FORENSIC DNA ANALYSIS

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October 12, 2015
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Overview

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- The basics of DNA

- The process of DNA typing

- Results interpretation

  - Mixtures

- Statistics
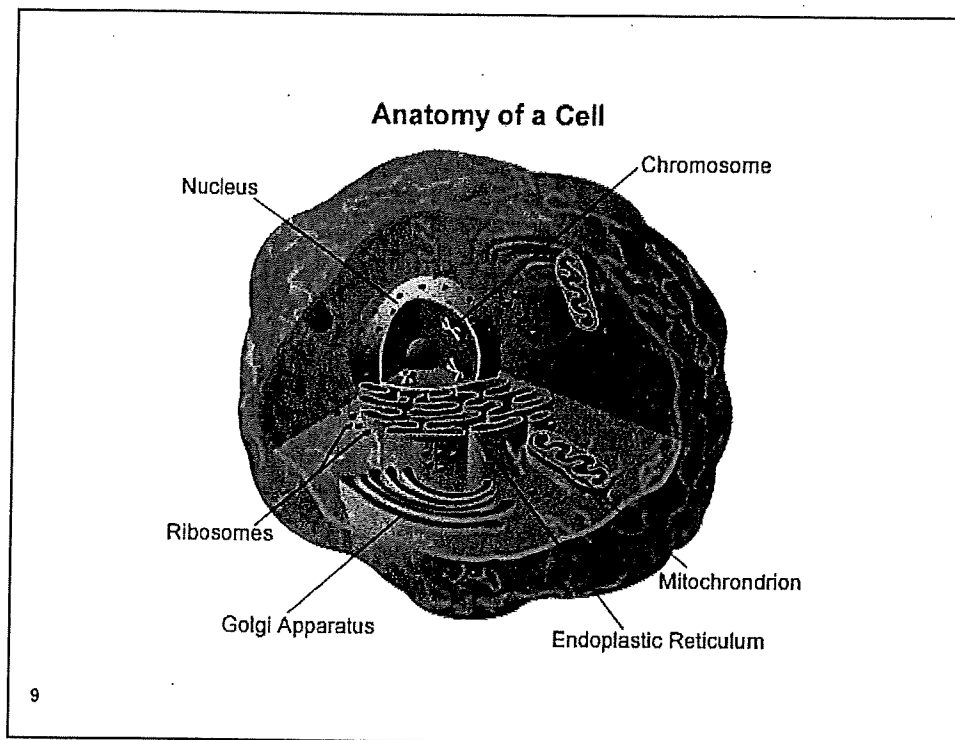
- Court testimony

What is DNA?

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- The code is a long sequence of side by side structural units (e.g. --- CGGCGAATGTACCT ---)

- 4 different structural units (called bases)
  - A = adenine
  - T = thymine
  - G = guanine
  - C = cytosine

- The order of the sequence codes the instructions

What is DNA?

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- A person's DNA is structurally arranged into 23 pairs of chromosomes

- Each chromosome is one long tightly packed strand of DNA

- The strand is in the form of a double helix

Anatomy of a Cell

Nucleus

Chromosome

Ribosomes

Golgi Apparatus

Mitochrondrion

Endoplastic Reticulum

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Where Does Your DNA Come From?

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- Your complete set of DNA is inherited from your parents in the form of chromosomes

- 23 <u>pairs</u> of chromosomes

  - 1 chromosome inherited from your mother
  - 1 chromosome inherited from your father

STRs

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- The variable regions of DNA that we type are referred to as short tandem repeats (STRs)

  - **Short**: each segment of the repeat is 4 units in length (e.g. TAGA)

  - **Tandem Repeats**: the repeating units are linked together

  - (e.g. ----TAGA-TAGA-TAGA--- 3 repeat units)

Locations of STRs

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- STRs are found at specific locations on the DNA strand called:
  - Loci (plural) or a locus (singular)

- In forensic DNA analysis, 15 different loci are typed and one sex determining locus is typed

  - Amelogenin (Amel) sex determining locus
    - X,X = female
    - X,Y = male

The Locus Genotype

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- At each locus a person's genotype is determined

- A genotype refers to both alleles that were inherited at a particular locus   12,15 = a genotype

- An allele is the name for different sequences of DNA that can exist at a locus   12(allele),15(allele) = a genotype

  - The alleles are what provide variability from person to person
  - Sometimes alleles result in obvious variation between people (i.e. eye color)
    - One allele codes for blue eyes, while a different allele codes for brown eyes

Allele vs. Genotype

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- Each locus has several different alleles that a person can inherit
- Example*

Locus	12 Different Alleles
D8S1179	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19

- Any combination of these alleles is possible
  - 12,14 or 8,10 or 15,18 (these are examples of genotypes)
  - An 11,11 is also possible (this means the same allele was inherited from both parents)
  - In this example, 78 different genotypes are possible
- * (using the alleles present in the Identifiler Plus allelic ladder)

Where does DNA evidence come from?

- Biological material containing cells
  - **Bloodstains**
  - **Saliva**
    - Drink containers
    - Cigarette butts
    - Masks
  - **Shed skin cells, sweat, transfer via contact**
    - Clothing
    - Handled objects
  - **Semen, vaginal fluid**

Where does DNA evidence come from?

- **DNA can be deposited on an item a variety of ways**
  - Shed skin cells from the hand
  - DNA in sweat and oils transferred from your face to your hand and then to an object
  - Secondary (or indirect) transfer of DNA from someone who has never touched the object
  - Talking, coughing, or sneezing over an object
  - Direct contact with a biological fluid (i.e. drop of blood, semen stain, etc.)
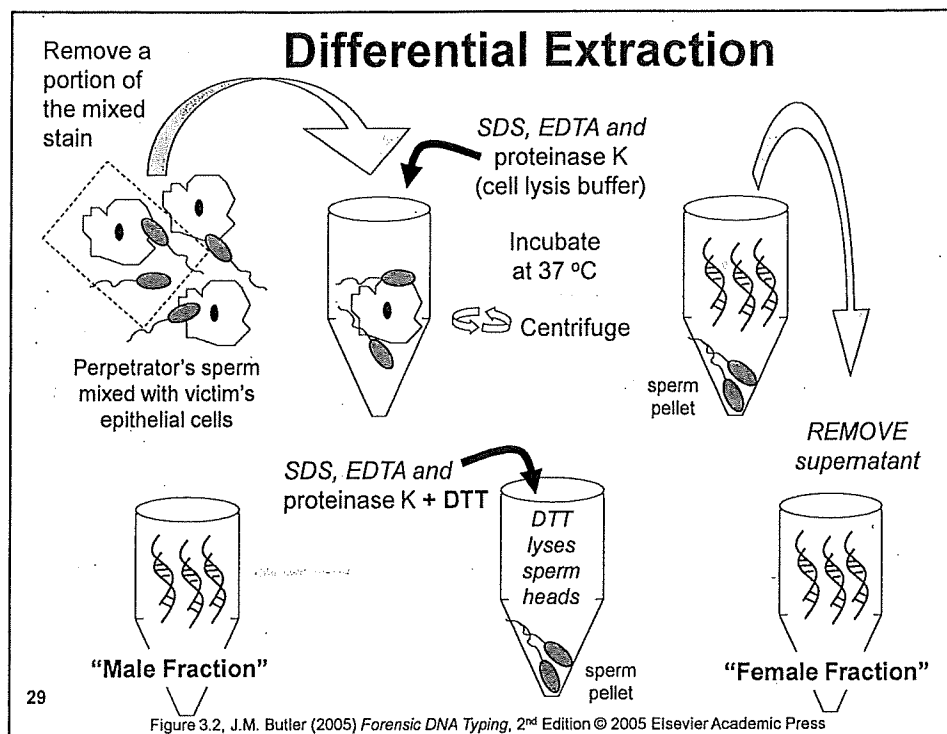
Sample Collection: Special Topic

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- **Evidence collection at autopsies**
  - Case dependent
  - Consider whether or not a sexual assault could have occurred

    - Potential items to collect would include
      - Fingernail clippings
      - If the nails are short, swab the finger tips (one swab per finger)
      - Vaginal or rectal swabs
      - Breast swabs

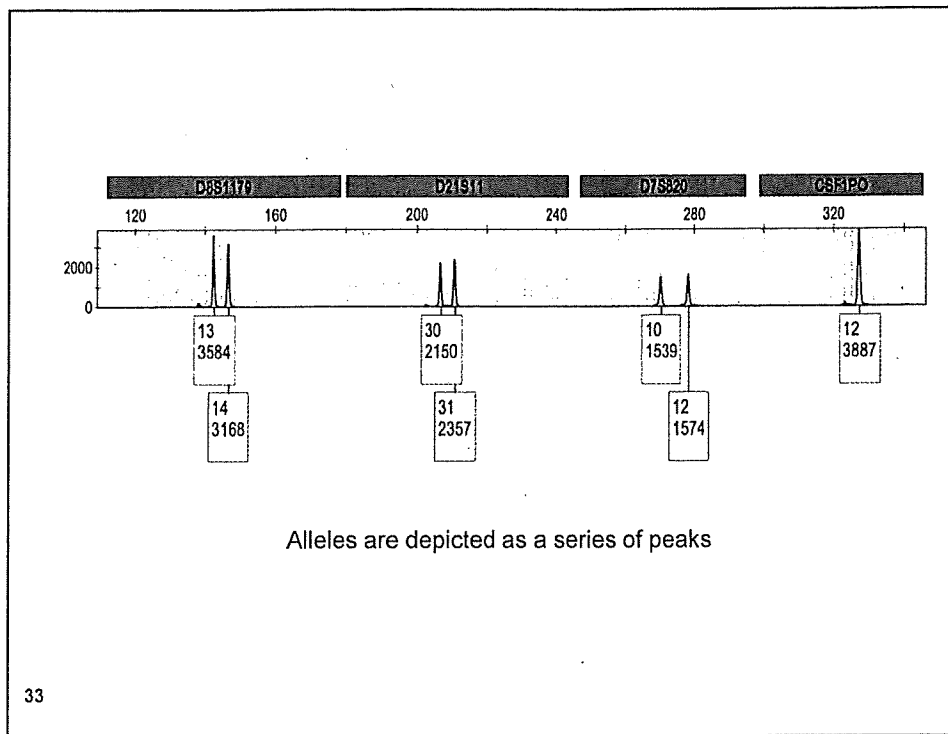## Sample Collection: Special Topic

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- **Evidence collection at autopsies**
  - blood reference for DNA analysis (reference DNA sample)
  - If the body is badly decomposed or there is not enough blood nails and teeth can be used in place of blood
- **Case dependent evidence to consider collecting**
  - blood and/or other samples for toxicology
  - hair references
  - any bullets or bullet pieces from the body
  - Clothing and other personal objects on the body
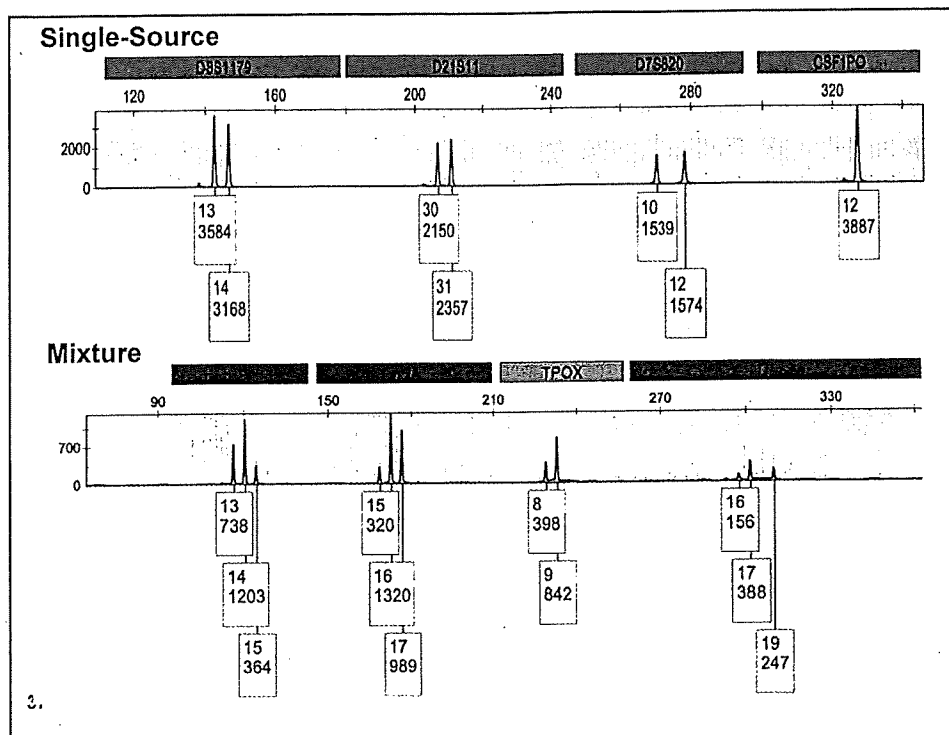 - Bugs (if applicable/needed for time of death)

Differential Extraction

Remove a portion of the mixed stain

SDS, EDTA and proteinase K (cell lysis buffer)

Incubate at 37 °C

Centrifuge

Perpetrator's sperm mixed with victim's epithelial cells

sperm pellet

REMOVE supernatant

SDS, EDTA and proteinase K + DTT

DTT lyses sperm heads

"Male Fraction"

sperm pellet

"Female Fraction"

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Figure 3.2, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

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DNA Quantification

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- **Provides the following information:**

  - An estimation of how much DNA was recovered
  - The quality of the recovered DNA
    - Is the DNA degraded?
      - Degraded DNA can result in incomplete DNA profiles (missing alleles)
    - Are DNA inhibitors present?
      - Inhibitors interfere with the amplification process and can result in incomplete DNA profiles or no results at all
  - Indicates if the sample has male or female DNA

Alleles are depicted as a series of peaks

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Break?

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Single-Source / Mixture electropherogram figure

Interpretation: Single-Source

- Was a complete profile recovered?

  - Are both alleles present at all 15 loci?

    - If the answer is "Yes" = complete profile
    - If the answer is "No" = partial profile

      - Partial profiles are usually recovered from:
        - old samples
        - samples with degraded DNA
        - samples with very small quantities of DNA

Interpretation: Report Table

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Complete

Locus	Item 1
D19S433	13.2,15.2
vWA	17,17
TPOX	8,11
D18S51	17,20

#,# indicates a genotype

Partial

Locus	Item 2
D3S1358	14,18
TH01	7,8
D13S317	11
D16S539	10

# indicates a detected allele only; a genotype was not determined

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Interpretation: Report Wording

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- A complete male DNA profile was recovered from the swab of blood from the desk.

- A male, partial DNA profile was recovered from the swab of the Pepsi can.

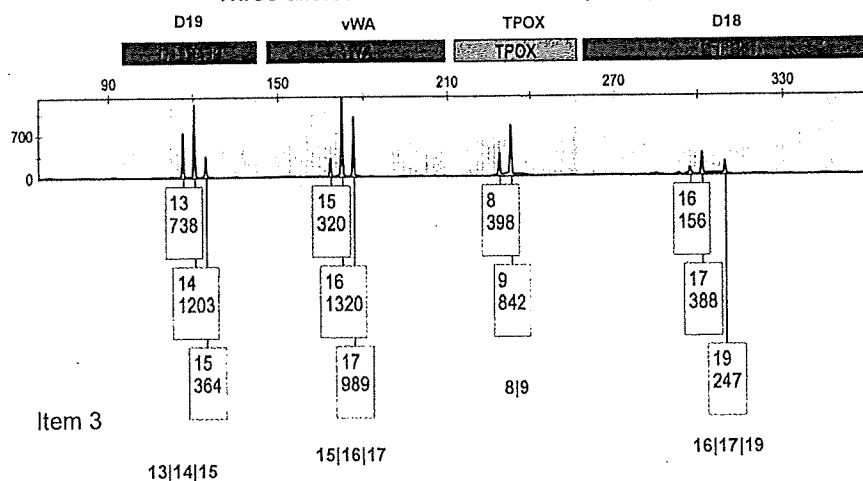# Interpretation: 2 Person Mixture

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How many people are in the mixture?

- What to look for: 2 person mixture

  - More than two peaks at more than one locus

  - No more than four peaks at a locus

Interpretation: 2 Person Mixture

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Three alleles were detected at D19, vWA, and D18

| D19 | vWA | TPOX | D18 |

90   150   210   270   330

700
0

13 | 15 | 8 | 16
738 | 320 | 398 | 156

14 | 16 | 9 | 17
1203 | 1320 | 842 | 388

15 | 17 | | 19
364 | 989 | 8|9 | 247

Item 3

13|14|15   15|16|17   16|17|19

Interpretation: 2 Person Mixture (with an assumed donor)

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- What is an assumed donor?
  - A DNA profile that is expected to be on an item
  - Most often assumed donors are either the victim or a consensual sex partner

- When is it appropriate to use an assumed donor during a mixture interpretation?
  - Any time you can reasonably assume a person's DNA could be present on an item

Interpretation: 2 Person Mixture (with an assumed donor)

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- Assumed donors are used for "intimate" samples
  - Samples taken directly from a person
    - Vaginal swabs, breast swabs, body swabs, etc.
  - Samples taken from personal items
    - Worn clothing, eyeglasses
  - Samples taken from items used to bind an individual
    - Duct tape, zip-ties, rope
- An assumed donor's DNA profile is used to help determine the genotypes of the "foreign contributor" to the mixture

Interpretation: 2 Person Mixture (major vs. minor)

Swab of a bandana

Listed genotypes are for the major contributor

* Minor allele
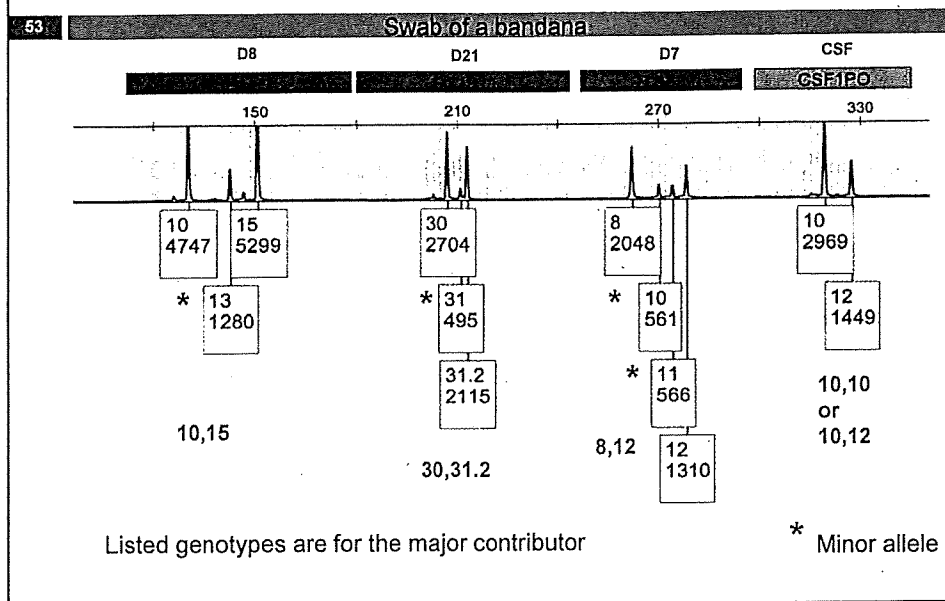
Interpretation: Report Table

Swab of a bandana

Locus	Detected alleles	Determined possible DNA types* of major contributor
D8S1179	10|13|15	10,15
D21S11	30|31|31.2	30,31.2
D7S820	8|10|11|12	8,12
CSF1PO	10|12	10,10 or 10,12

* Sometimes "DNA types" is used in place of "genotypes"

Interpretation: 2 Person Mixture (major vs. minor)

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- Why? (continued)

  - The quality of the minor component is poor.
    - The DNA is degraded and alleles could be missing

  - Not enough genetic information is present to make a comparison
    - Only a few alleles were detected

Interpretation: 2 Person Mixture (indistinguishable)

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- Indistinguishable Mixture
  - The peaks are all similar heights
  - The detected alleles can all pair with each other
  - Cannot determine which alleles belong to which person

  - Meaning: There are multiple genotypes that are possible for each person
   - e.g. there could be three possible genotypes at a locus instead of just one genotype

Interpretation: Report Wording

- A mixture of DNA consistent with two contributors was recovered.

- Assuming only two contributors are present in the mixture, the possible DNA types of both contributors were determined.

Interpretation: Mixtures that cannot be interpreted

Swab of a steering wheel of a communal car
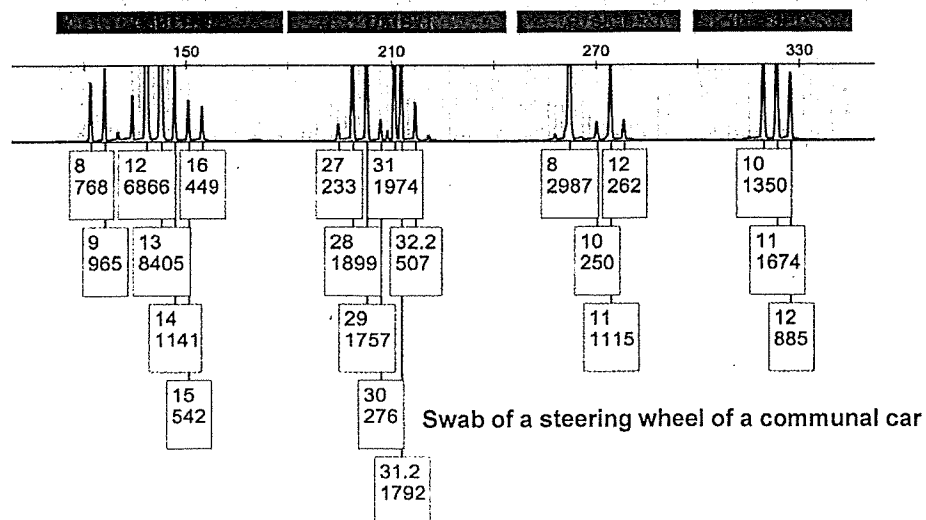
Interpretation: Mixtures that cannot be interpreted

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- Evidence items that most often produce results that cannot be interpreted
  - Swabs from any object that is small
  - Swabs from any object that was most likely held for a brief period of time
  - Swabs of objects frequently handled by multiple people
  - Flashlights, headlamps, lighters
  - Jewelry, wrappers, "smudges" on windows or walls
  - Baseball hats
  - Cartridge cases

Interpretation

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Important concepts to remember:

- It is possible to recover DNA from a lot of different types of items, but that doesn't always mean the recovered DNA is interpretable

- An interpretable DNA result (by itself) doesn't always provide meaningful information in the context of the case
  - For some types of items it is not possible to tell how or when the DNA was deposited

Results: Inclusion (mixture) aka "cannot be excluded"

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Locus	Item 6 Determined possible genotypes of the major male contributor	Item 7 Reference DNA sample
D8S1179	13,15	13,15
D21S11	28,F or 30,F	28,30
D7S820	10,11	10,11
CSF1PO	11,F	10,11

Results: Can Suspect 1 be excluded as a contributor to the mixture of DNA recovered from Item 3?

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Item 3: Two person mixture

Locus	Detected Alleles (mixture of DNA)	Determined possible genotypes for both contributors (mixture interpretation)	Reference DNA sample from Suspect 1
D19S433	13|14|15	13,F or 14,14 or 15,F	13,14
vWA	15|16|17	15,F or 16,16 or 17,F	15,17
TPOX	8|9	F,F	8,11
D18S51	16|17|19	16,F or 17,F or 19,F	17,17

Statistics: Random Match Probability

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- This DNA profile is estimated to occur at random, among unrelated individuals, in approximately 1 in 15 quintillion African Americans

Statistics: Random Match Probability

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This is estimating the chance of the following outcome occurring:

Scenario:

- A person is randomly picked from the African American population
- The DNA profile of this random person is determined

Outcome:

- The DNA profile of the random person turns out to be the same as the DNA profile recovered from the evidence sample

Statistics: Random Match Probability

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- The probability that a randomly selected, unrelated individual would by chance be included as the major contributor to this mixture was estimated to be approximately 1 in 30 quadrillion African Americans.

Statistics: Random Match Probability

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- Where do these numbers come from?

- Why are they so large?

Statistics: Allele Frequencies

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- CA DOJ BFS Labs use allele frequencies from African American, Caucasian, and Hispanic population data sets

- Why?
  - The race of the evidence profile is not known, therefore the frequencies of the three largest racial groups in the United States are reported

Court Testimony

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Refer to handout

Statistics: Allele Frequencies

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- Where do these numbers come from?

  - Use population data sets containing estimated allele frequencies

  - Allele frequencies = how often an allele was observed in the population data set

Statistics: Allele Frequencies

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- Why are these numbers so large?
  - Each locus has two alleles
  - The frequency of allele 1 gets multiplied by the frequency of allele 2
    - This gives you the genotype frequency at one locus
  - This calculation is repeated for all 15 loci
  - The genotype frequencies of all 15 loci then get multiplied together
    - This gives you the frequency of the entire DNA profile
    - Or all of the possible DNA types

Statistics: Random Match Probability

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What the RMP <u>does not</u> mean

- It is not the chance that someone else is guilty

- It is not the chance that someone else left their DNA

- It does not mean that out of 15 quintillion people, only one person would have that profile

Statistics: Random Match Probability

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- It is important to remember that this is an estimation of how rare the DNA profile is (the DNA profile from the evidence sample)

- For mixtures, we are often determining multiple possible genotypes rather than a single profile (one genotype per locus)

- The RMP is also used but the wording is a little different

Results: Inconclusive

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- If the DNA typing results cannot be interpreted

  - The results are inconclusive

  - Not suitable for comparison to any reference DNA samples

Statistics

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- If a person cannot be excluded as the source of the DNA
  - The strength of the match must be determined

  - This is done using a statistical calculation called the Random Match Probability (RMP)

  - The RMP is an estimate that expresses the significance of the match

Comparison/Results

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- If the DNA typing results are interpretable, the results are compared to reference DNA samples

  - Match (included or cannot be excluded)

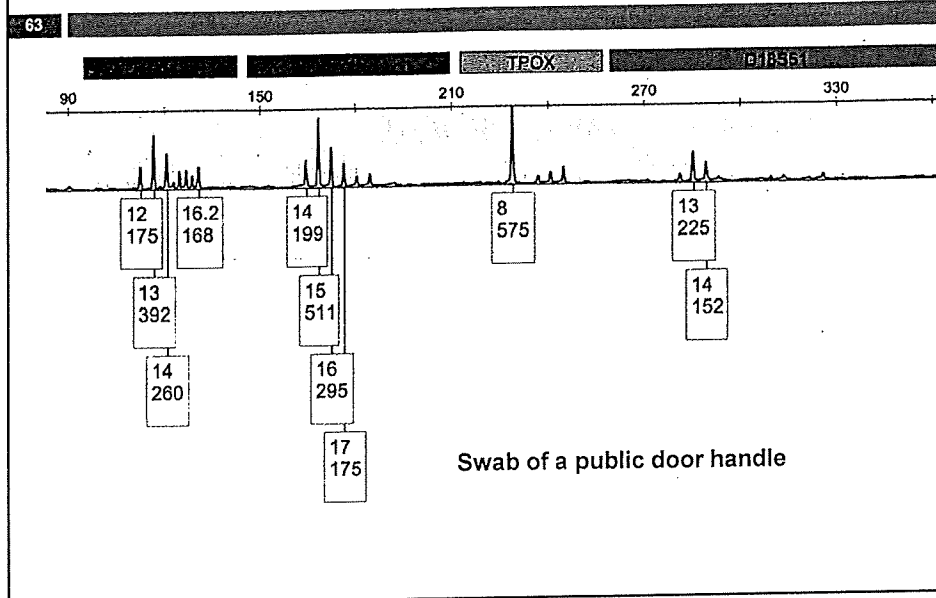 - No Match (excluded or eliminated)
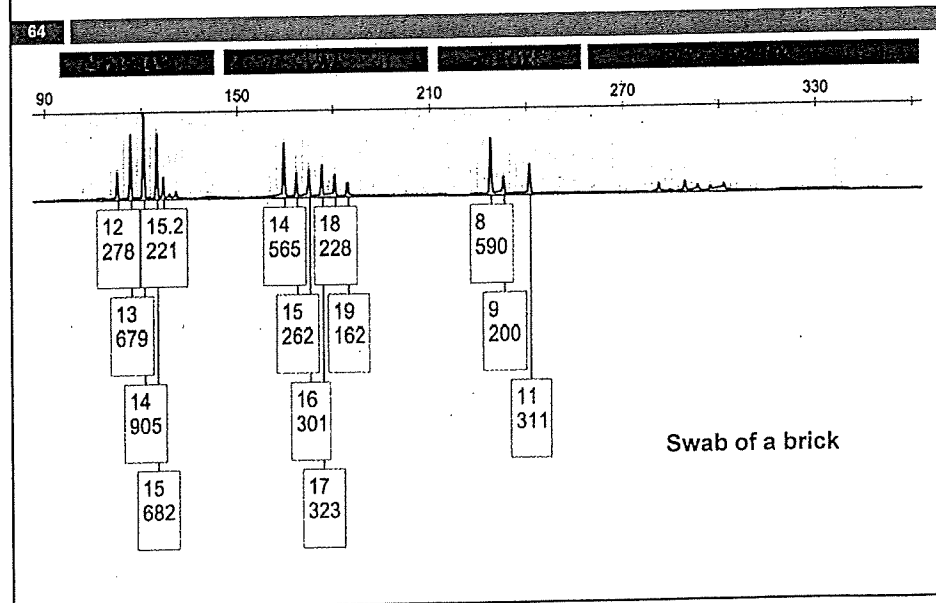
Results: Match (single-source)

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Locus	Item 4 Evidence Sample	Item 5 Reference DNA sample
D8S1179	12,14	12,14
D21S11	28,29	28,29
D7S820	10,12	10,12
CSF1PO	10,12	10,12

All genotypes have to be the same
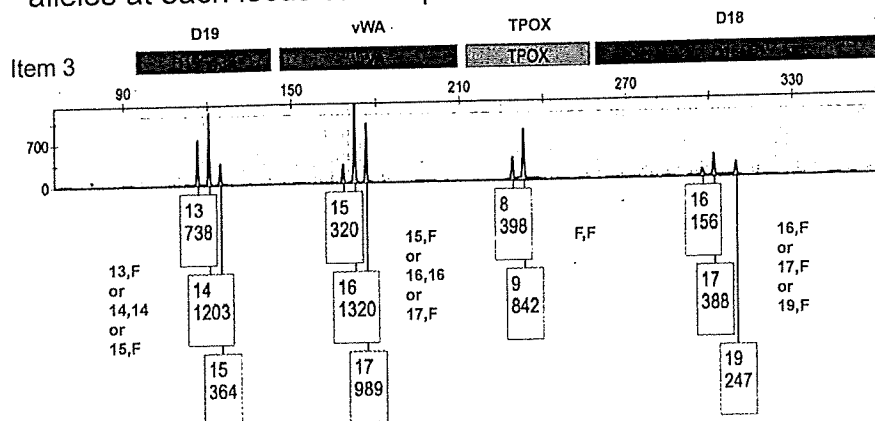
Interpretation: Mixtures that cannot be interpreted

Swab of a public door handle

Interpretation: Mixtures that cannot be interpreted

Swab of a brick

Interpretation: 2 Person Mixture (indistinguishable)

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Using validated thresholds it was determined that the detected alleles at each locus can all pair with each other

Item 3

| D19 | vWA | TPOX | D18 |

| 13 738 | 15 320 | 8 398 | 16 156 |

| 13,F or 14,14 or 15,F | 14 1203 | 15,F or 16,16 or 17,F | 16 1320 | F,F | 9 842 | 17 388 | 16,F or 17,F or 19,F |

| 15 364 | 17 989 | | 19 247 |

Interpretation: Report Table

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Item 3

Locus	Detected Alleles (mixture of DNA)	Determined possible genotypes for both contributors (mixture interpretation)
D19S433	13|14|15	13,F or 14,14 or 15,F
vWA	15|16|17	15,F or 16,16 or 17,F
TPOX	8|9	F,F
D18S51	16|17|19	16,F or 17,F or 19,F

- Numbers separated by a comma indicate a determined genotype (e.g. 14,14)
- An "F" allele designation indicates any allele is possible at that locus (e.g. 15,F or F,F)
- Numbers separated by a vertical bar indicate a detected allele only; genotypes were not determined (e.g. 13\|14\|15)

Interpretation: Report Wording

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- A mixture of DNA consistent with one major male contributor and one minor contributor was recovered from the swab of the bandana.

- Assuming only two contributors are present, the possible DNA types of the major contributor were determined and compared to available reference DNA samples.

Interpretation: 2 Person Mixture (major vs. minor)

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- **Sometimes only the major component of a mixture can be interpreted while the minor component cannot**
  - Why?
    - Cannot determine how many minor contributors are present

    - The minor component is too "low-level"
      - The quantity of the DNA from the minor contributor(s) is too small

Interpretation: Mixture with an assumed donor

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Sample from inside crotch area of Victim's underwear

Locus	Detected Alleles	Reference DNA sample from Victim	Determined possible DNA types for the male contributor	Reference DNA sample from Suspect
D8S1179	13|14	14,14	13,13 or 13,14	13,13
D21S11	28|29|30|32.2	29,32.2	28,30	28,30
D7S820	10|11|12	10,10	11,12	11,12

#,# indicates a genotype

Numbers separated by a vertical bar indicate a detected allele only; genotypes were not determined (e.g. 13|14|15)

Interpretation: 2 Person Mixture

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- Major contributor vs. Minor contributor
  - Major contributor = more of this person's DNA is present in the mixture than the other person's DNA (minor contributor)

  - Can tell major vs. minor by looking at the heights of the peaks that were detected
    - Significant difference between peak heights

  - Use thresholds to determine if alleles can pair with one another to determine genotypes

Interpretation: Report Table

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- Two person mixture before determining genotypes

Detected Alleles

Locus	Item 3
D19S433	13|14|15
vWA	15|16|17
TPOX	8|9
D18S51	16|17|19

Numbers separated by a vertical bar indicate a detected allele only; genotypes were not determined (e.g. 13|14|15)

Interpretation: Report Wording

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- A mixture of DNA consistent with two contributors was recovered.

Interpretation: Mixture

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Questions to Answer

- How many people are in the mixture?

- Are there major contributors vs. minor contributors in the mixture?
  - Meaning: Is one person's DNA present at a higher concentration than another person's DNA?

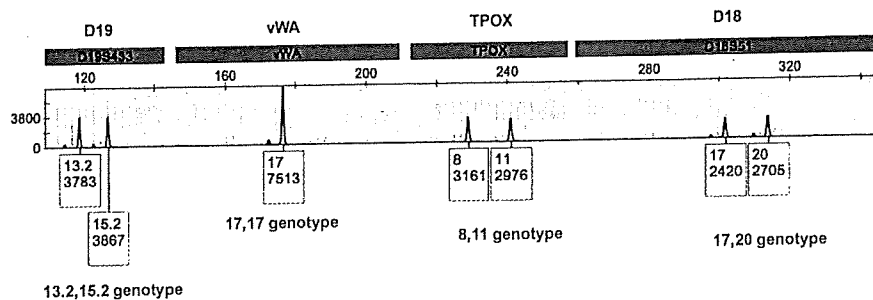## Interpretation: Mixture

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- Are the contributors to the mixture indistinguishable from one another?

- Can genotypes be determined for each contributor in the mixture?
  - Meaning: Were all alleles detected for each contributor or are some alleles missing?

- Can the mixture be interpreted?

Interpretation: Single-Source Complete

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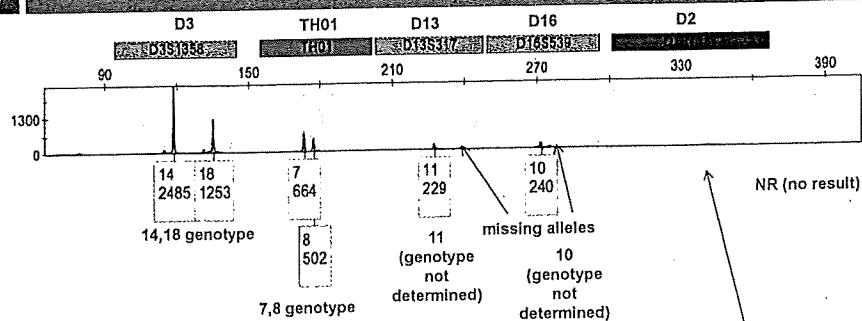Both alleles were detected at all 4 loci

D19	vWA	TPOX	D18

| 120 | 160 | 200 | 240 | 280 | 320 |

3800

| 13.2 | | 17 | | 8 | 11 | | 17 | 20 |
| 3783 | | 7513 | | 3161 | 2976 | | 2420 | 2705 |

15.2
3867

17,17 genotype

8,11 genotype

17,20 genotype

13.2,15.2 genotype

Item 1   Two 17 alleles were detected at vWA
(determined by looking at the height of the peak)

Interpretation: Single-Source Partial

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D3	TH01	D13	D16	D2

| 90 | 150 | 210 | 270 | 330 | 390 |

1300

| 14 | 18 | 7 | | 11 | | 10 | | NR (no result) |
| 2485 | 1253 | 664 | | 229 | | 240 | | |

14,18 genotype

8
502

11
(genotype
not
determined)

missing alleles

10
(genotype
not
determined)

7,8 genotype

Only one of two alleles were
detected at D13 and D16

No alleles were detected at D2

Item 2

Interpretation

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- Is the recovered DNA from one person or more than one person?

  single-source (one person)
  vs.
  mixture (more than one person)

- This is determined by the number of peaks and the heights of the peaks detected at a locus

  - peaks = alleles

Interpretation: Mixture or Single-Source?

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- **Is this a mixture?**
  - Look at how many peaks are present at each locus

    - Typically, if the recovered DNA is from one person there should be no more than two peaks at a locus

    - More than two peaks at more than one locus = mixture

DNA Amplification

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- A process called **Polymerase Chain Reaction (PCR)** is used to make millions of copies of the extracted DNA

- Only the DNA at the 15 loci used for forensic DNA typing is amplified during this process
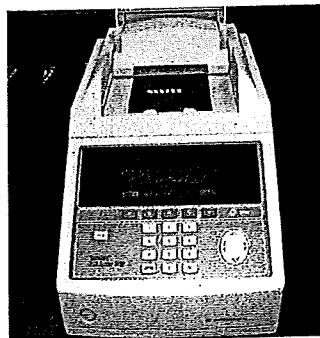
DNA Typing

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- The amplified DNA is then analyzed using an instrument called a genetic analyzer which separates and detects the specific regions of DNA for each sample

  - The alleles for each locus are detected as a series of peaks

  - The heights of the peaks are proportional to the amount of DNA that was detected

    - Taller peaks = more DNA

DNA Extraction

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- **Goal:** Remove DNA from the cells

- Extract a portion of the stain

or

- Extract a portion of the swab used to potentially collect DNA from an item

DNA Extraction: If semen is present

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- **Differential Extraction**

- Separates sperm from other body fluids

- Start with one sample and end with two fractions of the original sample
  - Sperm fraction (male component or sperm donor)

  - Non-sperm or epithelial cell fraction (female component or non-sperm donor)
    - Epithelial cells line body cavities such as the mouth and vagina

Where does DNA evidence come from?

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- **Important note:** for "touch DNA" type samples
  - Swabs of items that may have been handled by the suspect

- It is **not possible** to tell **how or when** DNA was deposited on an item

Sample Collection

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- **Collect sample** for DNA analysis

  - Take a direct cutting of a stain

  - Use a swab to collect DNA from an item

Allele vs. Genotype

- Everyone has two alleles at a locus (one is inherited from your mother and one from your father)

- For simplicity, alleles and genotypes are sometimes referred to as DNA types

  e.g.   Locus   DNA Type (aka "genotype")
         D16S539   5,7

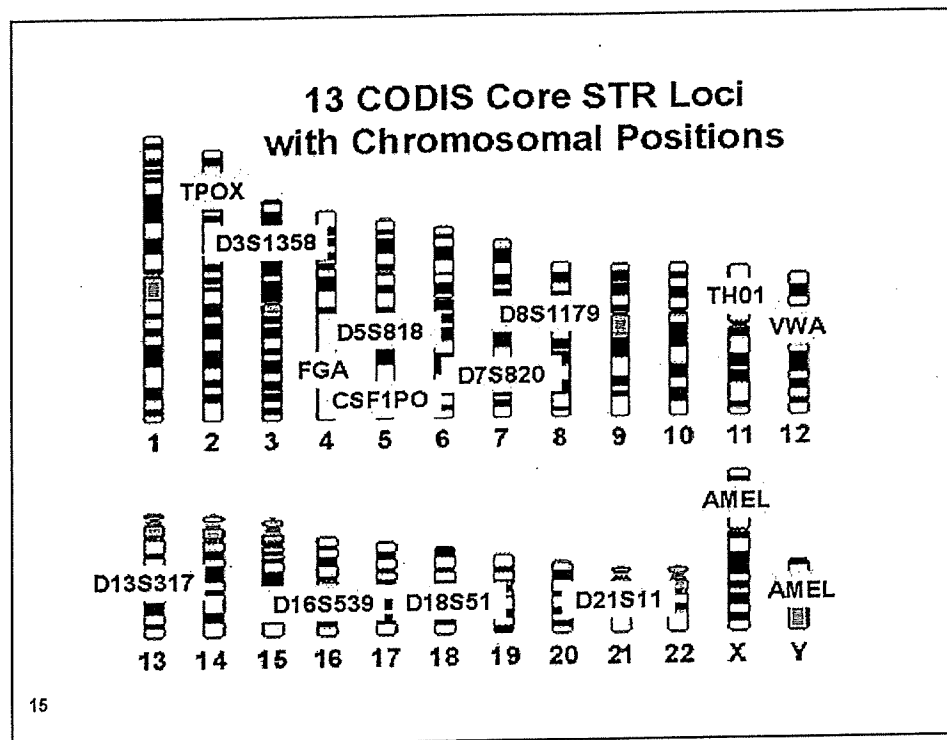- The numbers (5,7) correspond to the number of STR sequence repeats

      GATA-GATA-GATA-GATA-GATA = 5 repeats
      GATA-GATA-GATA-GATA-GATA-GATA-GATA = 7 repeats

DNA Typing Workflow

- Case Evaluation
- Sample collection
- Extraction
- Quantification
- Amplification
- Typing
- Interpretation
- Comparison/Results

13 CODIS Core STR Loci with Chromosomal Positions

A 15 Locus DNA Profile

A "DNA profile" refers to all 15 loci

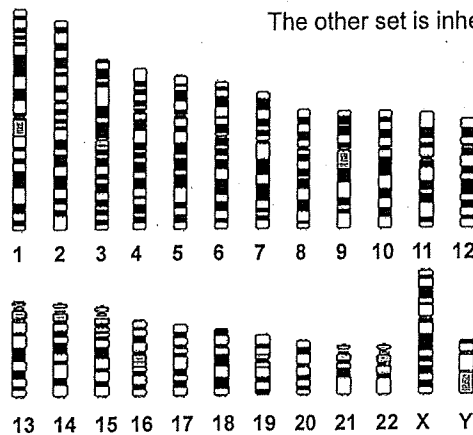| Locus	Item 1
D8S1179	15,15
D21S11	28,30
D7S820	11,11
CSF1PO	9,11
D3S1358	15,16
TH01	6,9.3
D13S317	9,11
D16S539	10,11
D2S1338	19,25
D19S433	13,16
vWA	16,18
TPOX	8,8
D18S51	12,17
Amelogenin	X,X
D5S818	12,13
FGA	21,25

Where Does Your DNA Come From?

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1 Set of Chromosomes

One set is inherited from your mother
The other set is inherited from your father

The nucleus of each cell
(except sperm and egg cells)
has two sets

1   2   3   4   5   6   7   8   9   10 11 12

13 14 15 16 17 18 19 20 21 22 X   Y

How is DNA Used in Forensic Science?

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- The vast majority (>99%) of everyone's DNA is the same

- It is only a very small portion (<1%) of each person's DNA that differs from other people

- These different or variable regions of DNA are what we type in Forensic DNA analysis

The Structure of DNA

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Base pairs

Adenine   Thymine

Guanine   Cytosine

Sugar phosphate
backbone
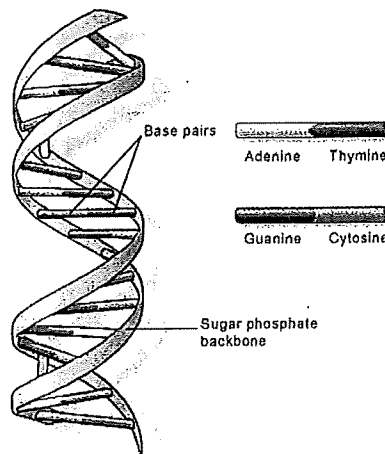
U.S National Library of Medicine

Where is DNA Found?

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- DNA is found inside the nucleus of a cell

  - Not found in red blood cells (they don't have a nucleus)

  - White blood cells provide the DNA that is obtained from blood samples

The Basics of DNA

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- What is DNA?

- Where is DNA found?

- Where does your DNA come from?

- How is DNA used in forensic science?

What is DNA?

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- deoxyribonucleic acid (DNA)

- A genetic template or code that contains the instructions responsible for directing cell activity

- Cellular activity is responsible for:
  - Development
  - Growth
  - Daily functions