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DNA 101:Introduction to Forensic DNA Testing & Reporting

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Overview

Basics

- Molecular biology, Short Tandem Repeat (STR) testing

Source of DNA

- Biological fluids, recovery, common items

DNA Workflow Components

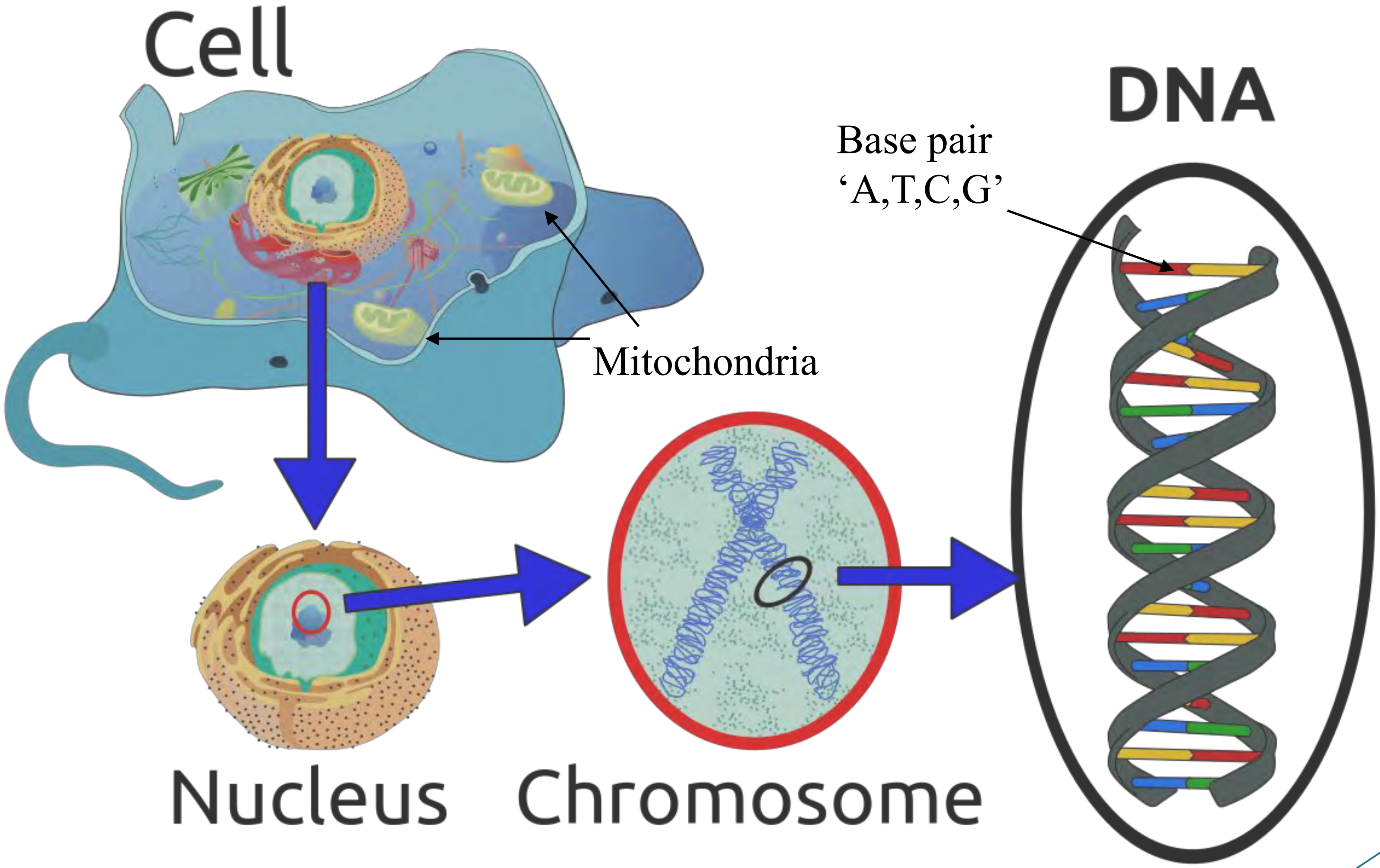
- Common steps, Sexual Assault Kit (SAK) workflows

Reporting

- Common language, use of cite words to guide interpretation

Mock Case Example

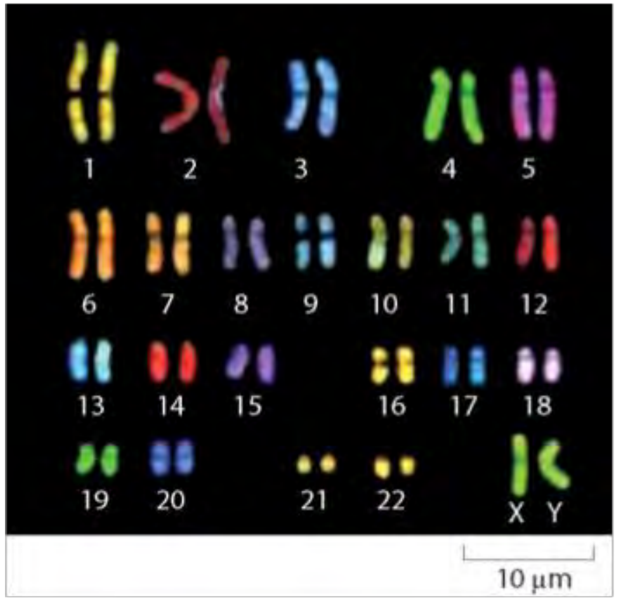
Molecular Biology in Two Slides



Molecular Biology in Two Slides

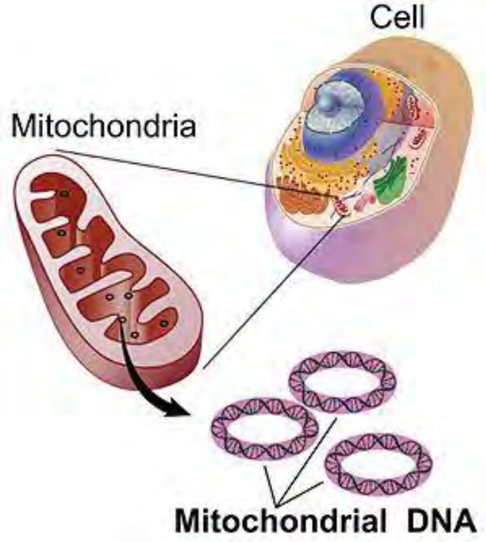
Nuclear DNA:
22 pairs of autosomal chromosomes and 1 pair of sex determining chromosomes

½ DNA from Mom and ½ from Dad
Used for STR and Y-STR Testing



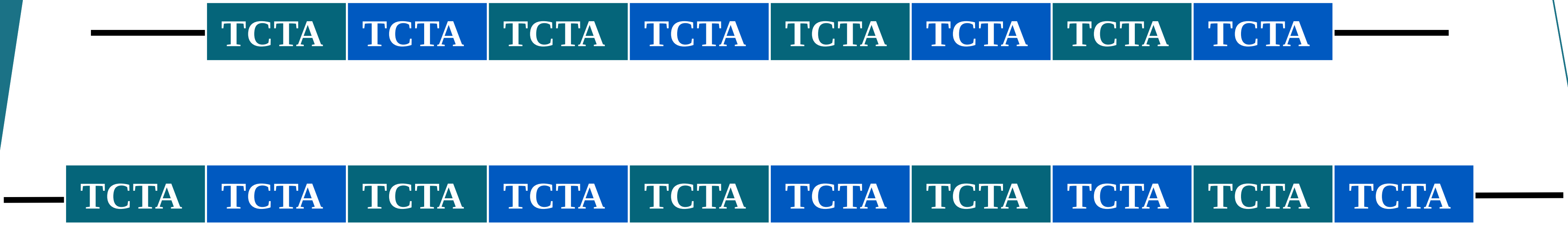
Mitochondrial DNA (mtDNA):
Maternally inherited DNA found in the mitochondria of the cell.

Hundreds of mitochondria are present in each cell therefore mtDNA testing can be good for compromised samples: bones, teeth, hair shafts



Short Tandem Repeats

STR Structure: D8S1179



DNA Profile: 8, 10

STR Advantages:

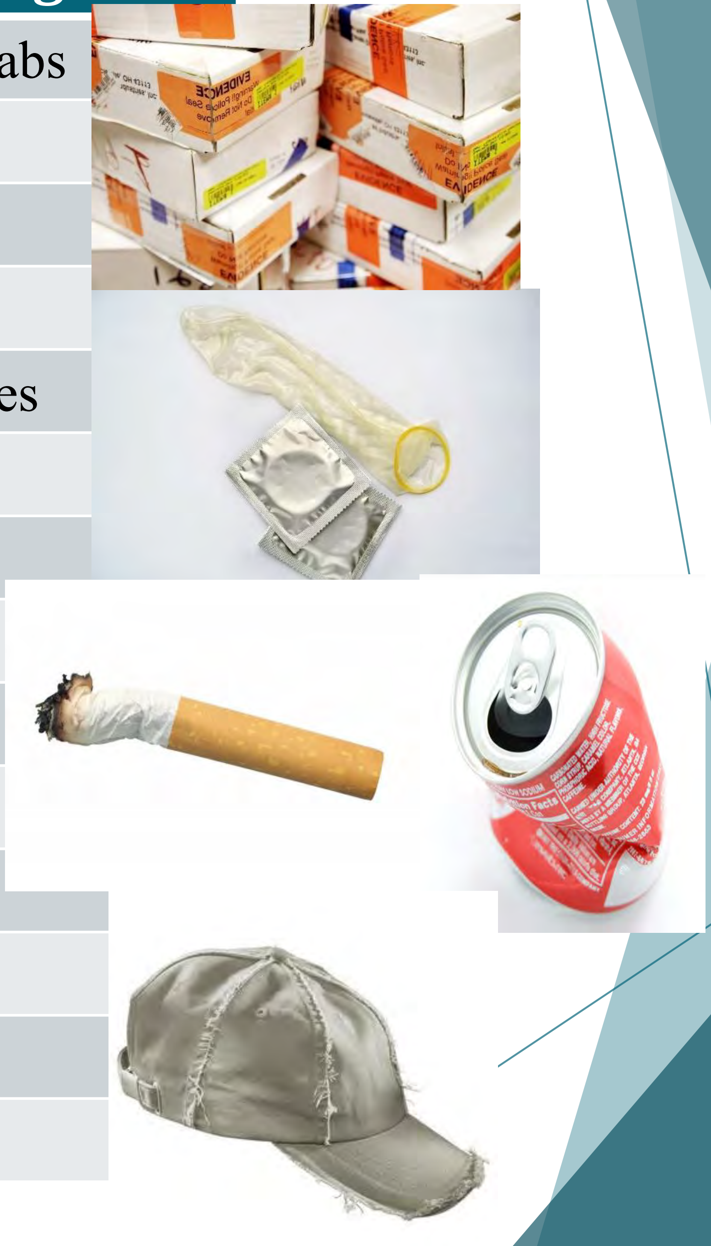
- 20+ STR locations tested simultaneously
- Small amount of sample (pin drop)
- Highly variable between humans
- Allows for data sharing (CODIS)
- Used in paternity, human ID, forensics

Sources of Biological Material

DNA is the same throughout a person's cells

Blood
Saliva
Tissue
Urine
Feces
Hair Roots
Skin Cells
Sperm Cells/Semen
Skeletal Remains/Teeth

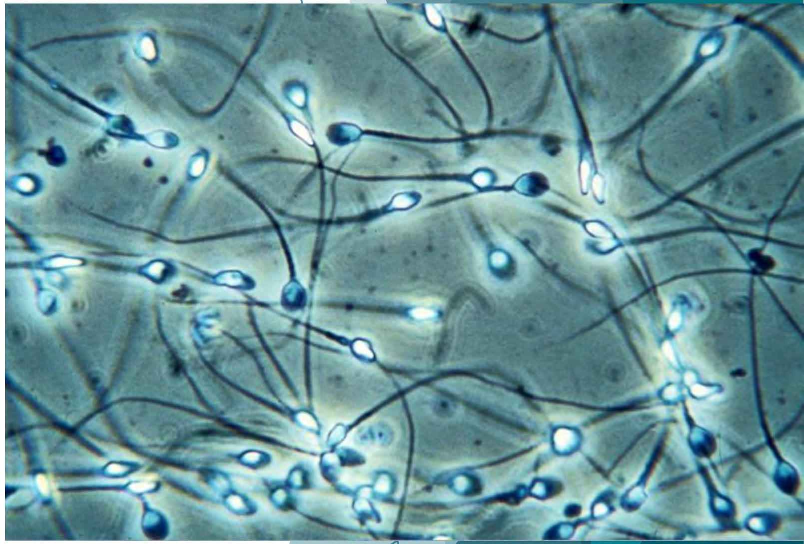
Items Commonly Submitted for DNA Testing
Sexual assault kits: vaginal swabs, anal swabs, oral swabs
Bedding
Condoms and wrappers
Swabs of possible stains: blood, saliva, semen
Clothing: hats, shirts, pants, sneakers, underwear, gloves
Cigarette butts
Bottles, straws, cups
Fingernail scrapings
Hair
Skeletal remains (bones/teeth)
Swabs from firearms
Ligatures
Swabs from surfaces: window, steering wheel, door
Weapon handles



Factors Influencing Recovery of Spermatozoa/DNA in SAK

- condom use
- ejaculation
- sperm count
- vasectomized
- azoospermic

- hygiene/cleaning
- menstruation
- bowel movements
- time since assault
- areas sampled
- kit storage



DNA Laboratory Steps

Screen	Extract	Quant	Amplify	Analyze	Review	Report
Detect biological fluid OR DNA	Remove possible DNA from substrate	Amount of human DNA is determined	DNA is copied by targeting STR regions	STR Regions are separated and with aid of computer software DNA profile is generated	100% of DNA exams undergo technical and administrative review	Results, comparisons, conclusions, and statistics are summarized in a forensic case report

DNA Detection through Semen ID

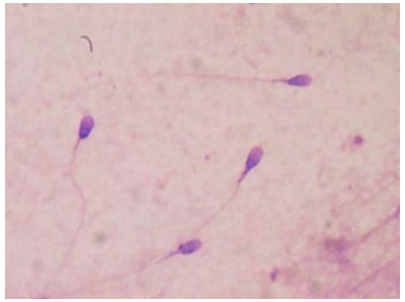
Presumptive Exams

Color changing or antibody tests that detect specific enzyme levels present in semen

Provide an indication of possible semen

Microscopic Exams

Visually confirms the presence of sperm heads under a microscope



Provides confirmation of spermatozoa

Biological Screening Reporting-Presumptive Exams

Examples:

The vaginal swab tested presumptive positive for the presence of semen.

Presumptive testing for the presence of semen on the vaginal swab was negative.

Semen was indicated on the item vaginal swab.

Words… ‘presumptive and indicated’ are NOT conclusive statements

Biological Screening Reporting- Confirmatory Exams

Examples:

Semen was confirmed on the vaginal swab.

Spermatozoa were confirmed on the vaginal swab.

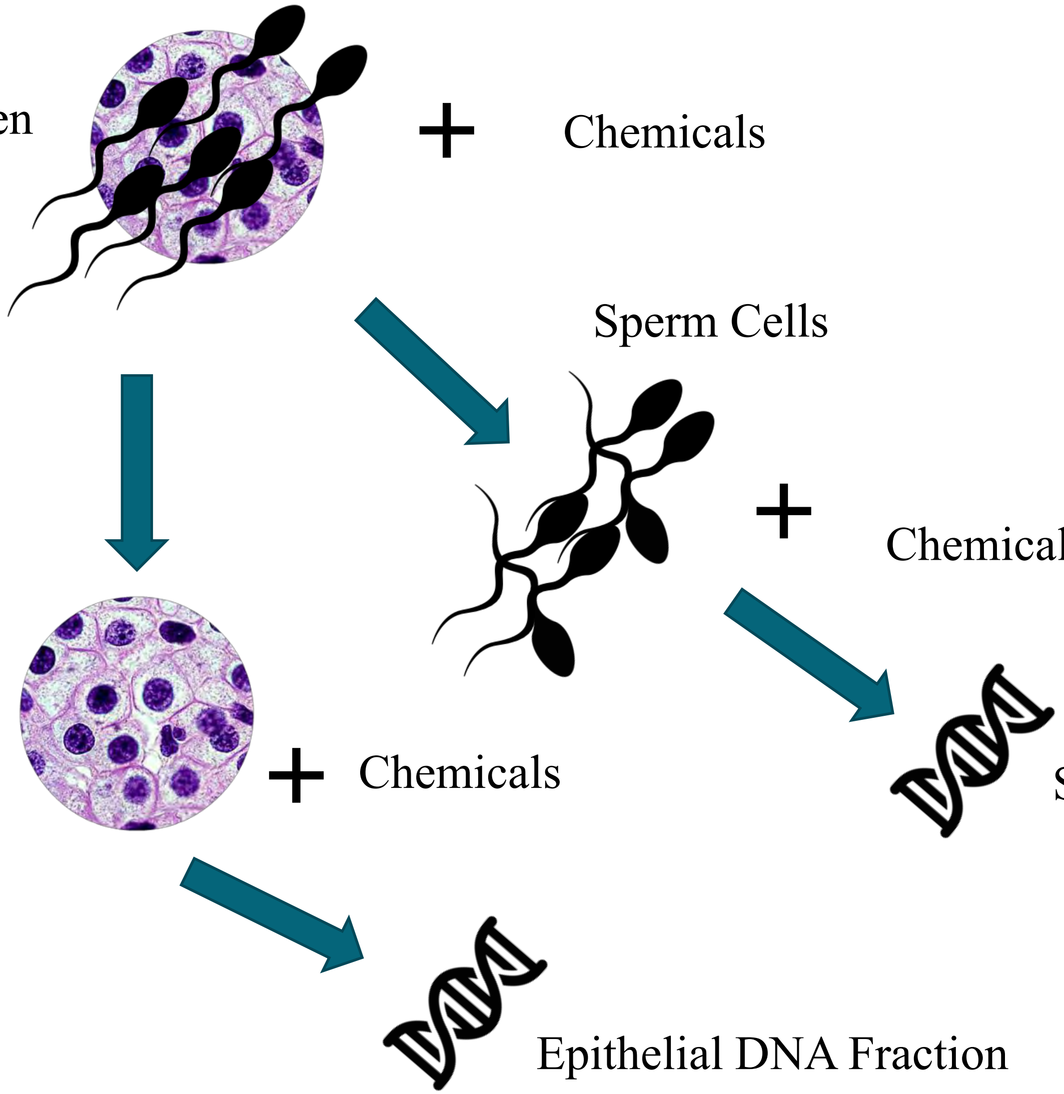
Presumptive tests for the presence of semen was inconclusive for the vaginal swab. Confirmatory testing was negative for the vaginal swab.

DNA Detection Through Male Screening

AKA: Direct to DNA, Y-Screening, Y-Chromosome Screening

- Shifts case assessment from biological fluid screening to DNA Quantification
- Best sample used for DNA testing
- Can detect DNA from vasectomized males
- Better at detecting male DNA from vaginal swabs, external genitalia and dried secretions
- Reduces turnaround time

Differential Extraction



Sample with
Possible Semen

+ Chemicals

Sperm Cells

+ Chemicals

Sperm DNA Fraction

Epithelial Cells

+ Chemicals

Epithelial DNA Fraction

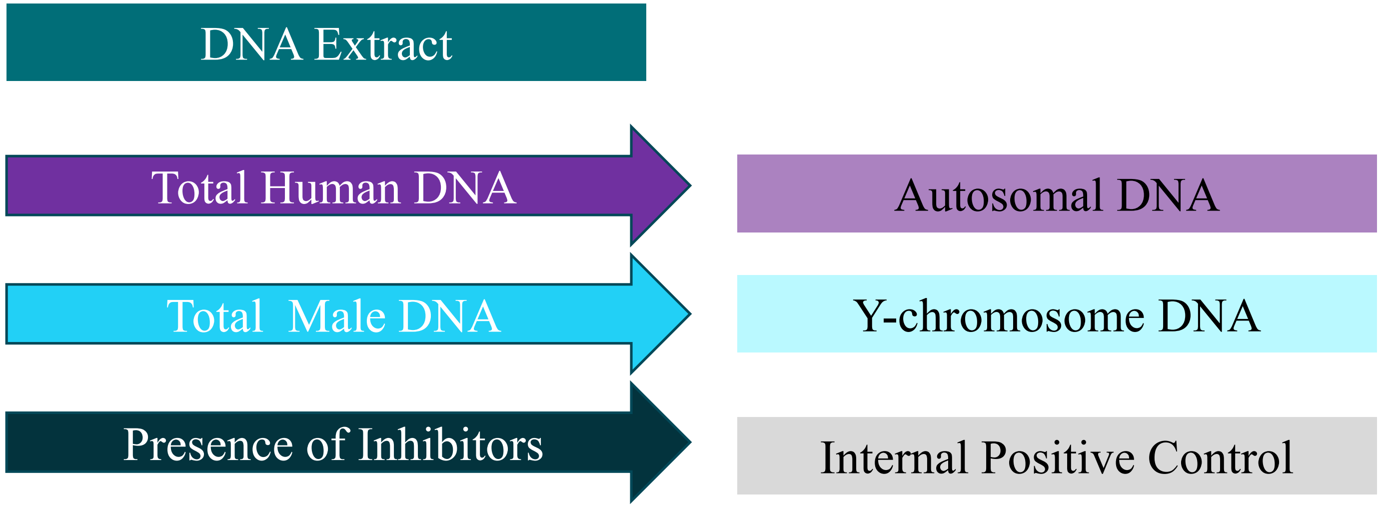
DNA Detection Through Male Screening

Screen BEFORE Extraction	Screen AFTER Extraction
A small cutting is taken from a sample. A fast/crude DNA extraction is performed then the sample proceeds to quantification with human/male detection	A larger cutting is taken for DNA testing. A differential extraction is performed then the sample proceeds to quantification with human/male detection

Human/Male DNA Quant

Aids in Decision Making

- Determine if enough DNA to proceed with downstream processing
- Evaluate mixture ratios to determine if enough male DNA to proceed with STRs or YSTRs
- Evaluates quality of DNA extraction



DNA Extract

Total Human DNA → Autosomal DNA

Total Male DNA → Y-chromosome DNA

Presence of Inhibitors → Internal Positive Control

Male DNA Screening Reporting

Examples:

Male DNA was present on the vaginal swab, this sample was processed further for STR testing.

Male DNA was not present on the vaginal swab, this sample did not proceed with further testing.

Male DNA was inconclusive on the vaginal swab. This sample was not processed further at this time. (i.e. might be a good Y-STR candidate)

Male DNA was present on the vaginal swab, but due to high levels of total human DNA, this sample was not processed further for STR testing. (i.e. might be a good Y-STR candidate)

Testing Approaches

STRs

- Default Test
- Targets human DNA
- DNA passed down from both parents
- Can discriminate between individuals

Y-STRs

- If requested
- Targets male specific DNA
- DNA passed down from father
- Relatives in same paternal line may have same Y-STR profile

Combined

- STRs/YSTRs given equal weight to solving crime
- Help resolve mixtures
- Provide clarity when there is little detectable male DNA or too much female DNA

Commercial STR Kits

Examples:

PowerPlex®Fusion

PowerPlex®Fusion 6C

AB GlobalFiler™

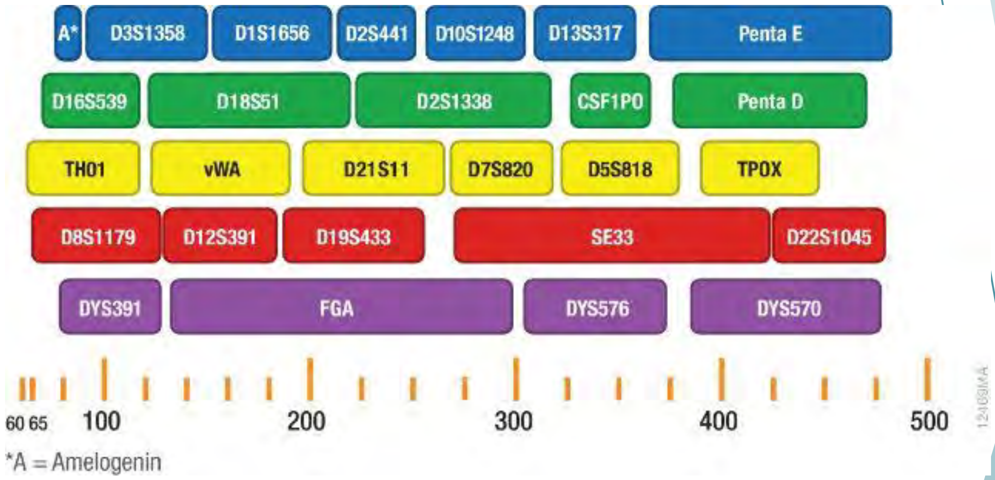
QIAGEN Investigator® 24plex

- Over 20 locations are copied at once
- Fluorescent dyes help distinguish different locations and alleles
- Can detect down to 0.5 ng of DNA

Commercial Kits

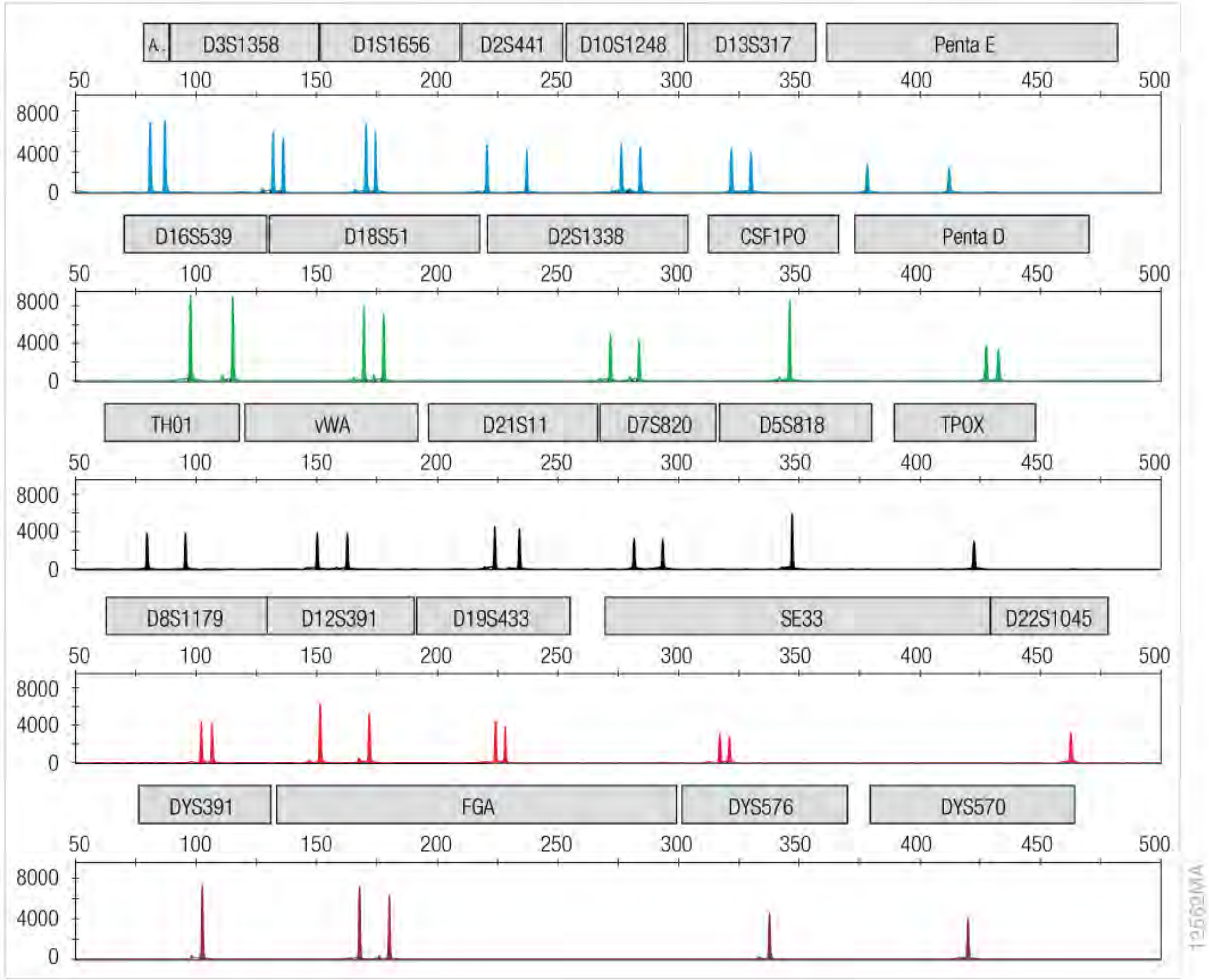
“Megaplex” kits offer:

- More discrimination power due to more loci
- Improved degraded sample recovery due to smaller STR loci
- Improved inhibited samples due to enhanced chemistry
- Increased recovery due to increased sensitivity



Example: PowerPlex® Fusion 6C

STR DNA Profile



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

Analysis

Evidence samples analyzed always before knowns by examining data

- Determine profile quality
- Determine number of contributors
- Deduce profile: major, minor, foreign
- Results generally summarized in table format
- Reduces possibility of bias

Analysis

Single source profile: DNA from one contributor. Generally recognized by the presence of no more than two alleles at each location tested and overall good quality

Mixture profile: DNA from more than one contributor. Generally recognized by the presence of three or more alleles at each location tested and or peak height imbalance. Mixtures are generally reported as

… 2 people

… at least 2 people

… 3+ people

Mixtures in SAK

Mixtures may arise due to:

- Any combination of victim, perpetrator, and consensual partner
- Multiple perpetrators
- Poor differential extraction efficiency (i.e carry over of EF into SF)
- Contamination (i.e. crime scene, police, collection staff, laboratory staff)

Analysis

Partial Profile: Information is missing from some or most of the locations typed.

> This can be indicative of a sample that needs additional testing, is degraded, is inhibited, or of limited quantity.

No Results: No DNA was detected at any of the locations typed.

Analysis

When analysis of evidence is completed then analyze known samples (i.e. victim, suspect, elimination)

- Known samples will be processed at a separate time and space from evidence samples
- Known samples should be single source
- Victim’s profile can be important to establish chain of custody, deduce out a male component
- Elimination samples such as consensual partners should be submitted for comparison
- Generally results will be summarized in same allele table as the evidence

Report Features

- Title
- Name and address of lab performing the work
- Case identifier
- Name and address of client
- Description of evidence
- Dates items were received and tested
- Description of the technology
- Locus of amplification system
- Results and/or conclusions
- Quantitative or qualitative interpretation statement
- Date issued
- Deposition of evidence
- Signature/title

Report Features- Comparative Key Words

Inclusion, cannot be excluded, match:

Refer to possibility that a known sample could have contributed to the DNA profile obtained from the evidence sample.

Exclusion, cannot be included, does not match:

Refers to a known sample that could not have contributed to the DNA profile obtained from the evidence sample.

Report Features- Comparative Key Words

Inconclusive, uncertain:

Cannot draw an absolute conclusion of inclusion or exclusion regarding the known sample contributing to the DNA profile obtained from the evidence sample.

Possible reasons for an inconclusive result should be explained such as:

Evidence data is of poor quality, uninterpretable, unsuitable for comparison.

Mock Case

Scenario:

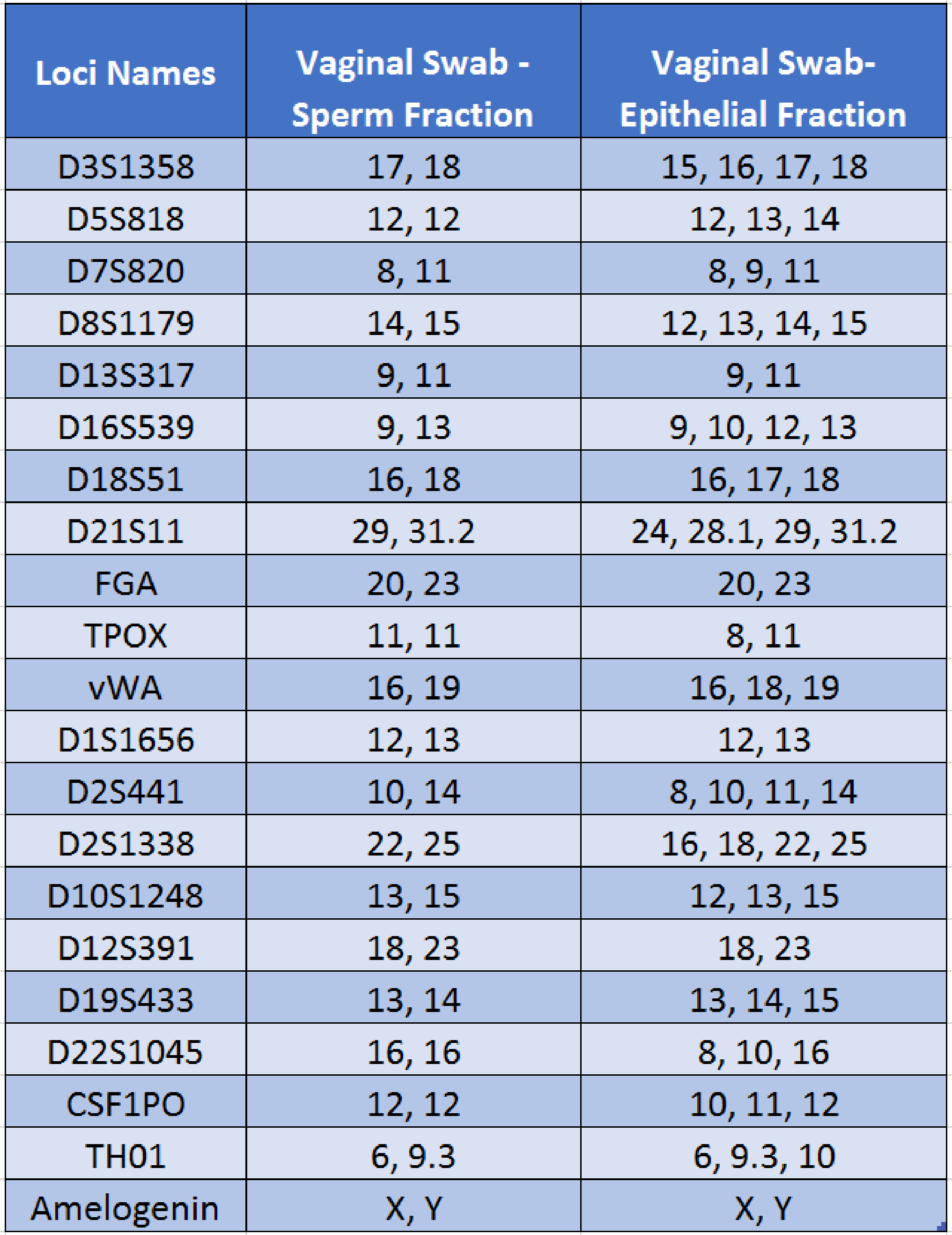
A single vaginal swab from a sexual assault collection was submitted to the crime laboratory for rapid DNA processing. Ideal turnaround time was 15 days.

The victim and possible suspect knowns were also submitted for comparison, if applicable.

Mock Case

Laboratory Steps

1) Cut ½ vaginal swab
2) Perform differential extraction
3) Quantify the sperm fraction and epithelial fraction
4) Amplify SF and EF with a minimum of 20 loci



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

Number of Contributors

Sperm Fraction:
Single Source Profile.
Indicated by presence of no more than 2 different alleles at each location tested.

Epithelial Fraction:
Mixture Profile.
Indicated by presence of 3-4 alleles at each location tested.
Data analysis may indicate a mixture of 2 people.

If applicable could determine if victim is contributing to the EF and deduce out foreign DNA profile.

Evidence Result Statements

Example Reporting (SF)

A DNA profile was obtained from the sperm fraction of the vaginal swab.

The DNA profile obtained from the sperm fraction of the vaginal swab is consistent with a single contributor.

Evidence Result Statements

Example Reporting (EF)

The DNA profile obtained from the epithelial fraction of the vaginal swab is consistent with a mixture of two individuals.

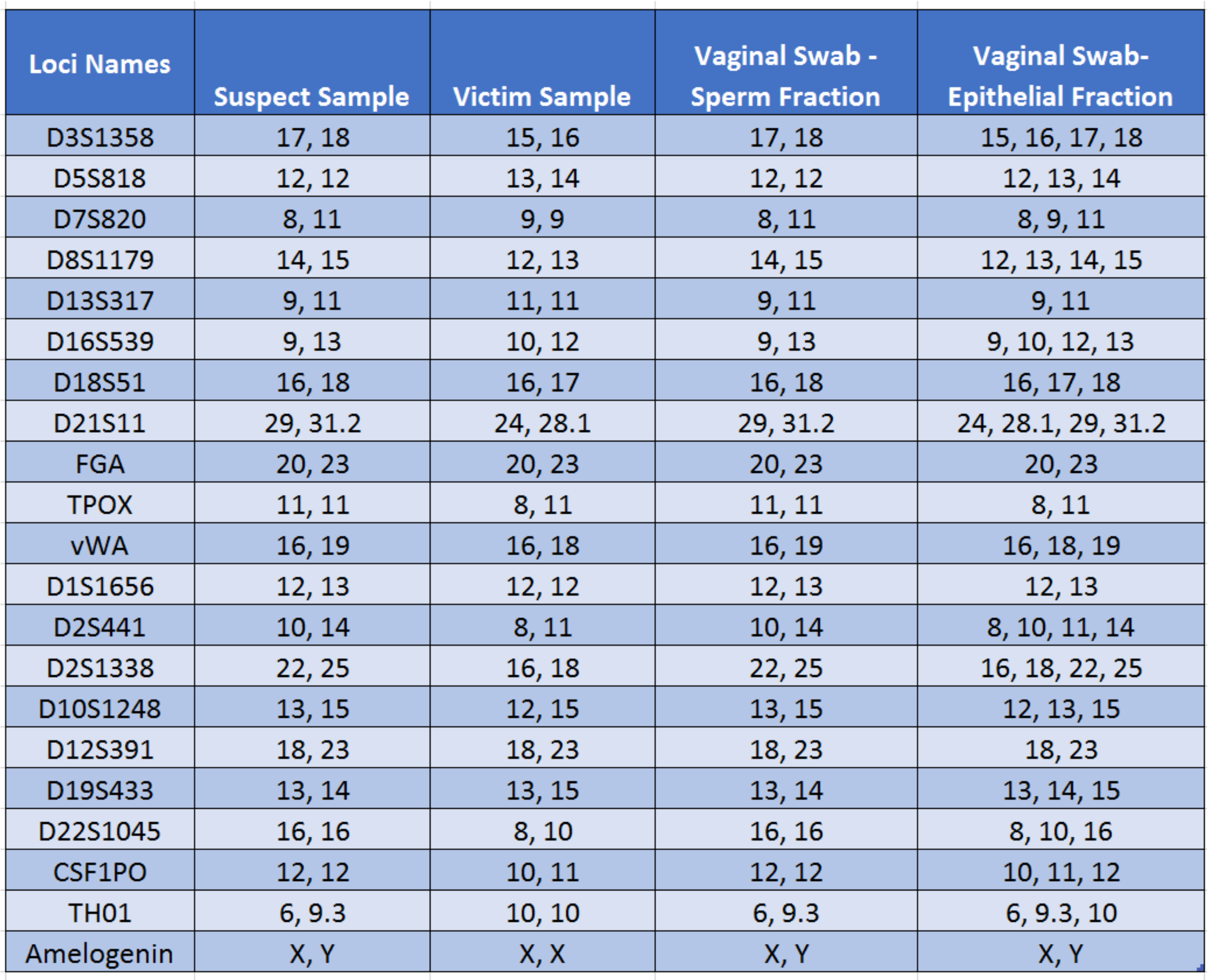
A mixture DNA profile of two individuals was obtained from the epithelial fraction of the vaginal swab.

Mock Case

Laboratory Steps

1) Cut ¼ of each submitted known to processes separately vaginal swab
2) Perform extraction
3) Quantify the victim and suspect standards
4) Amplify with same 20 loci as vaginal swab

Example- Summary of Results



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

Analysts will use electropherogram data outputs to make comparisons.

A summary of results can be a helpful visual, especially for court, but should not be solely relied upon for data comparison as it does not accurately represent data quality.

Comparative Statement

Example Conclusion-SF:

A DNA profile, consistent with the suspect, was obtained from the sperm fraction of the vaginal swab.

The DNA profile obtained from the sperm fraction of the vaginal swab is consistent with the suspect.

Comparative Statement

Example Conclusion-EF:

The DNA profile obtained from the epithelial fraction of the vaginal swab is consistent with a mixture of two individuals. The victim and suspect cannot be excluded as possible contributors to the mixture.

A mixture DNA profile of two individuals was obtained from the epithelial fraction of the vaginal swab. The victim and suspect cannot be excluded as possible contributors to the mixture.

Frequency Estimates

A quantitative statement shall be addressed to provide the weight of a possible ‘inclusion’ scenarios.

The rarity statement is determined:

- based on alleles present in the profile
- the population allele frequencies used
- genetic formulas that account for population substructure/relatedness

Reporting Frequency Estimates

The frequency estimate will be a numerical value.

Generally for a variety of population groups (Caucasian, Hispanic, African American)

Single source example expressed as:

The probability of randomly selecting an unrelated individual with this DNA profile is:

1 in XXX in the US Caucasian population

1 in XXX in the US African American population

1 in XXX in the US Hispanic population

Reporting Frequency Estimates

Mixture example expressed as:

The estimate of the proportion of individuals in the general population that would be included as possible donors to the mixture is:

1 in XXX in the US Caucasian population

1 in XXX in the US African American population

1 in XXX in the US Hispanic population

Totality of evidence

Lack or presence of DNA should always be examined within the totality of all the evidence in an investigation

DNA Does Not	**Active Areas of Research**
Statistic does not express if the person did or did not commit the crime	Age of stain and age of donor cannot currently be determined from DNA
DNA cannot convey guilt or innocence	

Summary

Basics

- Molecular biology, Short tandem repeat testing

Source of DNA

- Biological fluids, recovery, common items

DNA Workflow Components

- Common steps, SAK workflows

Reporting

- Common language, use of cite words to guide interpretation