. allele call) based on how many repeats are represented at each location tested

The cumulation of results at each STR location tested is known as a DNA profile

DNA Profile:

TPOX 8,9.3

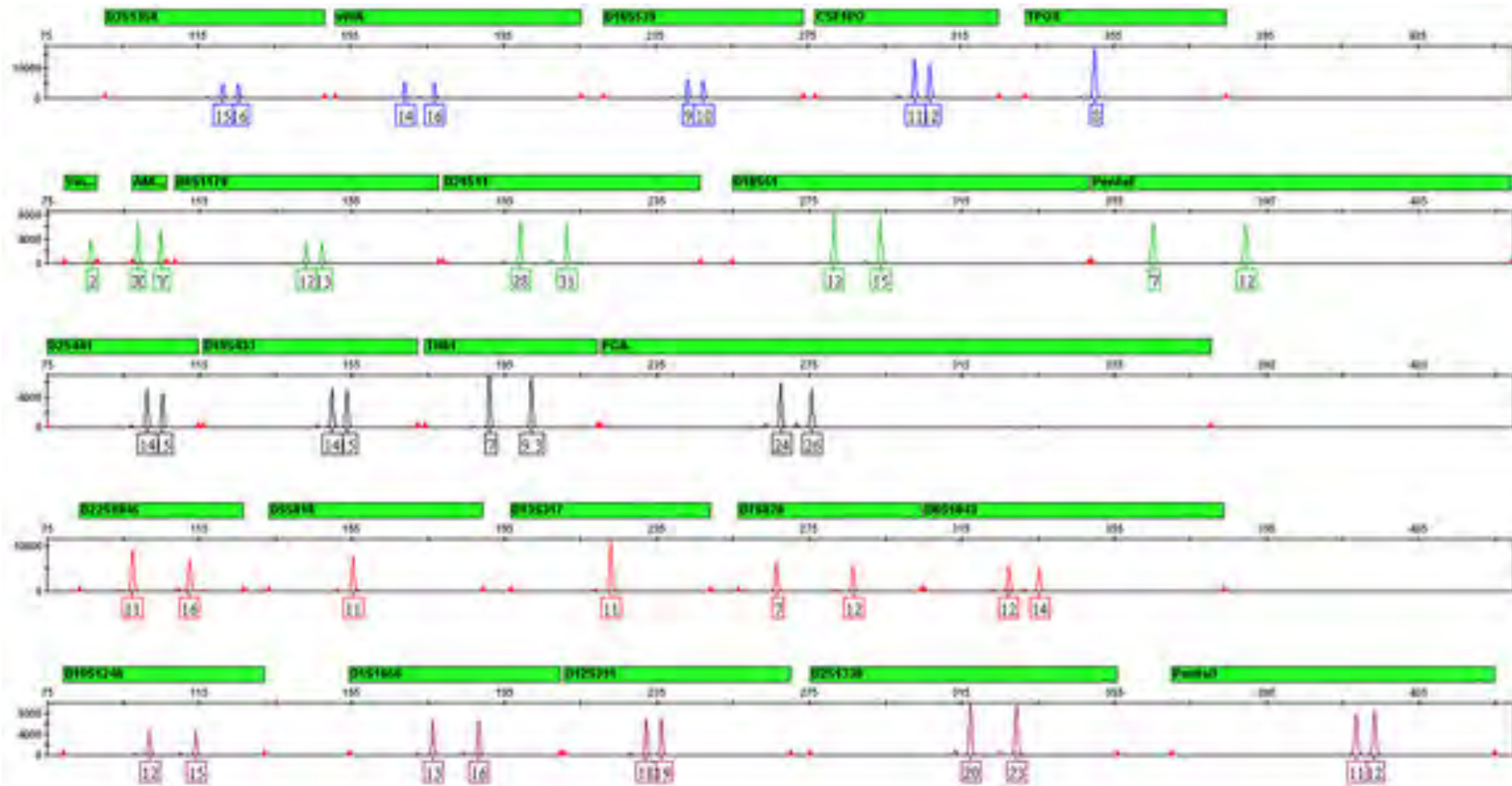
FGA: 23,28

D7: 9,13

AMEL: X, Y



STR Profile



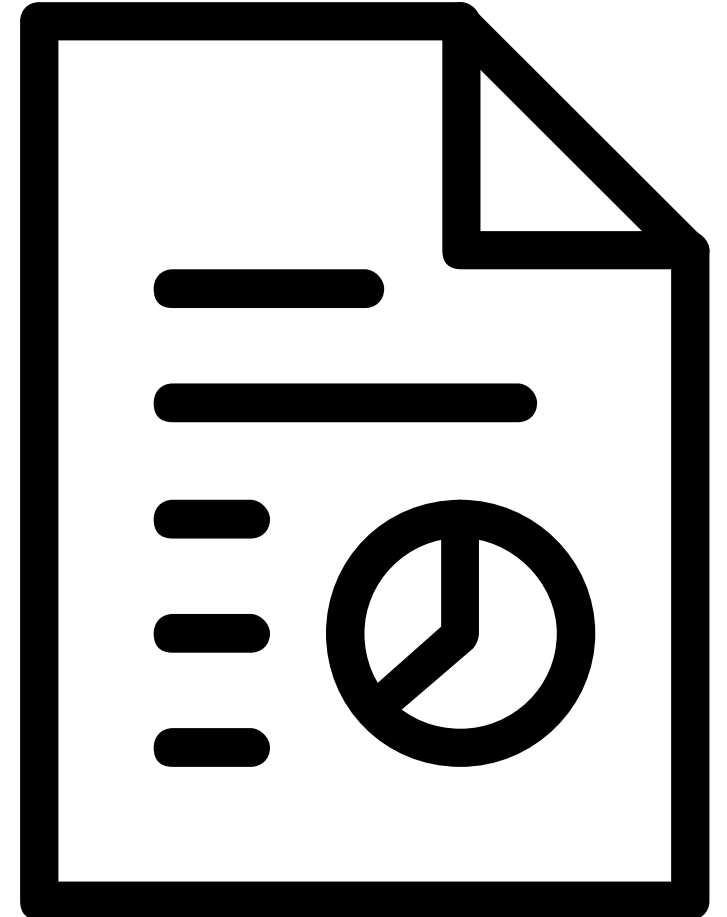
Example allele table

Loci Names	Suspect Sample	Victim Sample	Vaginal Swab - Sperm Fraction	Vaginal Swab- Epithelial Fraction
D3S1358	17, 18	15, 16	17, 18	15, 16, 17, 18
D5S818	12, 12	13, 14	12, 12	12, 13, 14
D7S820	8, 11	9, 9	8, 11	8, 9, 11
D8S1179	14, 15	12, 13	14, 15	12, 13, 14, 15
D13S317	9, 11	11, 11	9, 11	9, 11
D16S539	9, 13	10, 12	9, 13	9, 10, 12, 13
D18S51	16, 18	16, 17	16, 18	16, 17, 18
D21S11	29, 31.2	24, 28.1	29, 31.2	24, 28.1, 29, 31.2
FGA	20, 23	20, 23	20, 23	20, 23
TPOX	11, 11	8, 11	11, 11	8, 11
vWA	16, 19	16, 18	16, 19	16, 18, 19
D1S1656	12, 13	12, 12	12, 13	12, 13
D2S441	10, 14	8, 11	10, 14	8, 10, 11, 14
D2S1338	22, 25	16, 18	22, 25	16, 18, 22, 25
D10S1248	13, 15	12, 15	13, 15	12, 13, 15
D12S391	18, 23	18, 23	18, 23	18, 23
D19S433	13, 14	13, 15	13, 14	13, 14, 15
D22S1045	16, 16	8, 10	16, 16	8, 10, 16
CSF1PO	12, 12	10, 11	12, 12	10, 11, 12
TH01	6, 9.3	10, 10	6, 9.3	6, 9.3, 10
Amelogenin	X, Y	X, X	X, Y	X, Y

REPORTING

Not processed further

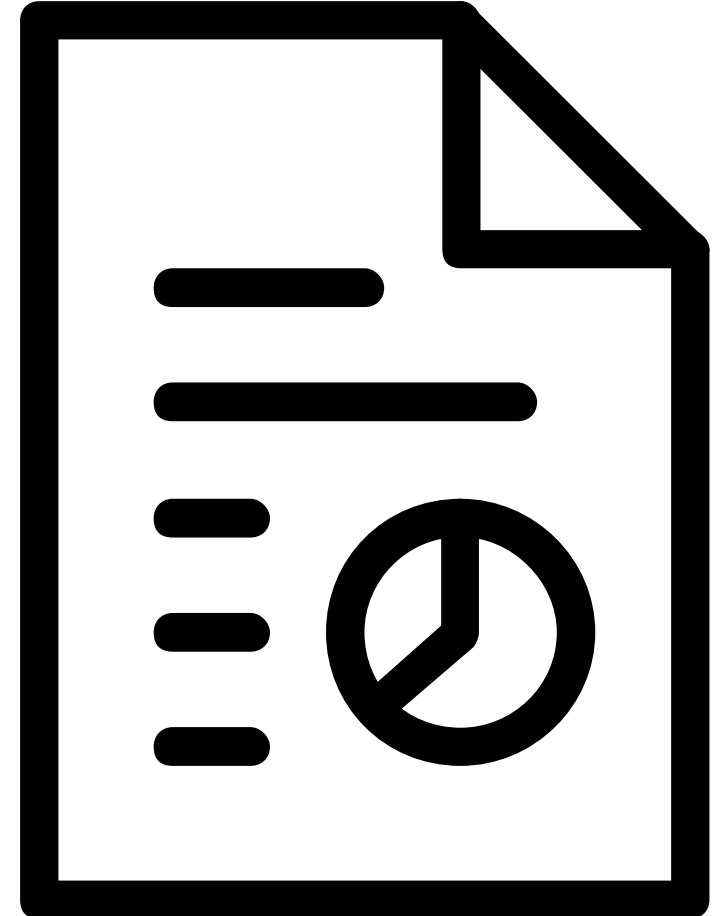
Insufficient biological fluid or male DNA exists to warrant DNA testing



REPORTING

No results

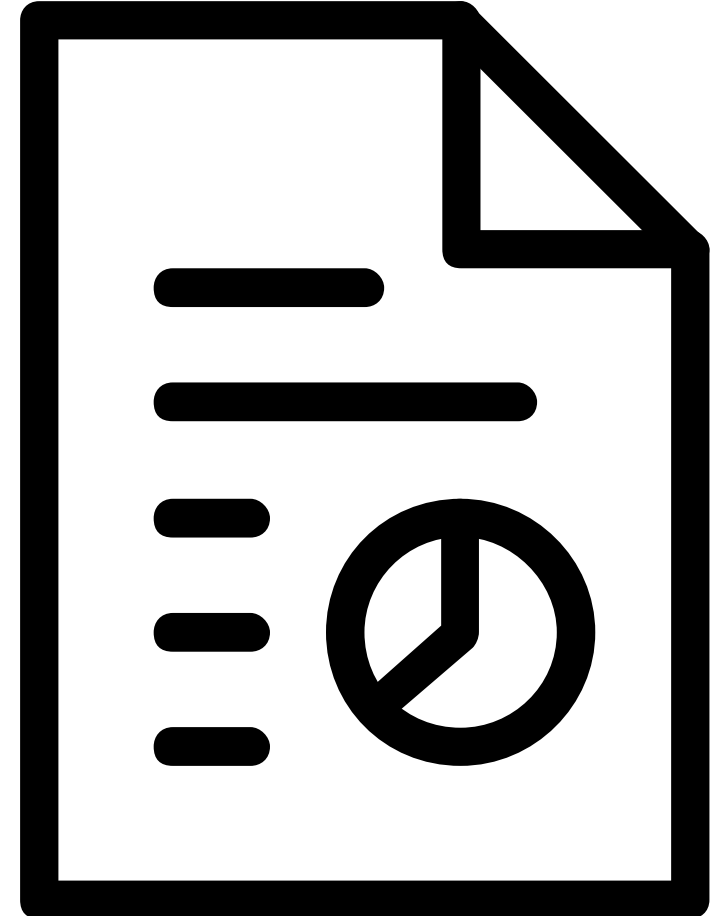
Insufficient biological material exists to generate a DNA profile



REPORTING

Partial profile

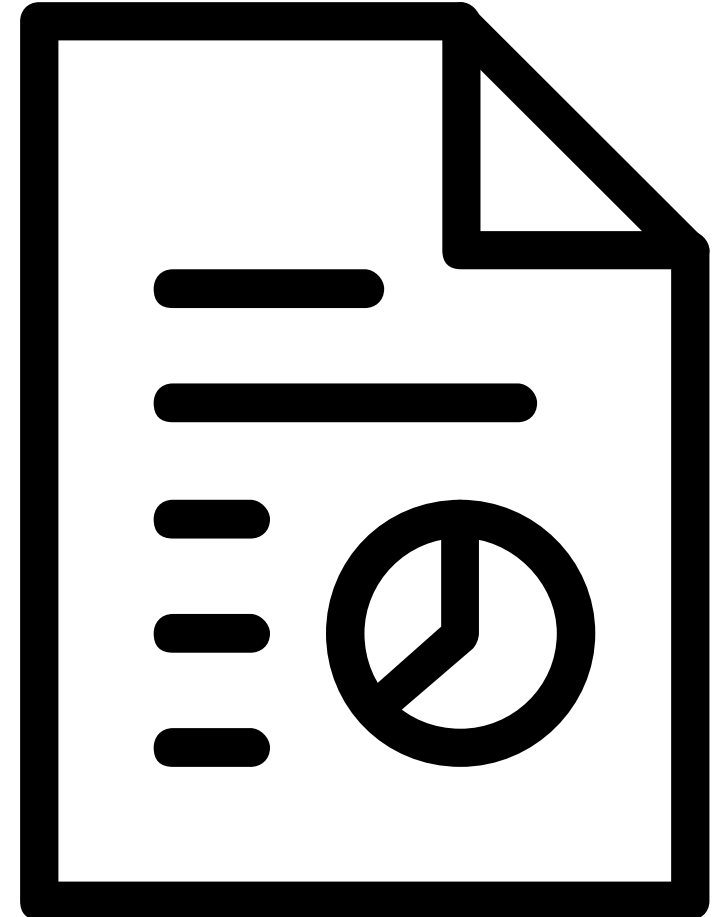
Genetic information was obtained from some of the STR regions tested



REPORTING

Full profile

Genetic information was obtained from all STR regions tested

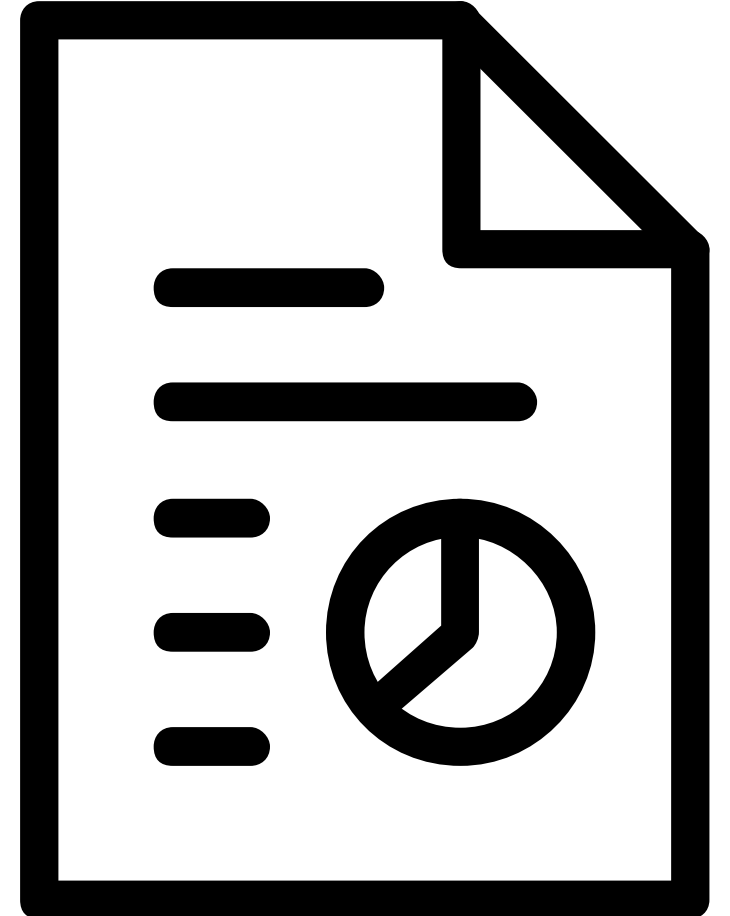


REPORTING

Mixture profile

Sample contains DNA from more than one contributor

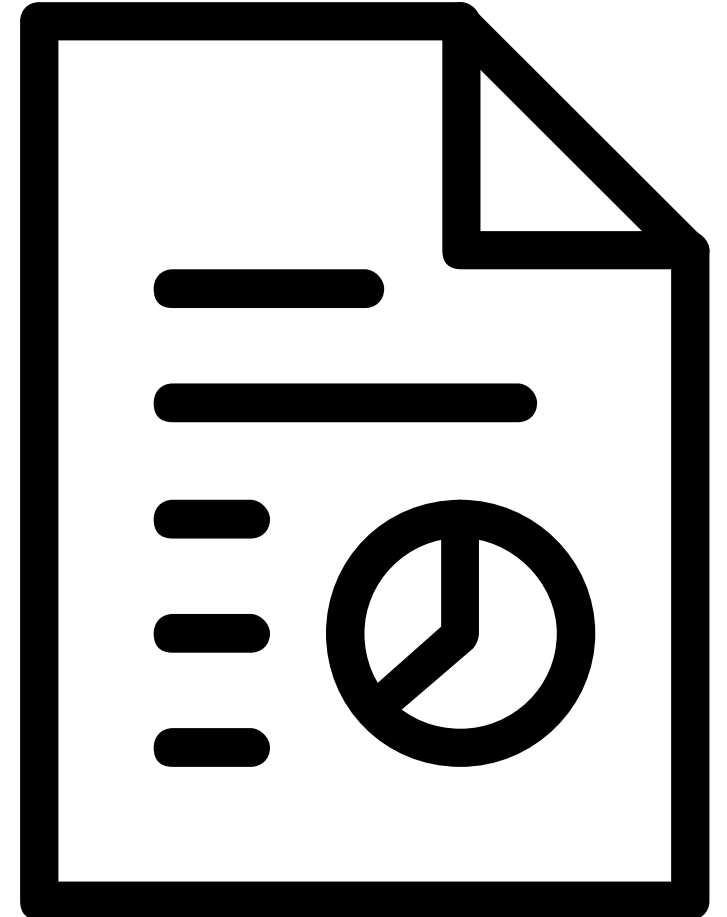
- # of contributors
- Major/minor profiles
- Assumptions



REPORTING

CODIS eligible

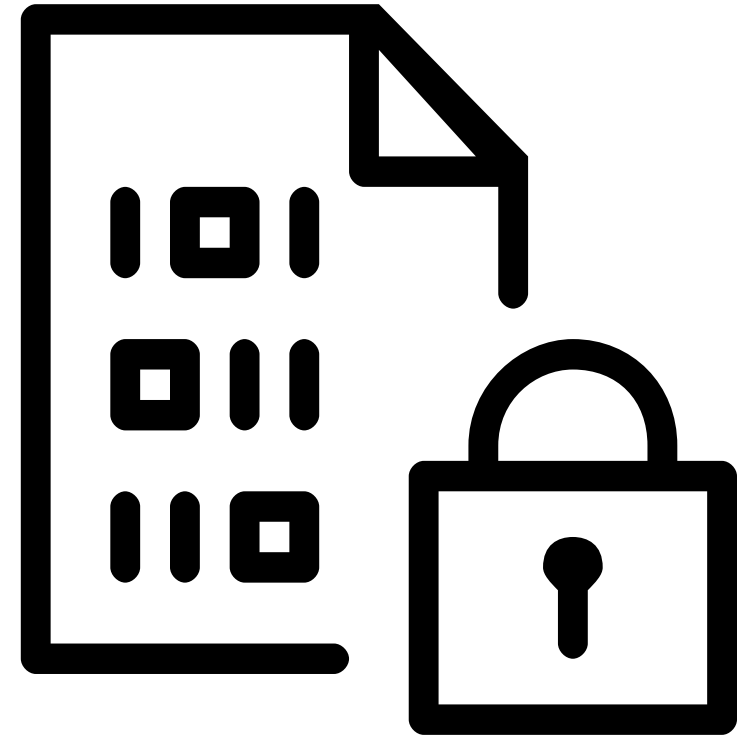
DNA profile meets the quality requirements for entry and search in CODIS



CODIS UPLOAD

The Combined DNA Index System administered by the FBI.

- Links DNA evidence obtained from crime scenes, thereby identifying serial criminals.
- Compares crime scene evidence to DNA profiles from offenders, thereby providing investigators with the identity of the putative perpetrator.



CASE STUDY- REPORT

Date: April 20, 2018

Victim name: Katie Wells

Agency Case Number: 12-00104

Laboratory Case Number: 2018-02100

Items submitted for testing on: February 2, 2018

Internal vaginal swabs (x2)	2018-02100-03
External vaginal swabs (x2)	Not tested
Neck swab (x1)	2018-02100-02
Breast swab (x1)	2018-02100-01
Underwear	Not tested
Fingernail clippings	Not tested
Blouse and jeans	Not tested
Known sample- Katie Wells	2018-02100-04

CASE STUDY- REPORT

MALE DNA SCREENING RESULTS

1. Sample 2018-02100-01 (breast swab) contains insufficient amount of male DNA. This sample was not processed for STRs.
2. Sample 2018-02100-02 (neck swab) contains male DNA. This sample was processed further.
3. Sample 2018-02100-03 (vaginal swab) contains male DNA. This sample was processed further.

CASE STUDY- REPORT

STR RESULTS AND CONCLUSIONS

1. A female profile was obtained from sample 2018-02100-04 (Katie Wells).
2. The partial DNA profile obtained from sample 2018-02100-02 (neck swab) is consistent with a male contributor (male 1).
3. A male DNA profile (male 1) was obtained from the sperm fraction of sample 2018-02100-03 (vaginal swab).
4. A DNA profile consistent with the victim was obtained from the epithelial fraction of sample 2018-02100-03 (vaginal swab).

CASE STUDY- REPORT

APPENDIX

1. The male DNA profile obtained from sample 2018-02100-03 (vaginal swab) was uploaded into the combined DNA index system (CODIS).
2. All controls produced the expected results.
3. The evidence and extracts will be returned to the submitting agency.

CASE STUDY- HIT REPORT

The purpose of this report is to inform an investigative lead

Example:

During a search of the Combined DNA Index System a match occurred between case number: 12-00104 and convicted offender (NAME).

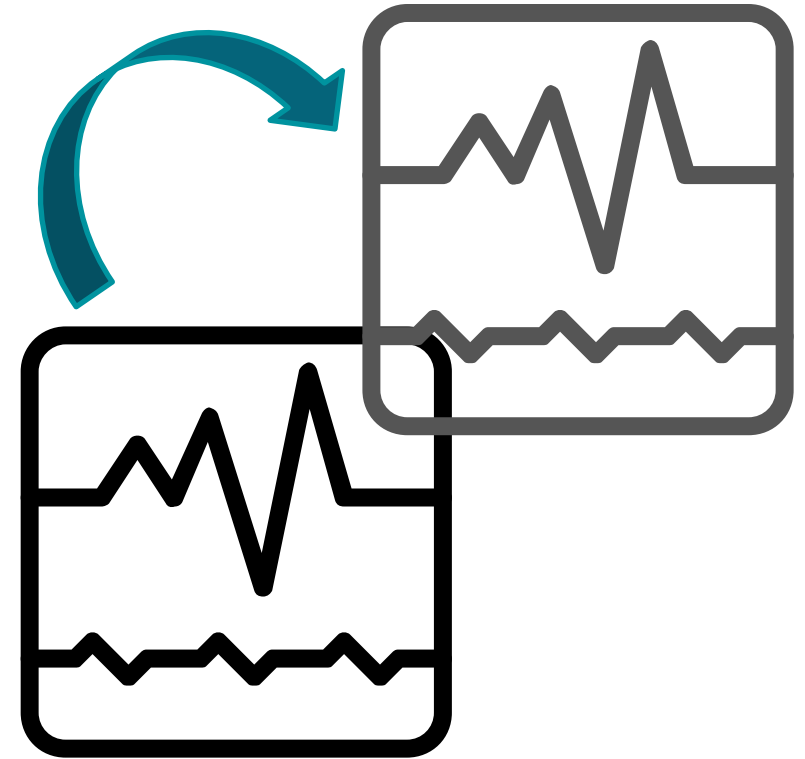
No comparisons will be made between the DNA profiles obtained in case 12-00104 without the submission of a buccal sample from (SUSPECT NAME).

CONCLUSIONS

Inconclusive

Unable to make a comparison:

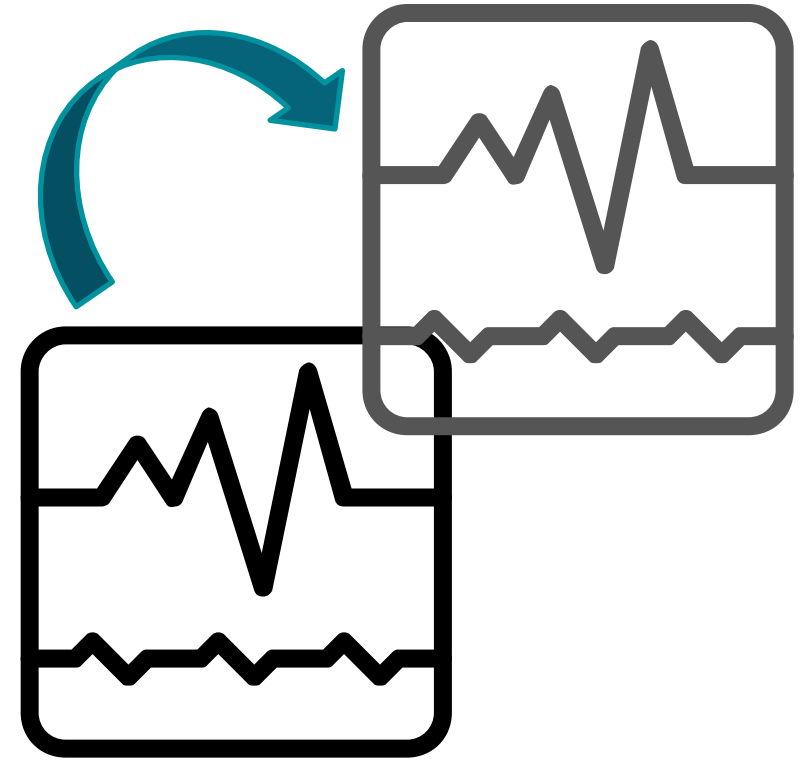
- Limited data
- Complexity of data
- Failed controls



CONCLUSIONS

Exclusion

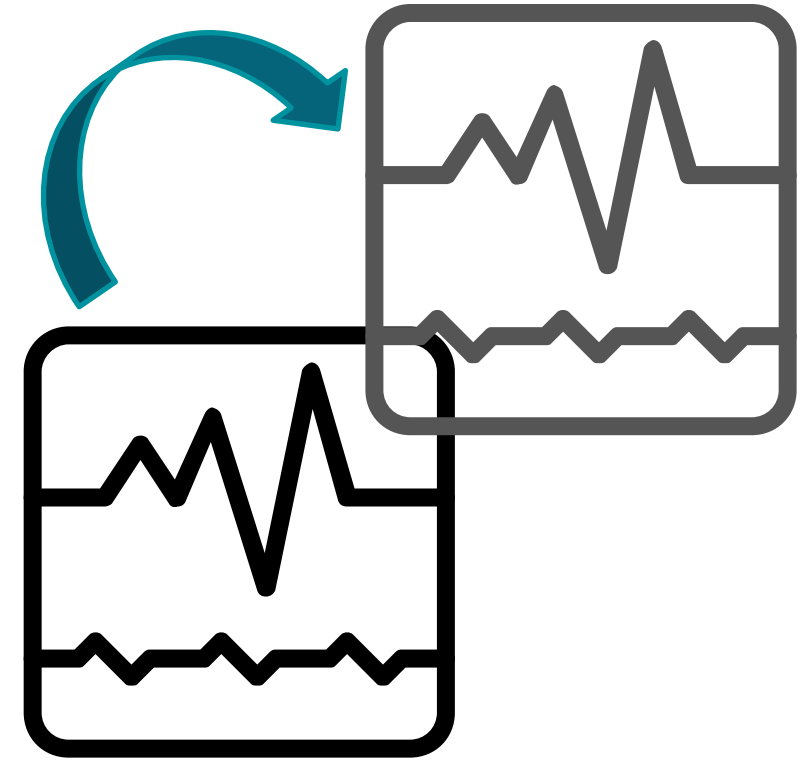
- Known sample is excluded from the DNA profile obtained
- Does not match
- Cannot be included



CONCLUSIONS

Inclusion

- Not eliminated from the DNA profile
- 'Match' to a single source
- Included in the mixture
- Support with a statistic to estimate the rarity of the inclusion



GENERATE A TESTING PLAN

Consider the results

- Input from law enforcement and legal system
- Test more items if necessary
- Determine possible suitability for Y-STR testing
- Determine need for biological fluid screening (if not performed initially)

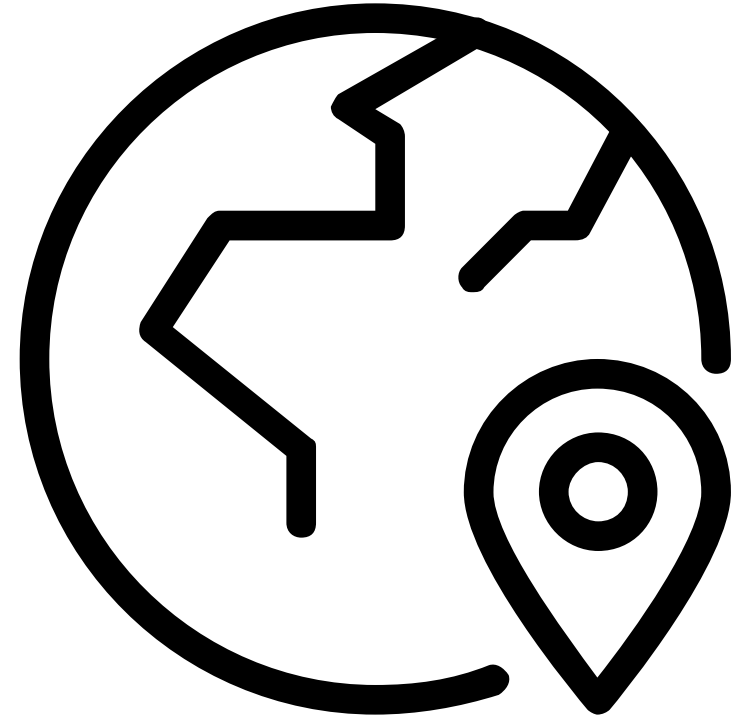
Goal:

Obtain additional information needed for investigation & prosecution

STATISTICAL ANALYSIS

Give meaning to the findings-
frequency of occurrence

- Should be provided for all 'inclusions'
- Need a database
- Use allele frequencies to calculate locus frequency

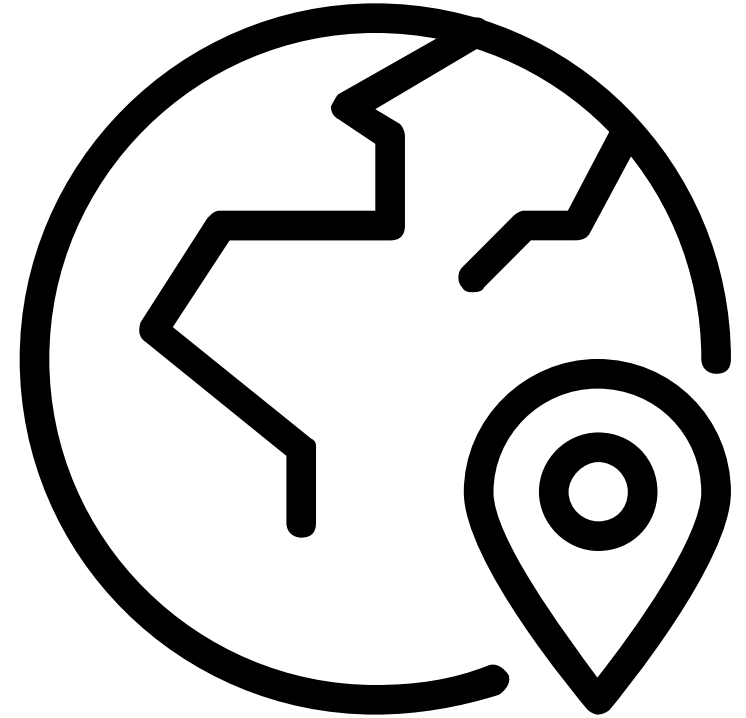


STATISTICAL ANALYSIS

Product Rule

The probability of several independent events occurring simultaneously is the product of the probability of each event.

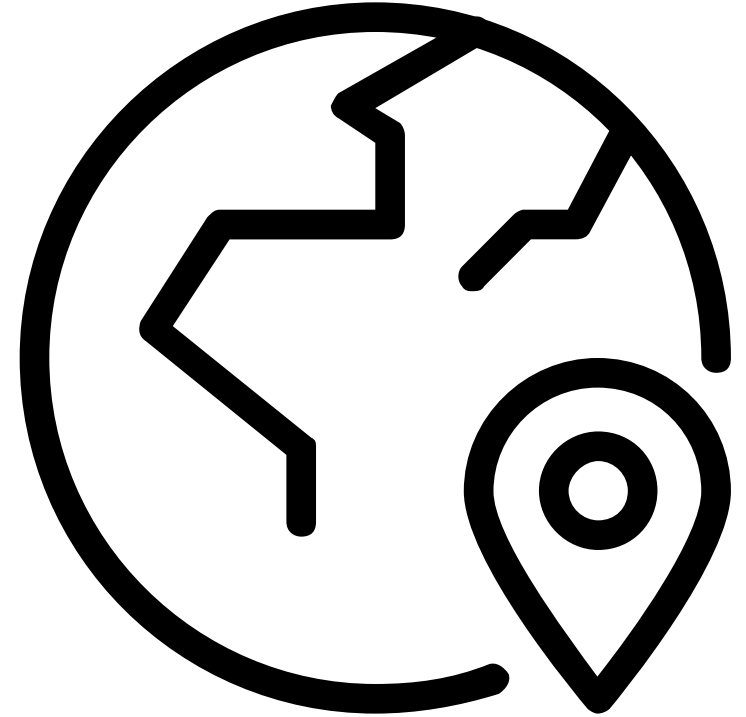
(Locus 1) X (Locus 2) X (Locus 3) X etc



STATISTICAL ANALYSIS

Random match probability

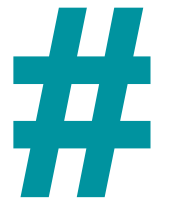
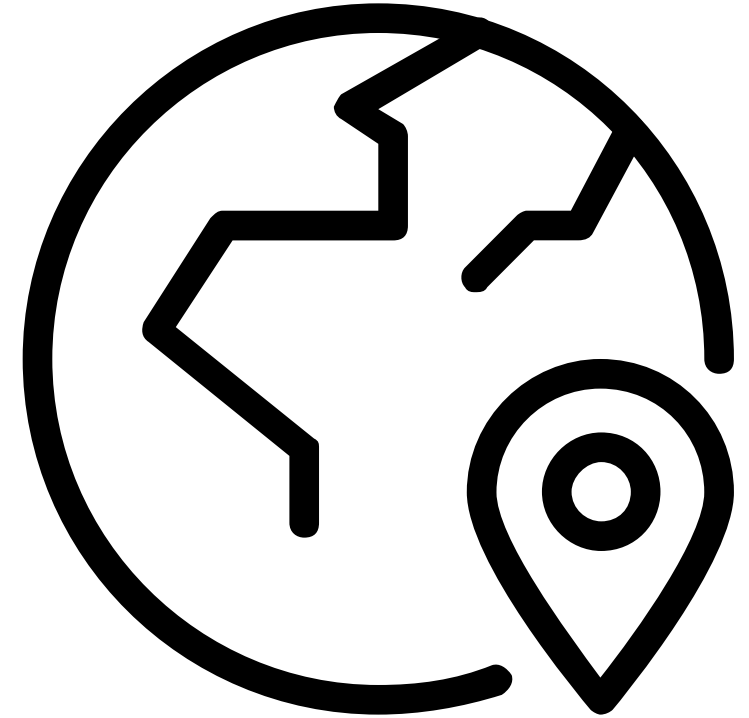
The probability that the DNA of a randomly chosen person has the same profile as the DNA of an evidentiary sample



STATISTICAL ANALYSIS

Probabilistic Genotyping

The use of biological modeling, statistical theory, computer algorithms, and probability distributions to calculate likelihood ratios (LRs) and/or infer genotypes for the DNA typing results of forensic samples.



CASE STUDY- COMPARISON REPORT

1. A male DNA profile (male 1) was obtained from the sperm fraction of sample 2018-02100-03 (vaginal swab). This profile is consistent with the DNA profile obtained from SUSPECT NAME.

The probability of randomly selecting an unrelated individual with this DNA profile at 24 of 24 loci tested is:

1 in 4.5 septillion in the U.S. Caucasian population

1 in 3.2 septillion in the U.S. Hispanic population

1 in 6.4 septillion in the U.S. African American Population

LABORATORY PROCESS TIMELINE -1 KIT

Crime analysts complete the following steps to test biological evidence from a victim's sexual assault kit (SAK).



SAMPLING: 1 hour
EXTRACTION: 2- 24 hours

QUANT:
2.5-5 hours

AMPLIFICATION:
3-6 hours

FRAGMENT
ANALYSIS:
2-4 hours

ANALYSIS:
1-3 hour

REPORTING:
1-3 hour

TECHNICAL REVIEW:
1-2 hour

2 business days
3 people

Access to all equipment
No reprocessing

LABORATORY SCALING- 100 KITS



Laboratory
processes

3 months
5 people
Batching

TURNAROUND TIME VARIABLES

Workflow

First pass success

Supplies/Equipment

Personnel

Quality Standards

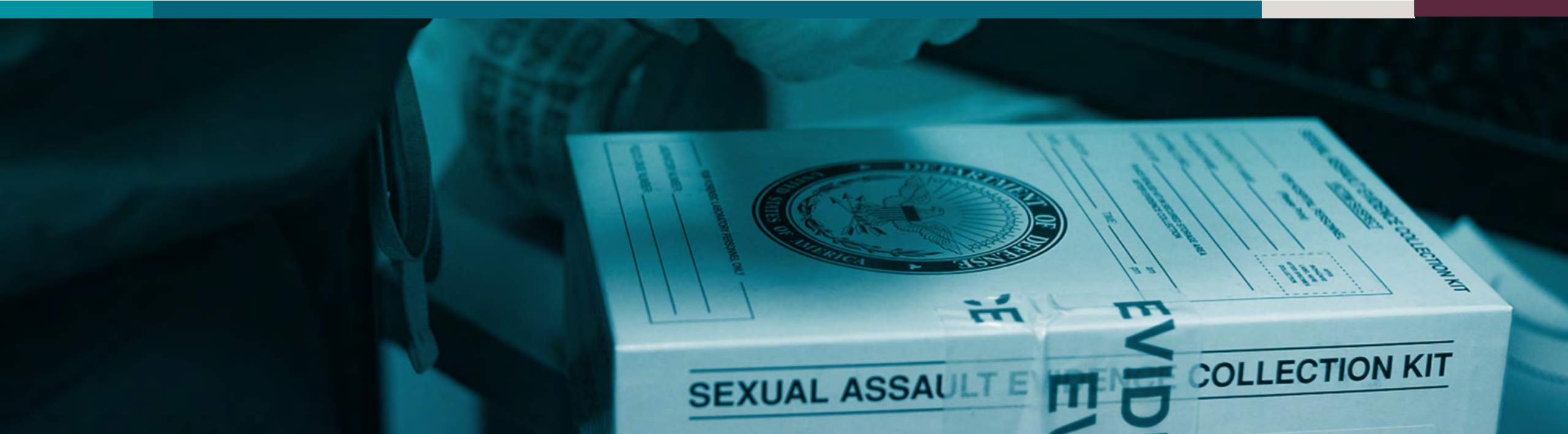
Communication

Screening method

Complexity of analysis

Additional testing

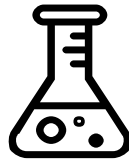
TOPIC REVIEW



DNA Typing



Case Study



Laboratory
Workflow



Reporting &
Conclusions



Timelines

Contact Information

Amy Jeanguenat

Principal Consultant

amy@mindgenllc.com

