# Forensic genetics

## An overview for medical students


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# Lecture overview

- Introduction to forensic genetics
- DNA sampling
- Individual vs. group identification
- Factors influencing the results of DNA analysis in forensic genetics

# *forensic*

1. Relating to, used in, or appropriate for courts of law or for public discussion or argumentation.
2. Of, relating to, or used in debate or argument; rhetorical.
3. **Relating to the use of science or technology in the investigation and establishment of facts or evidence in a court of law**
4. The word *forensic* comes from the Latin *forēnsis*, meaning "of or before the forum.“

# Forensic genetics?

„Application of genetics and molecular biology science to solve the questions raised by the legal system“

To link an individual to a crime scene/criminal act

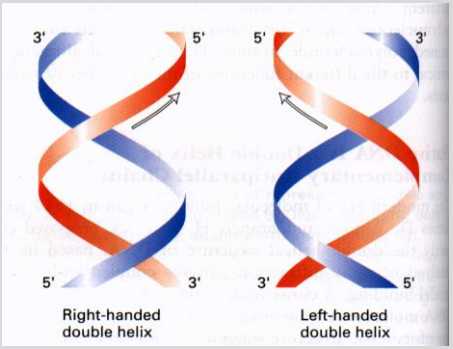
To identify victim

To exonerate suspect

To identify the animal/plany/microorganism

To perform kinship analysis

„To change history“



# The short history of forensic genetics

- 1980 – Ray White - The first RFLP marker

- 1985 – Alec Jeffreys - Multilocus VNTR probes
- 1985 – Kary Mullis - PCR (Nobel prize 1993)

- 1988 – FBI starts with DNA identifications
- 1995 – Forensic Science Service (UK) starts UK DNA database
- 1998 – FBI starts CODIS datbase

# The short history of CZE forensic genetics

~ 1990 –DNA analysis used to solve crime in Czechoslovakia (Doc. Ferak, UK Bratislava)

~ 1992 – 1st Police DNA laboratory in Czechoslovakia (Institute of Criminalistics Prague)

~ 1994 – 1st Police DNA laboratory in Slovakia

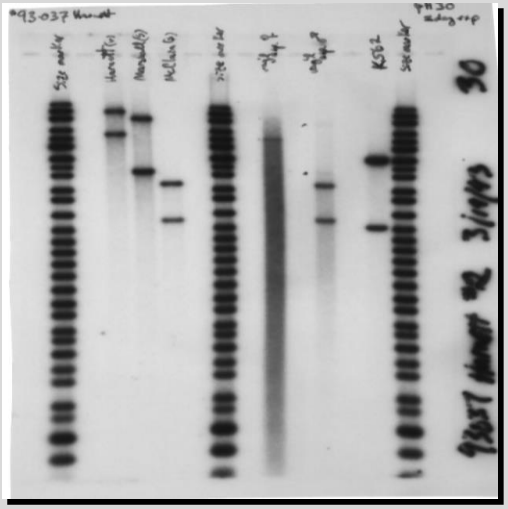
# Basic terminology: Genetics

- DNA Polymorphism (“many forms”)
  - Regions of DNA which differ from person to person
- Locus (plural = loci)
  - Site or location on a chromosome
- Allele
  - Different variants which can exist at a locus
- DNA Profile
  - The combination of alleles for an individual

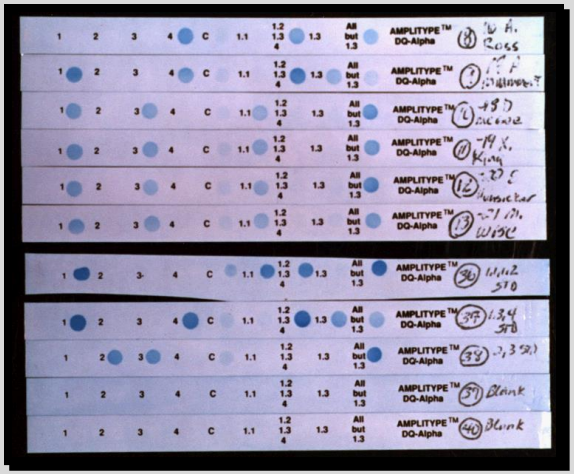
# Basic terminology: Technology

- Amplification or PCR (Polymerase Chain Reaction)
  - A technique for ‘replicating’ DNA in the laboratory (‘molecular Xeroxing’)
  - Region to be amplified defined by PRIMERS
  - Can be ‘color coded’
- Electrophoresis
  - A technique for separating molecules according to their size

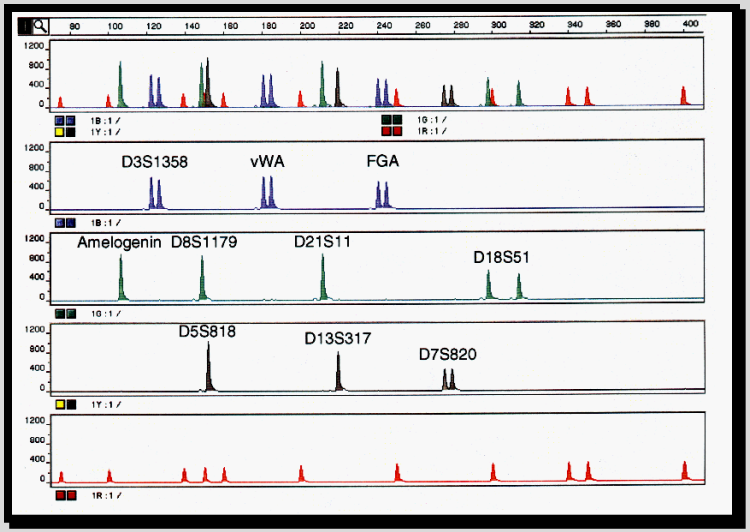
# Three generations of DNA testing



**RFLP**
**AUTORAD**
**Allele = BAND**



**DQ-alpha**
**TEST STRIP**
**Allele = BLUE DOT**



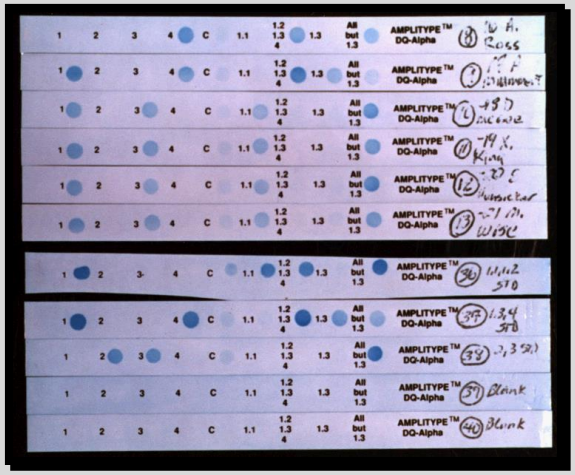

**Automated STR**
**ELECTROPHEROGRAM**
**Allele = PEAK**

# RFLP

- High starting amount of DNA needed
- Very high discrimination power
- Radioactive probes
- Labor intensive
- Non-uniform methodology/systems
- Limited potence for databasing

# Dot-blot

- Minimum starting amount of DNA needed (PCR amplification)
- Very poor discrimination power
- Labor intensive but can be automated
- Limited potence for databasing



# STR
# Short Tandem Repeat

- Good discrimination power
- Suitable for databasing
- Possibility of automation
- Uniform methodology/CODIS systems
- Very low demands on the quantity and quality of DNA

# Next generation sequencing

ion torrent

by life technologies™

VEROGEN

illumina®


Oxford NANOPORE Technologies

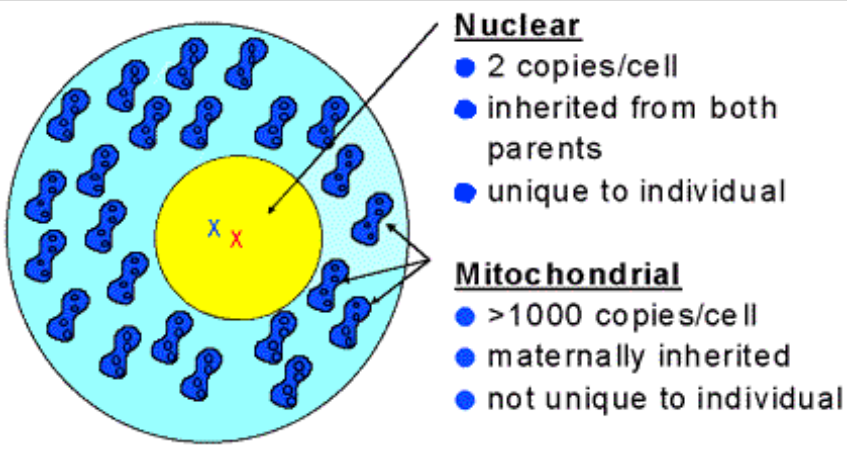
**(Next Generation Sequencing) Massively Parallel Sequencing**

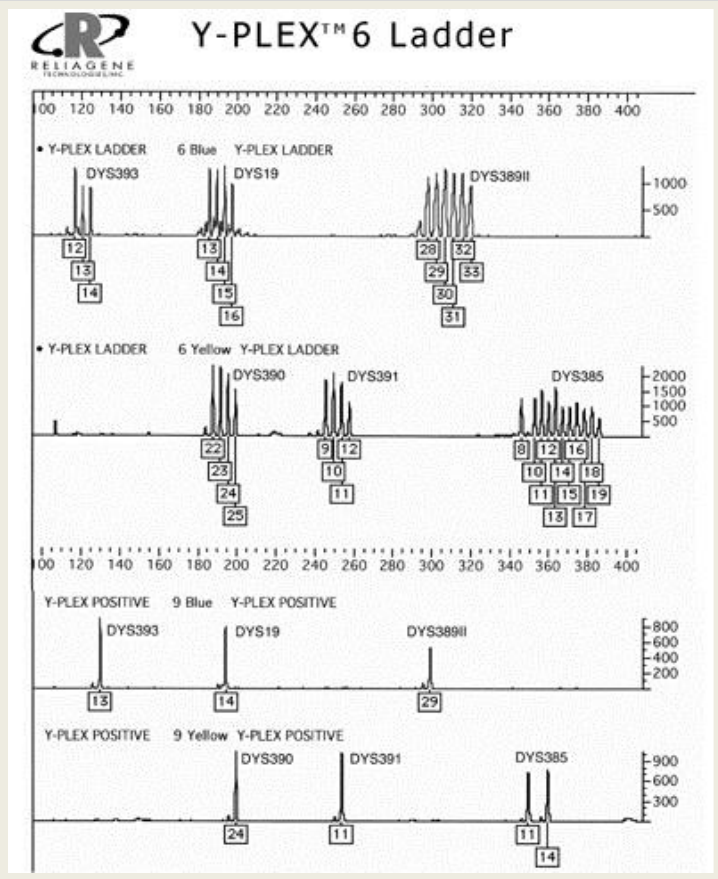
***IN SILICO* SEKVENCE**

**Sekvence**

# DNA tests for group identification

Lineage markers



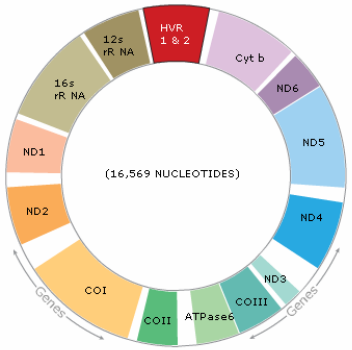

**Mitochondrial DNA**
**mtDNA sequence**
**Sensitive but not discriminating**

**Y-STRs**
**Useful with mixtures**
**Paternally inherited**

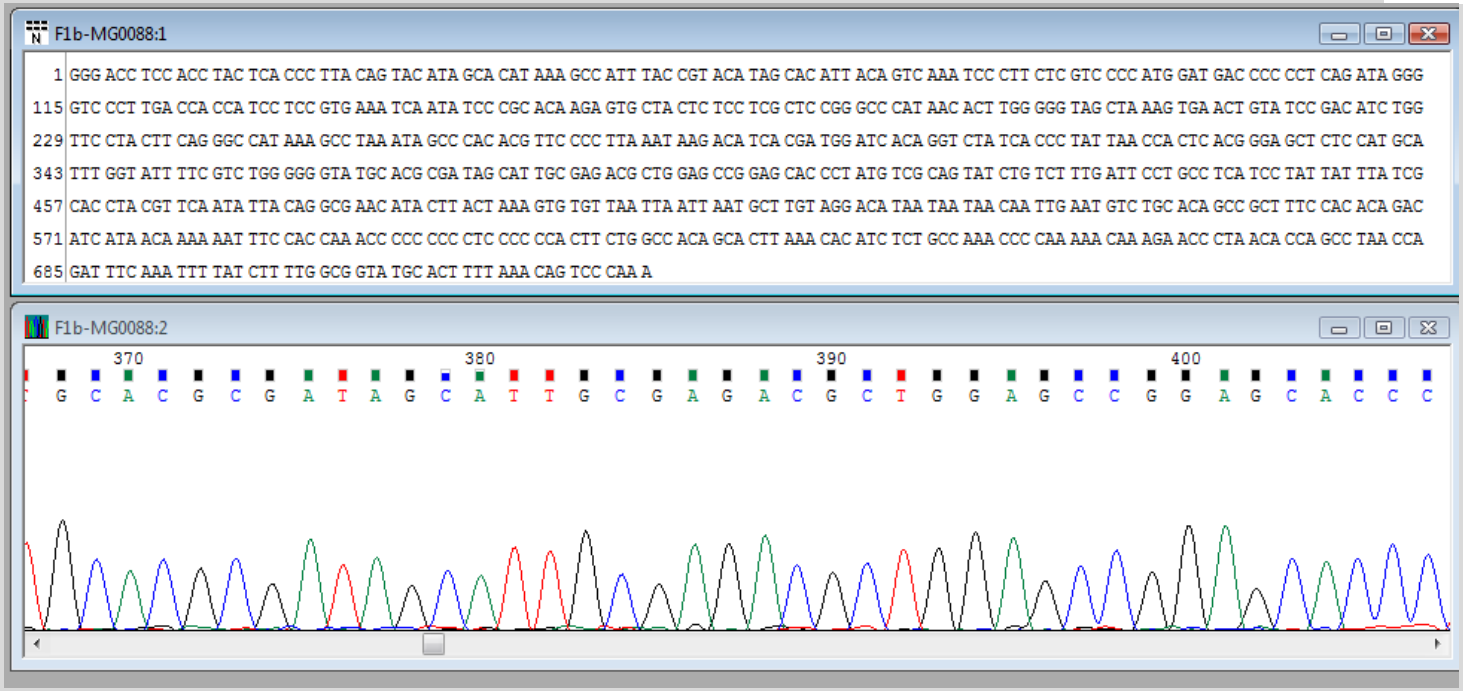
# Mitochondrial DNA sequencing

Hair shafts
Severely degraded bodies (HVR1+2)

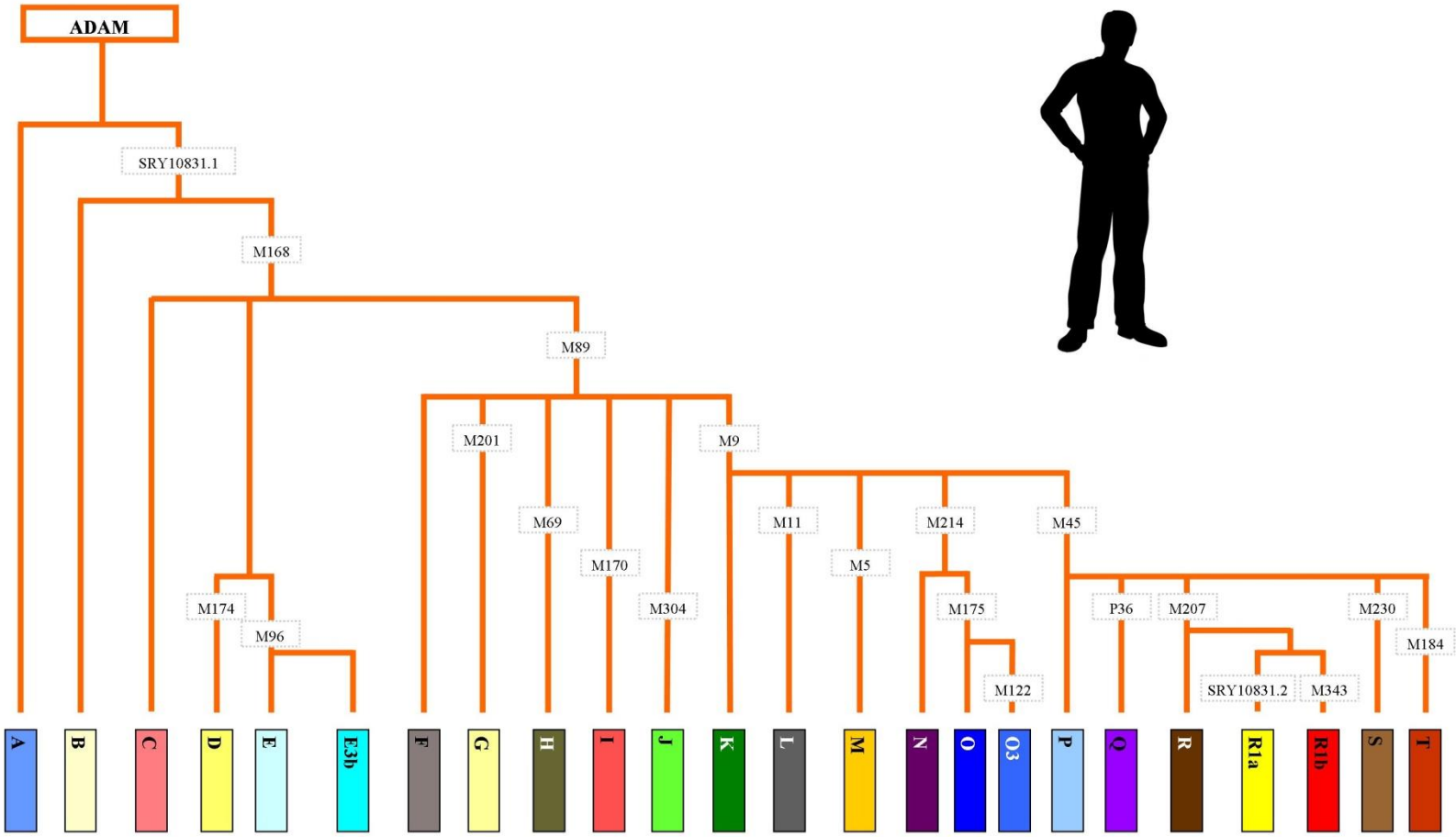
Species determination  (cytochrome b)

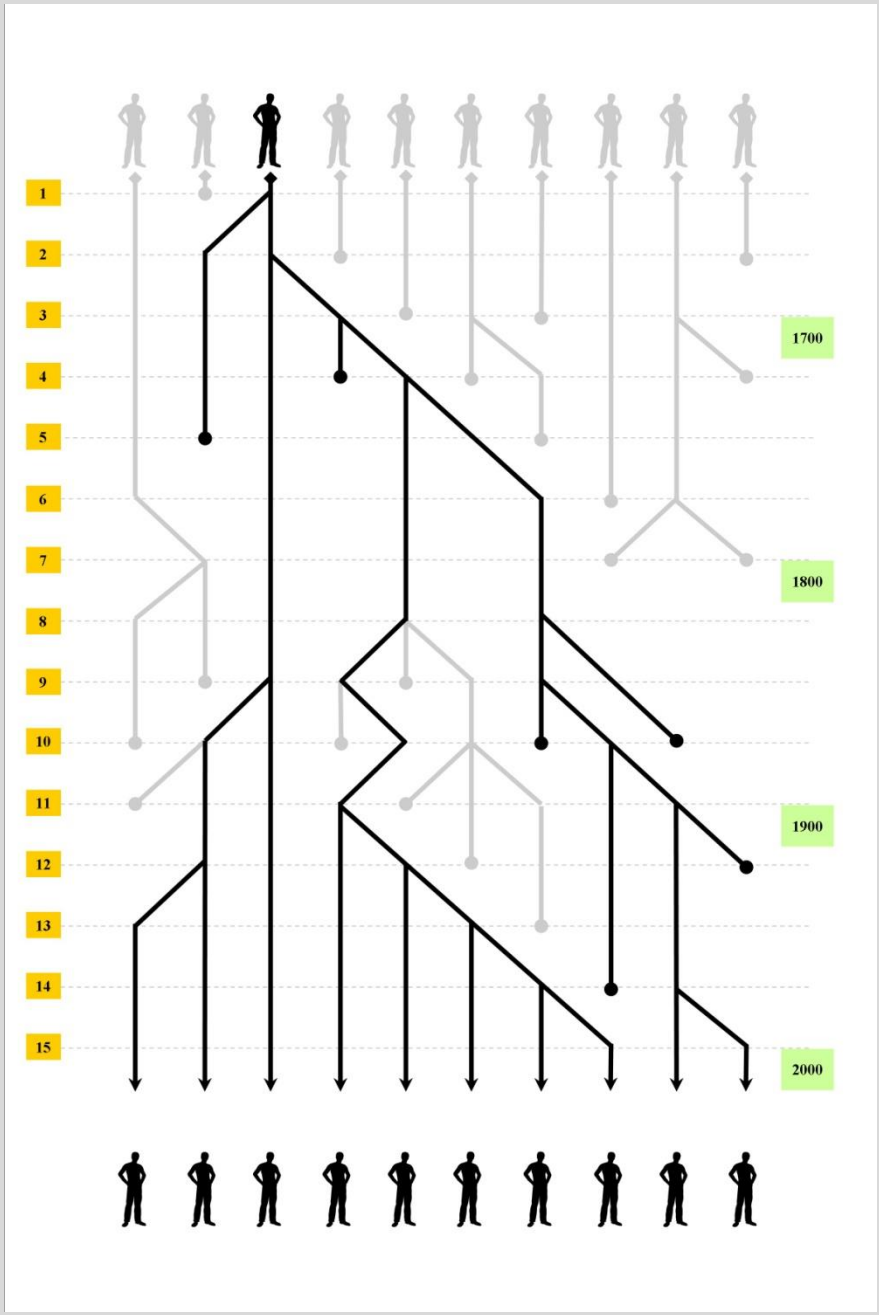



Maternally inherited



# Genetic genealogy






# Genetic genealogy

**Y-chromosome STRs:**

Mutation speed

Surname „specific“ haplotype

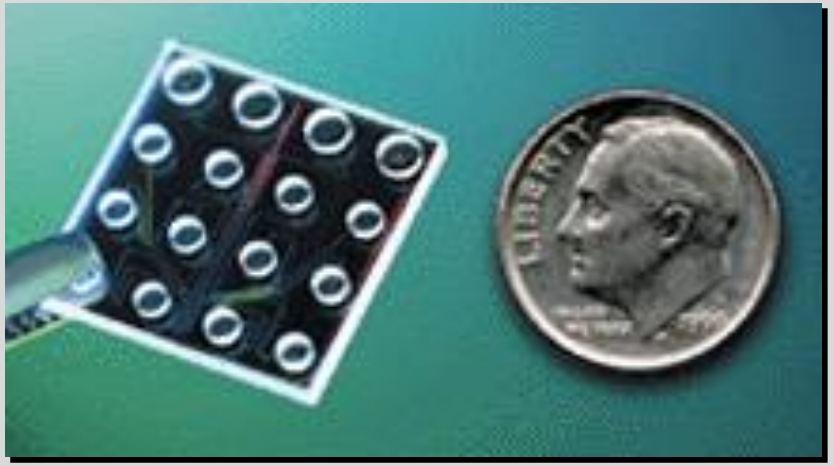
Geographic origin (SNP)

# SNP typing

- Geographic origin
  - Sub-Saharan African
  - East-Asian
  - Indo-European
  - Native-American
- Eye color
- Hair color
- Skin color
- Identification
- Family lineages

# Lab-on-a-chip/RAPID DNA

- All-in-one
  - Extract DNA
  - Quantify DNA
  - PCR amplify DNA
  - Capillary electrophoresis



# Scope of forensic DNA analysis

- Almost any kind of biological material (plant, animal, human, microbial)
- Identification = comparison of reference sample and unknown sample

# STR

- Short tandem repeat
- Describes a type of DNA polymorphism in which:
  - a DNA sequence repeats
  - over and over again
  - and has a short (usually 4 base pair) repeat unit
- A length polymorphism -- alleles differ in their length

**3 repeats: AATG AATG AATG**

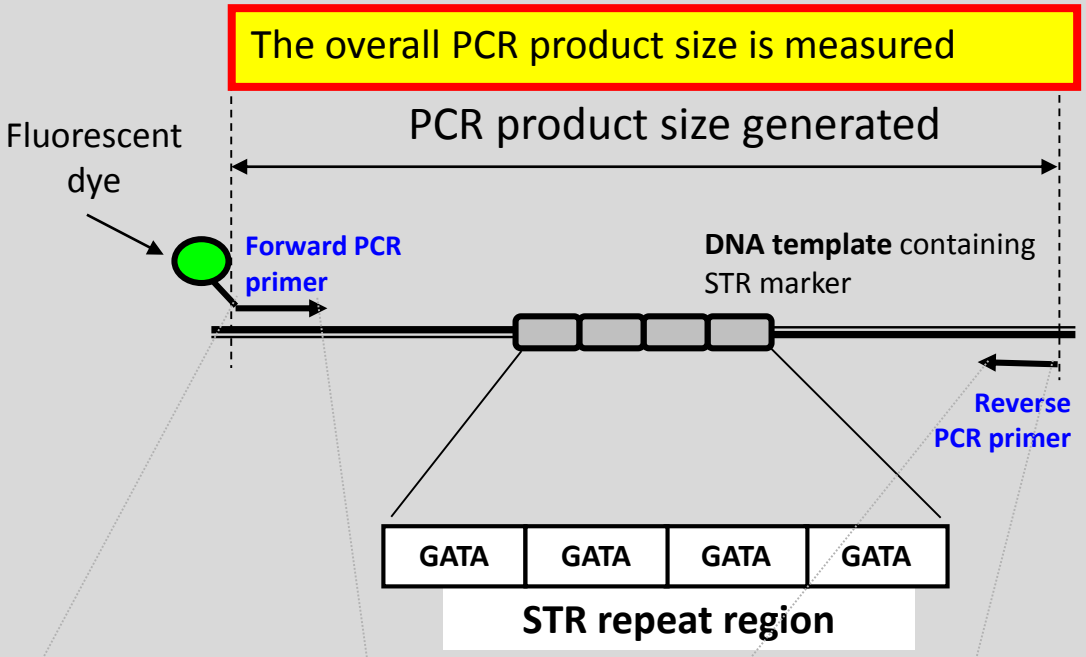
**4 repeats: AATG AATG AATG AATG**

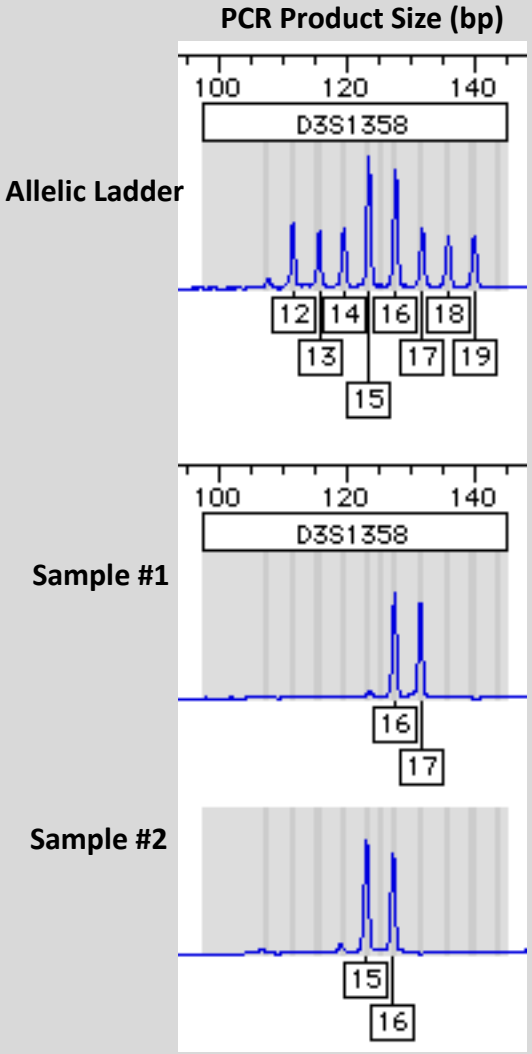
**5 repeats: AATG AATG AATG AATG AATG**

**6 repeats: AATG AATG AATG AATG AATG AATG**

# Short Tandem Repeat (STR) Markers

*PCR primers anneal to unique sequences bracketing the variable STR repeat region*

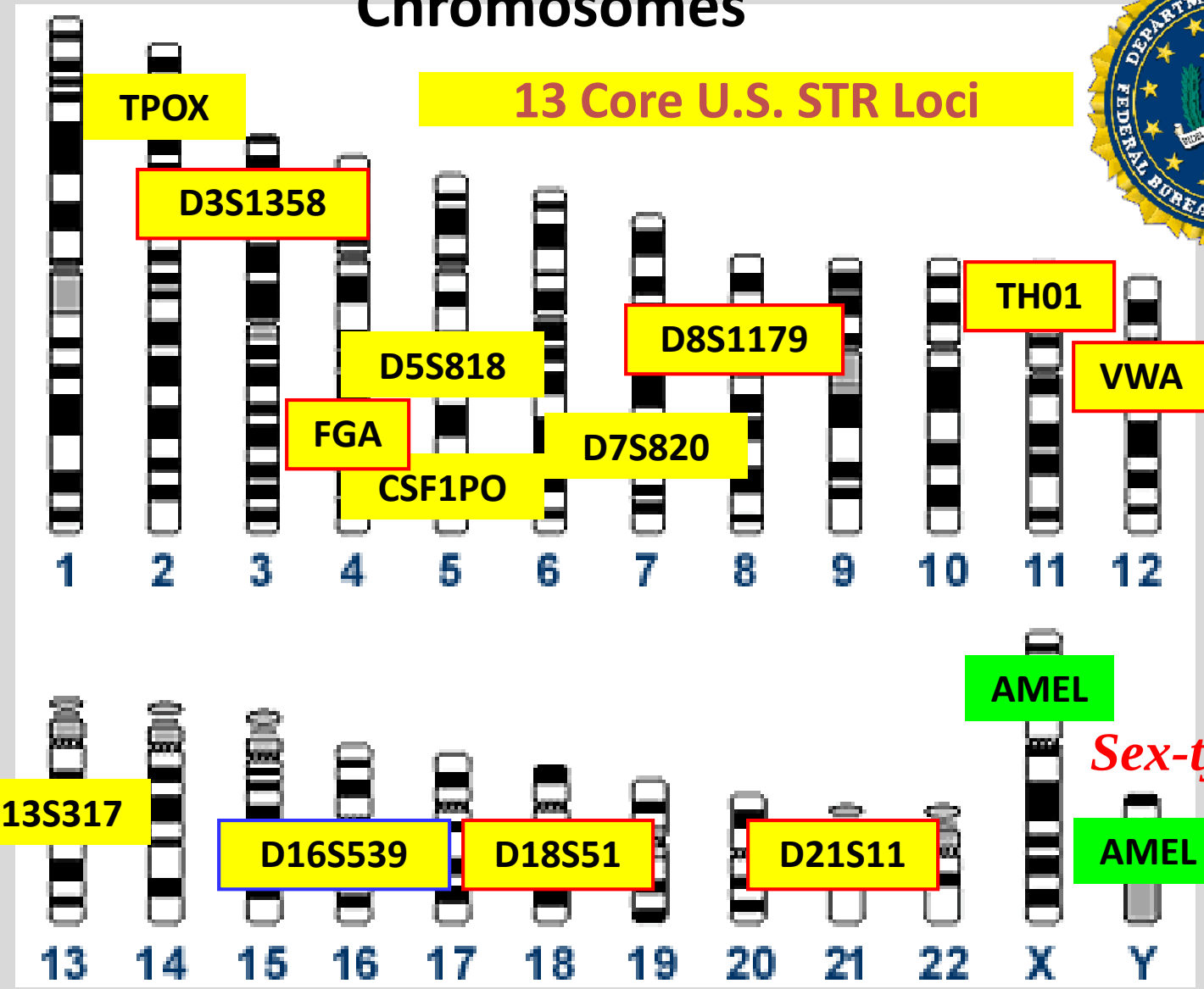



TCCCAAGCTCTTCCTCTTCCCTAGATCAATACAGACAGAAGACAGGTG**G**
**ATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATA**TCATTG
AAAGACAAAACAGAGATGGATGATAGATACATGCTTACAGATGCACAC

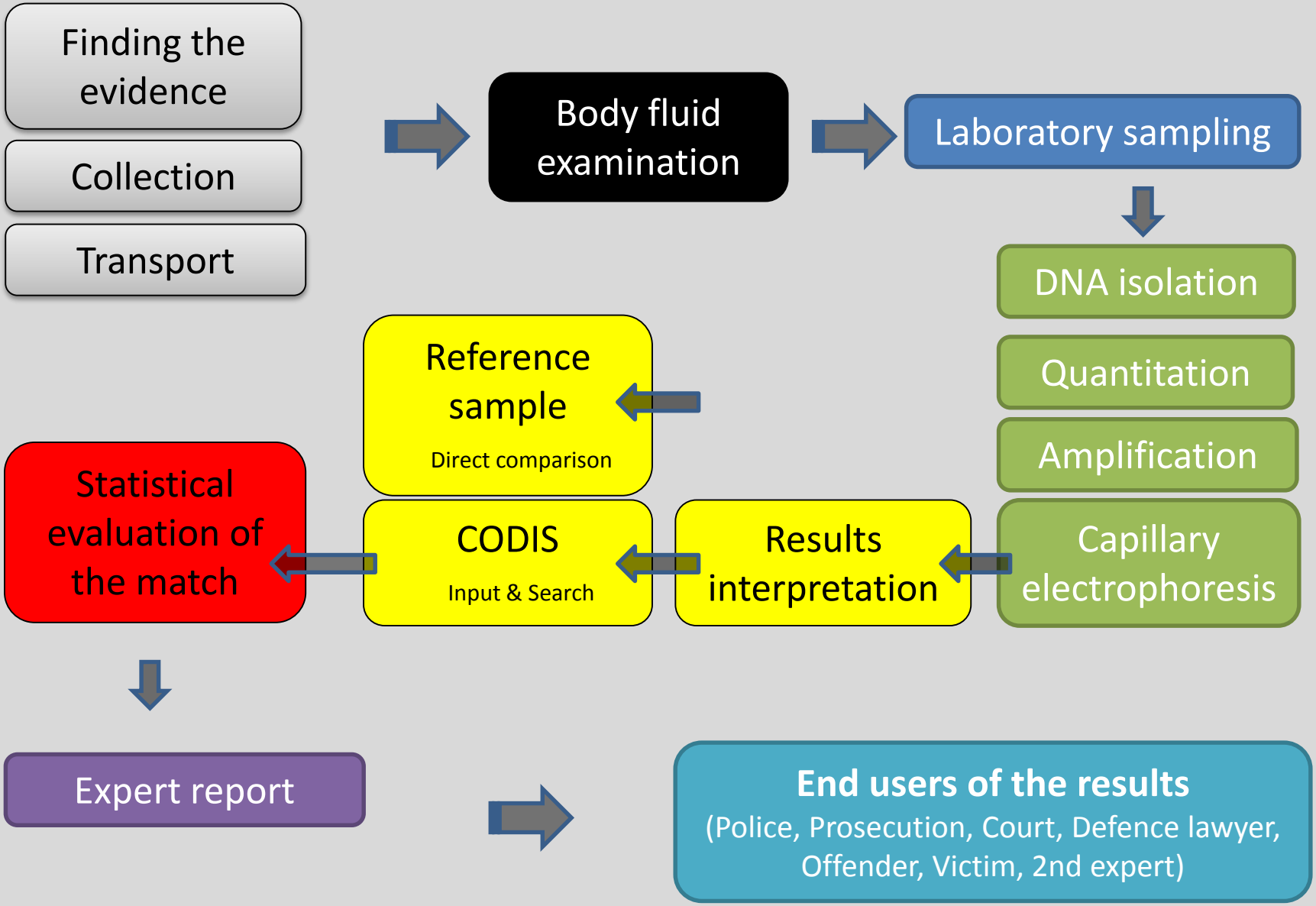
**= 11 GATA repeats ("11" is all that is reported)**

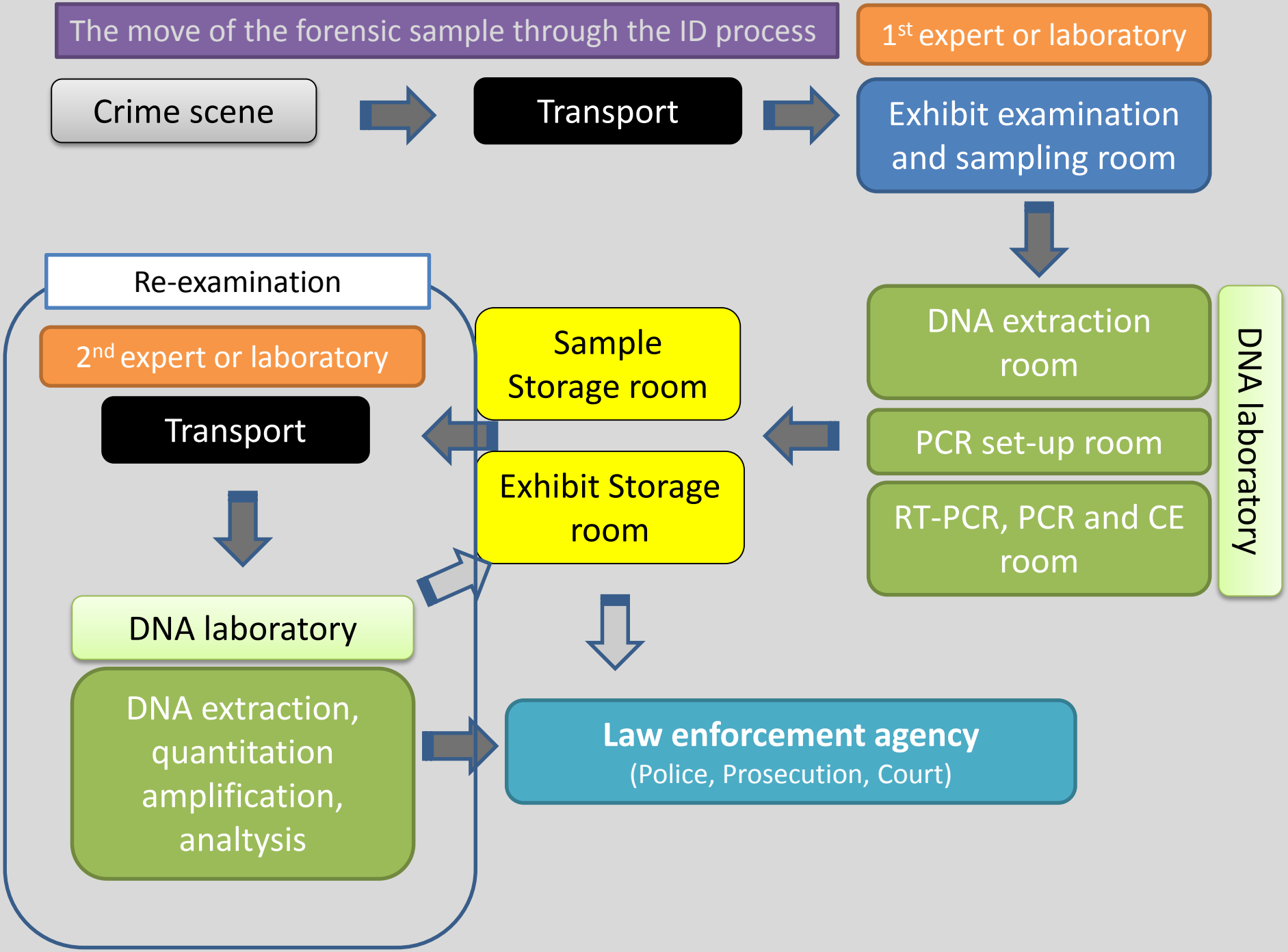
# Position of Forensic STR Markers on Human Chromosomes



The process of forensic DNA identification is not only about the technology used!!!

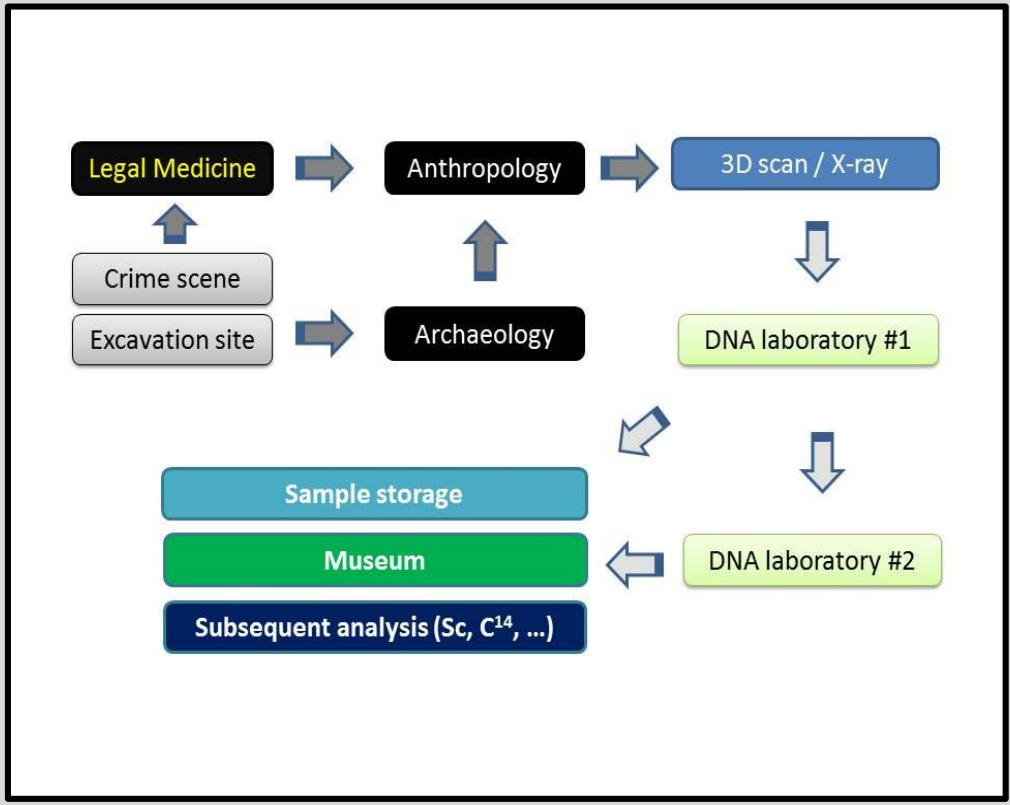
# The process of forensic DNA analysis




The move of the forensic sample through the ID process
1st expert or laboratory
Crime scene
Transport
Exhibit examination and sampling room
Re-examination
2nd expert or laboratory
Transport
Sample Storage room
Exhibit Storage room
DNA extraction room
PCR set-up room
RT-PCR, PCR and CE room
DNA laboratory
DNA laboratory
DNA extraction, quantitation amplification, analtysis
Law enforcement agency
(Police, Prosecution, Court)

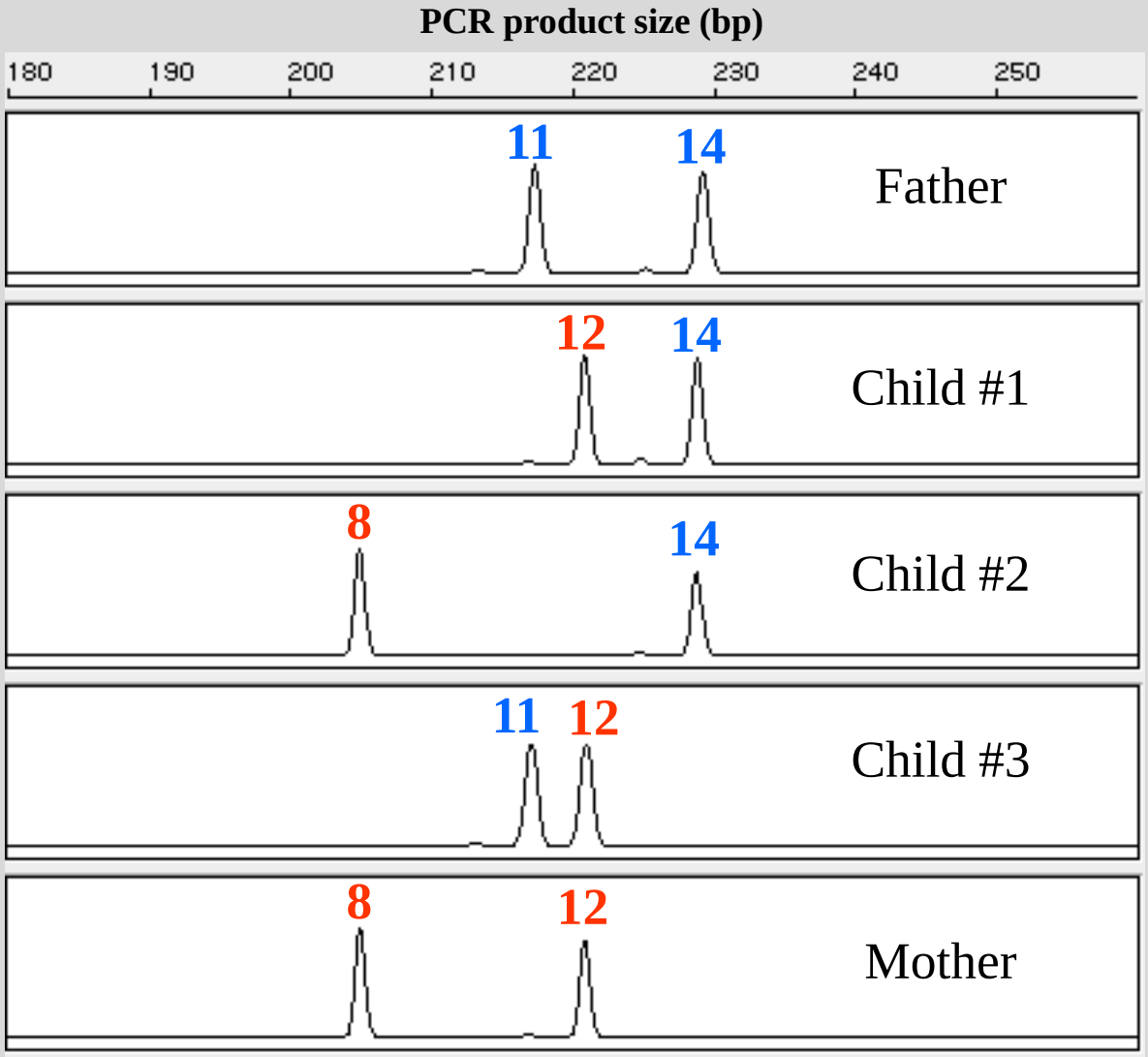
# Major external factors influencing the quality of DNA typing

- **UV light**
- **Humidity**
- **Radiation**
- **Chemicals**
- **Temperature**
- **Contamination**
- **Microorganisms**

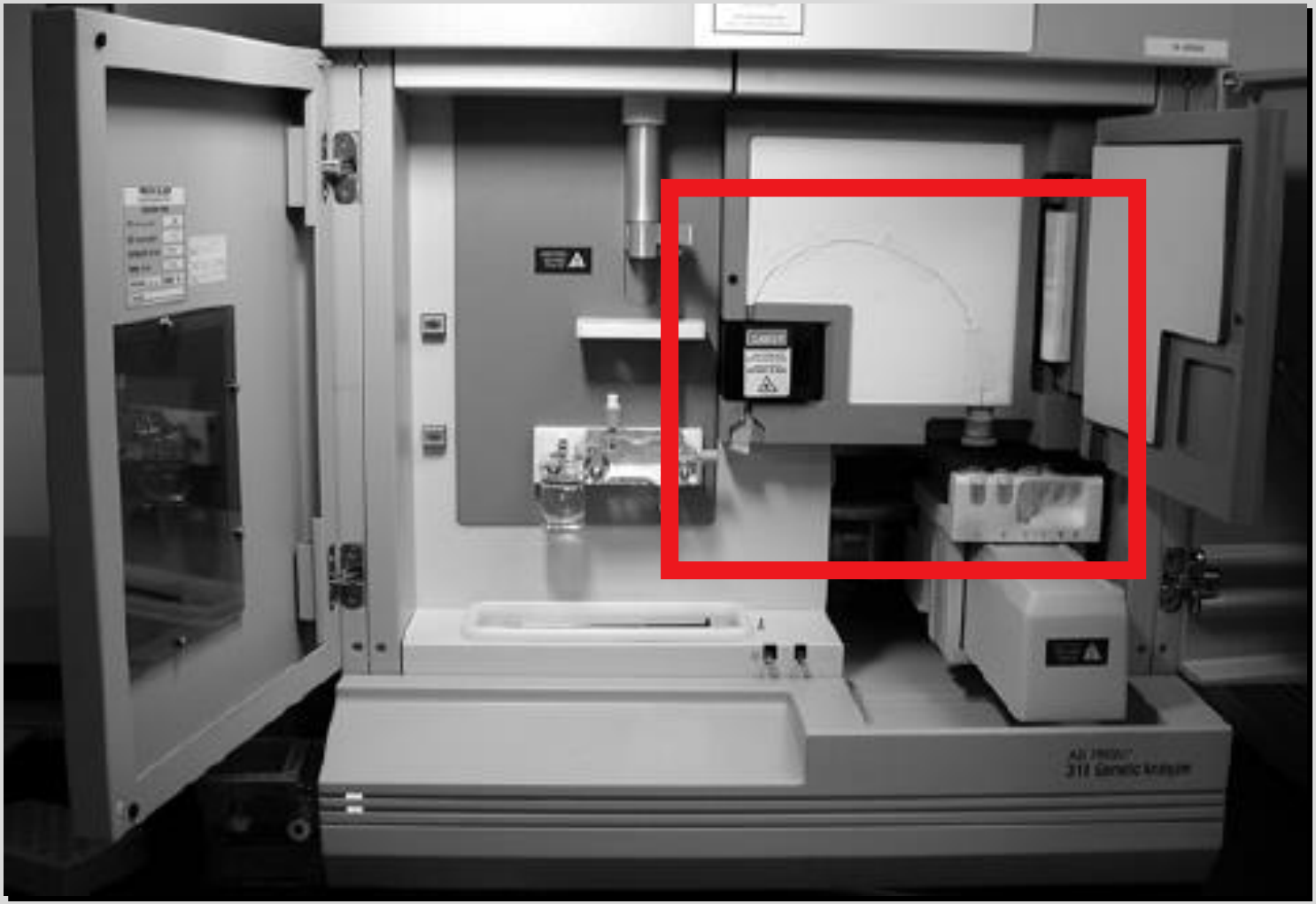


# PATERNITY TESTING

Family Inheritance of STR Alleles (D13S317)

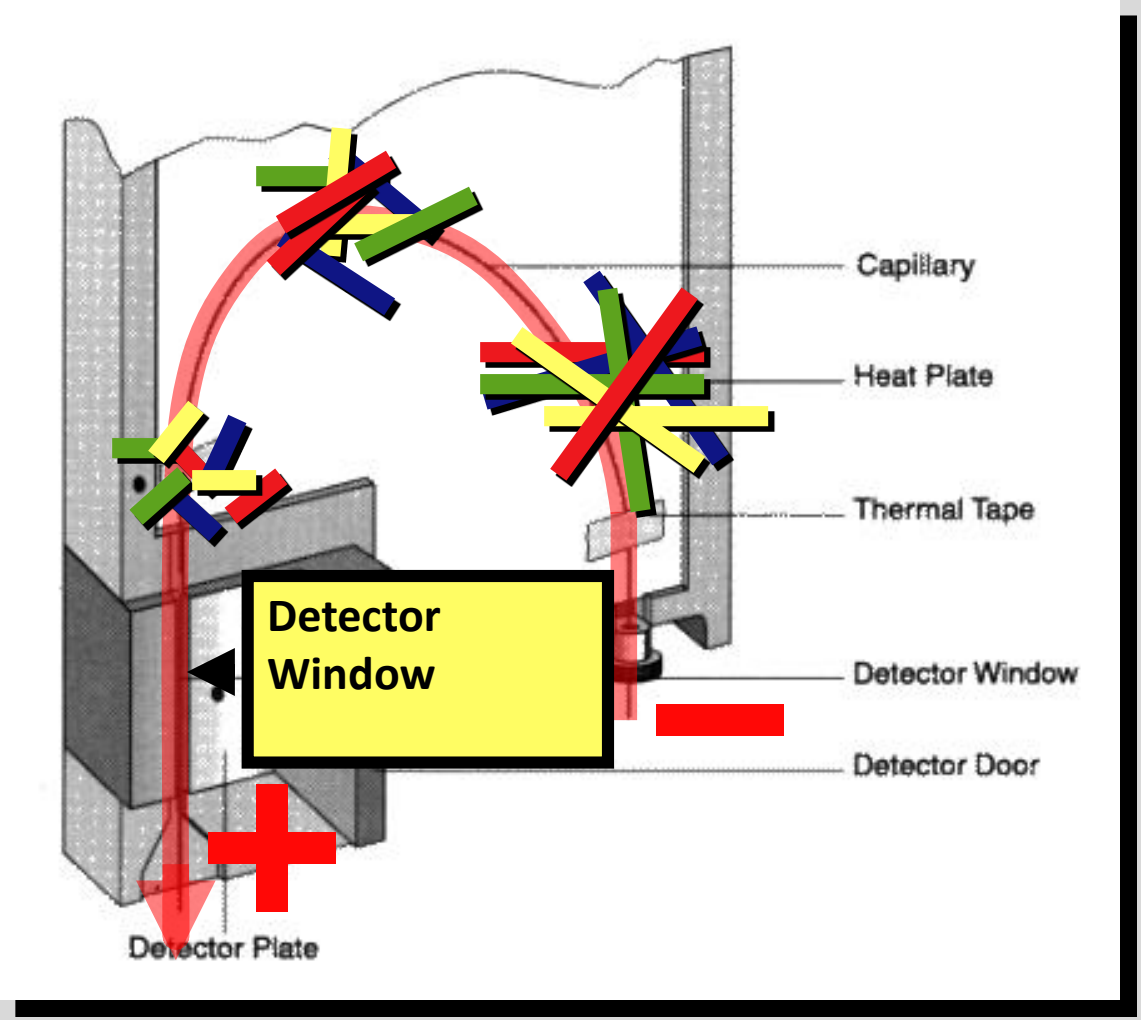


# The ABI 310 Genetic Analyzer: SIZE, COLOR & AMOUNT

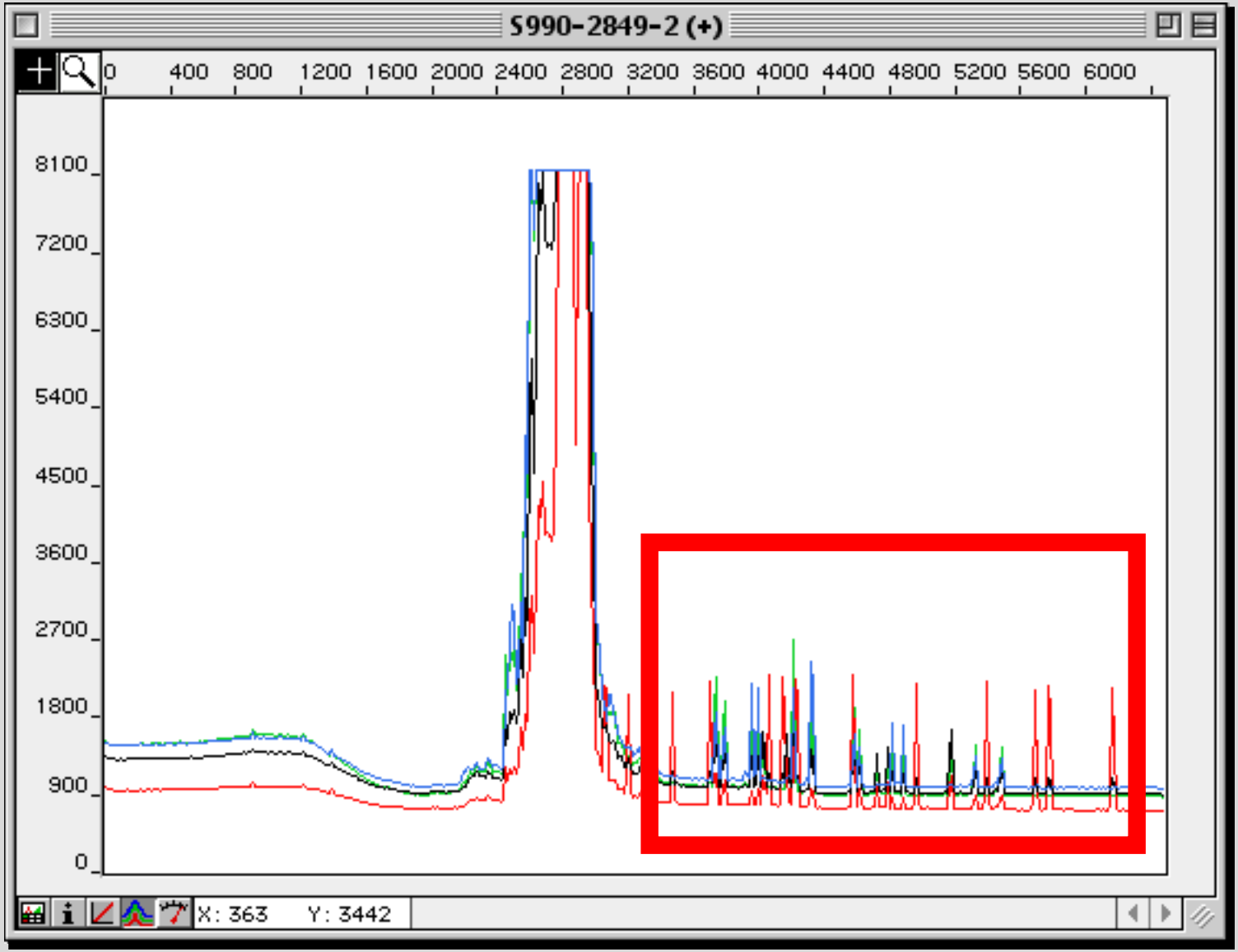


# ABI 310 Genetic Analyzer: Capillary Electrophoresis

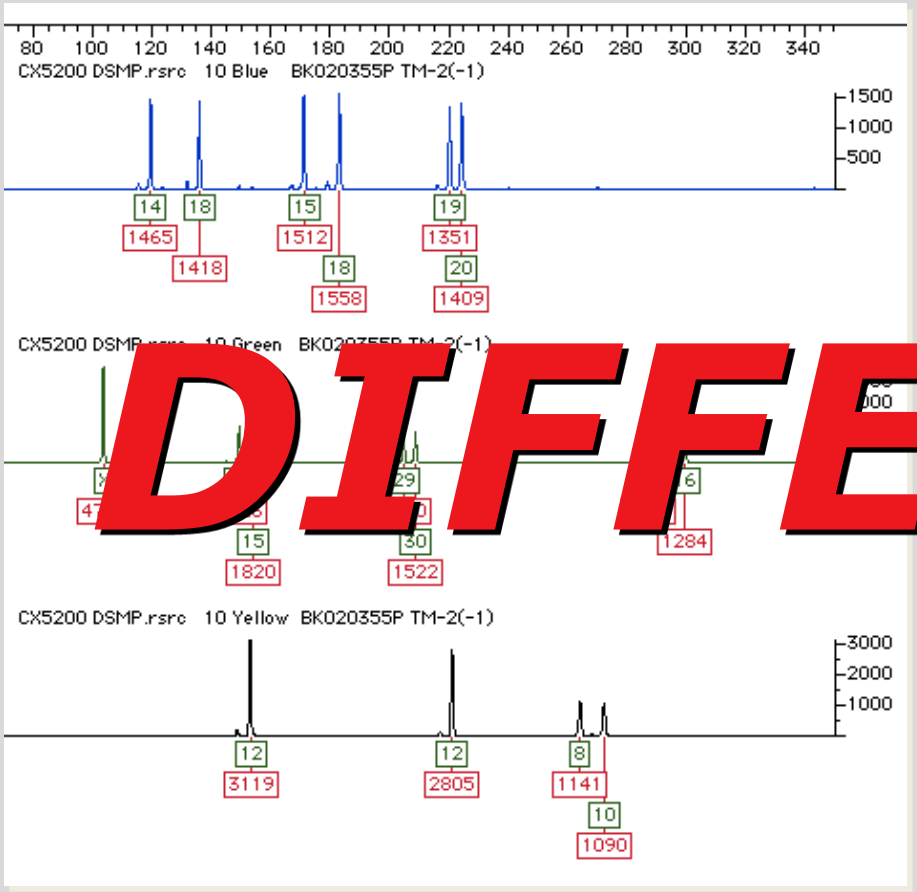
- Amplified STR DNA injected onto column
- Electric current applied
- DNA pulled towards the positive electrode
- DNA separated out by size:
  - Large STRs travel slower
  - Small STRs travel faster
- Color of STR detected and recorded as it passes the detector

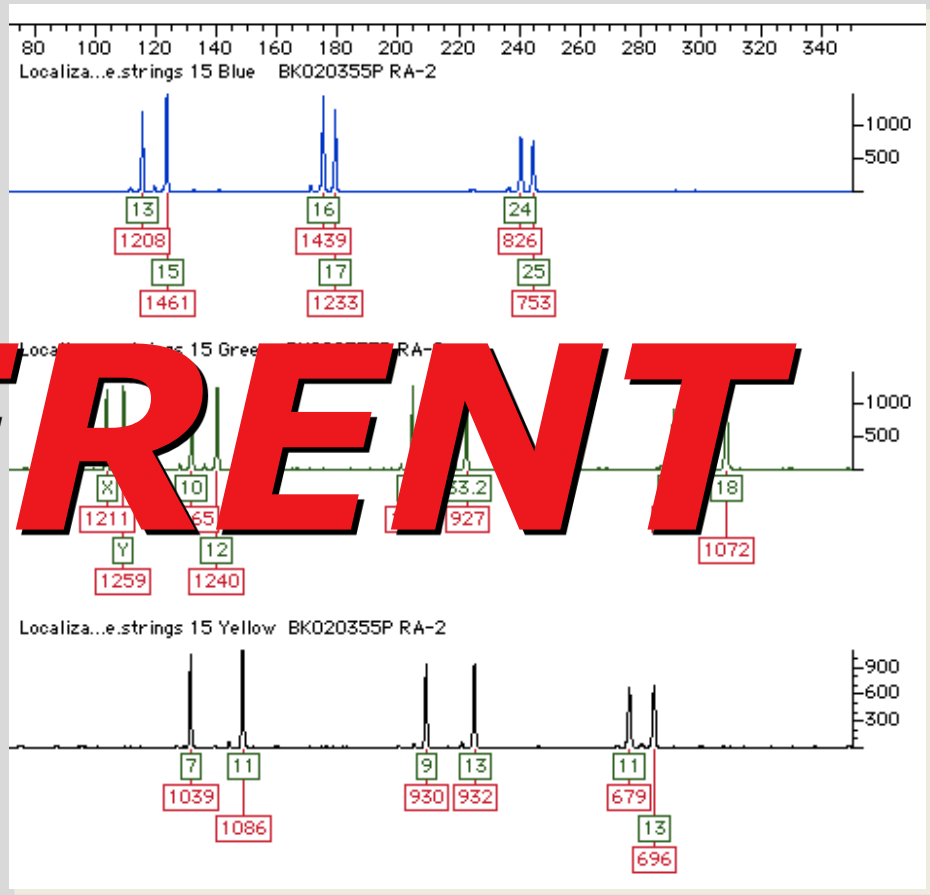


# Profiler Plus: Raw data



# Comparing electropherograms



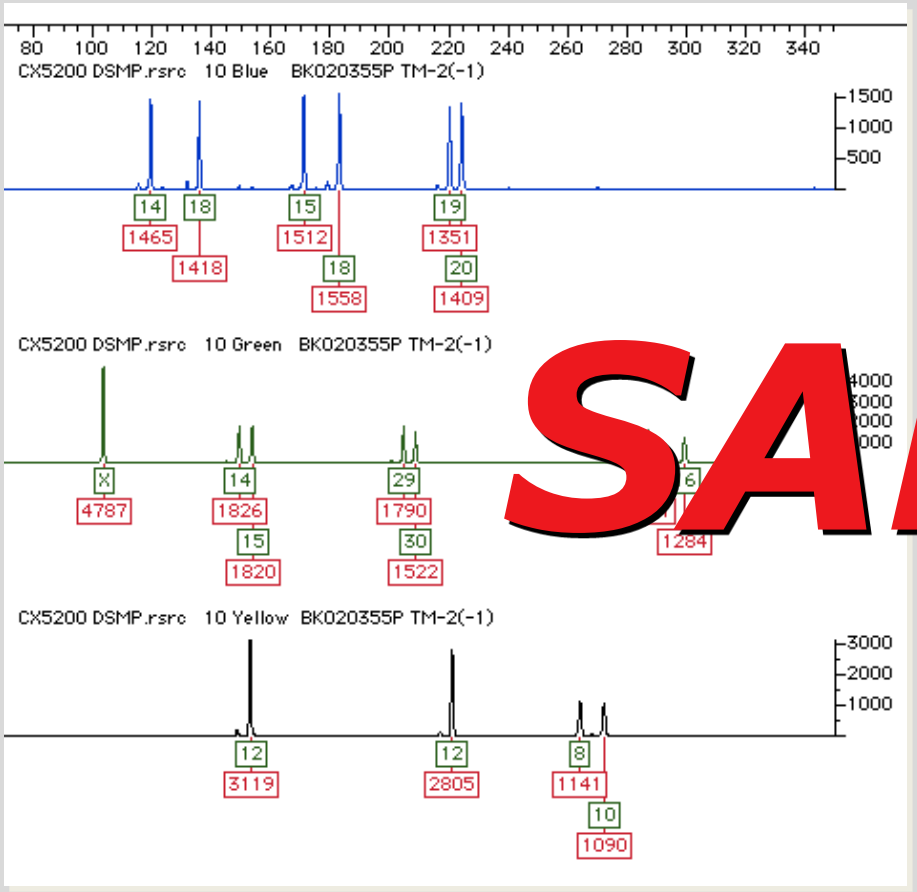



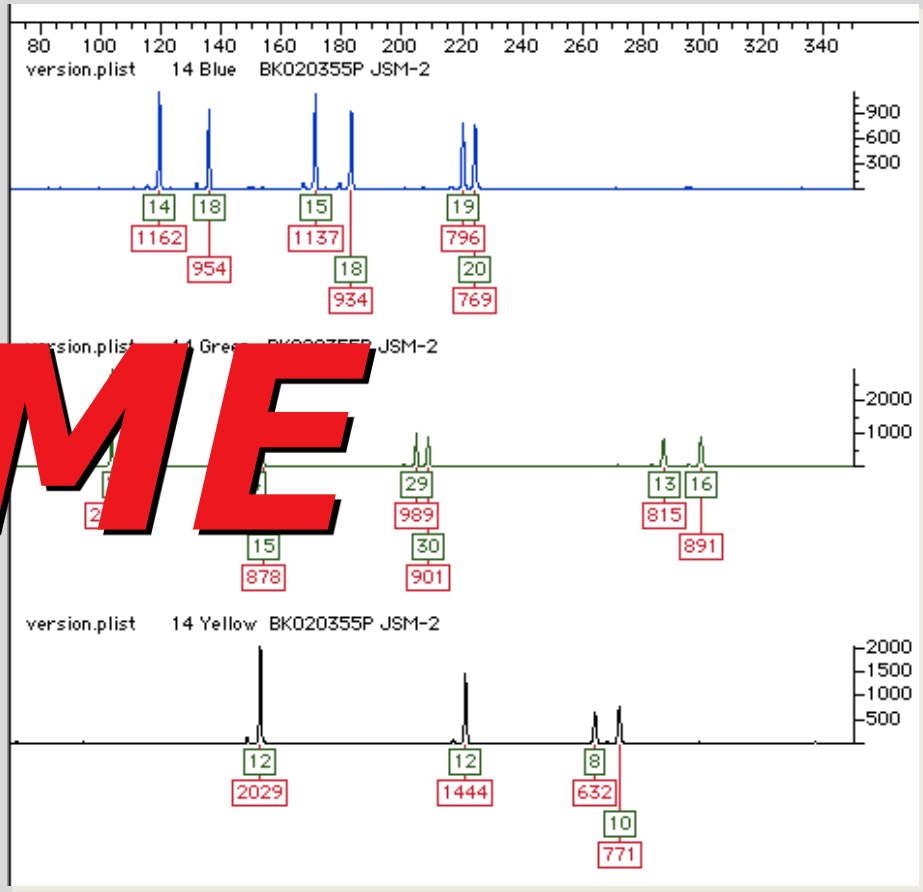

DIFFERENT

**Evidence (Bloodstain)**

**Suspect reference**

# Comparing electropherograms

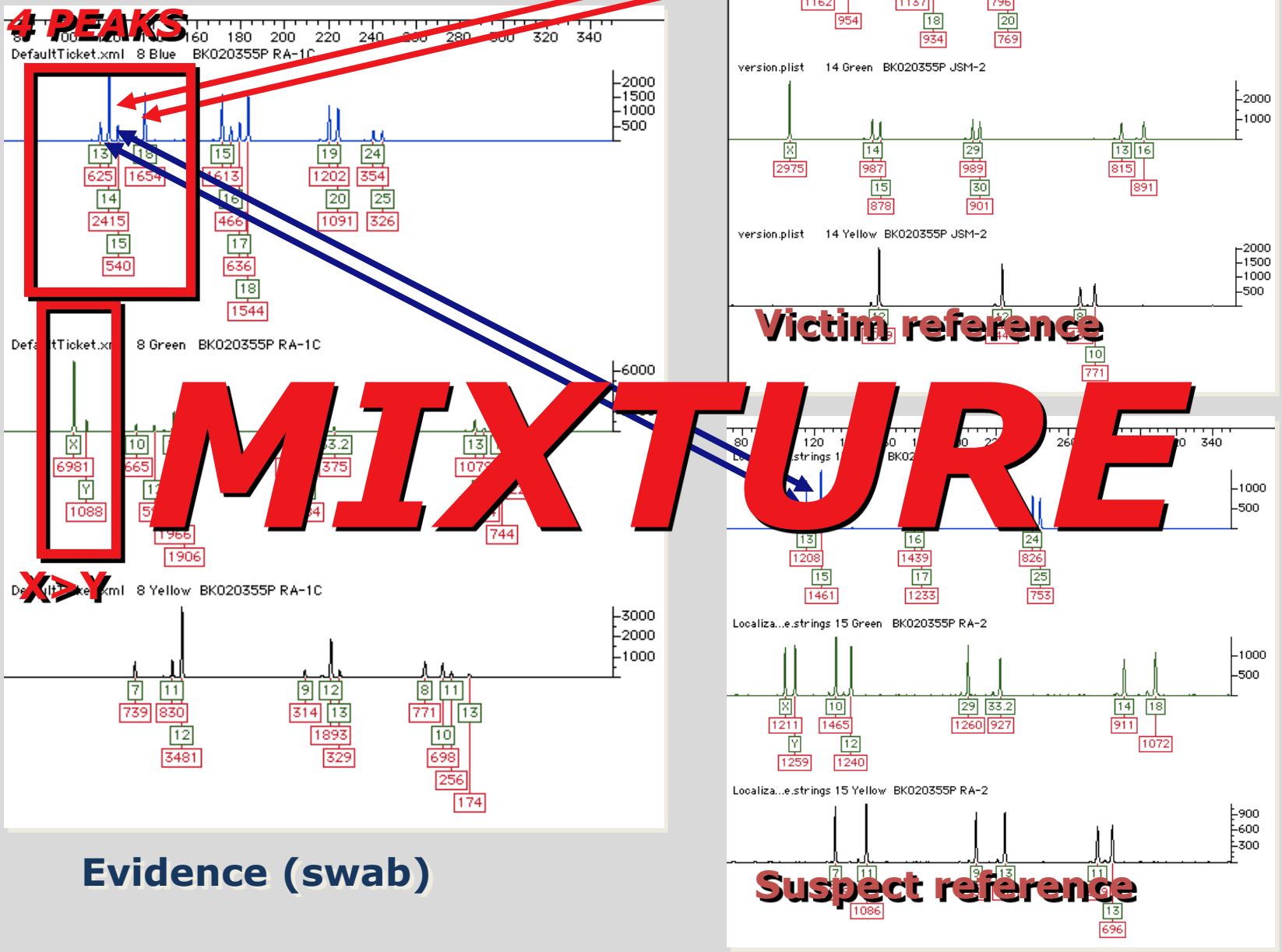



SAME

**Evidence (Bloodstain)**

**Victim reference**


4 PEAKS
MIXTURE
X>Y
Victim reference
Suspect reference
Evidence (swab)
DefaultTicket.xml 8 Blue BK020355P RA-1C
DefaultTicket.xml 8 Green BK020355P RA-1C
DefaultTicket.xml 8 Yellow BK020355P RA-1C
version.plist 14 Blue BK020355P JSM-2
version.plist 14 Green BK020355P JSM-2
version.plist 14 Yellow BK020355P JSM-2
Localiza...e.strings 15 Green BK020355P RA-2
Localiza...e.strings 15 Yellow BK020355P RA-2

# Statistical estimates: the product rule

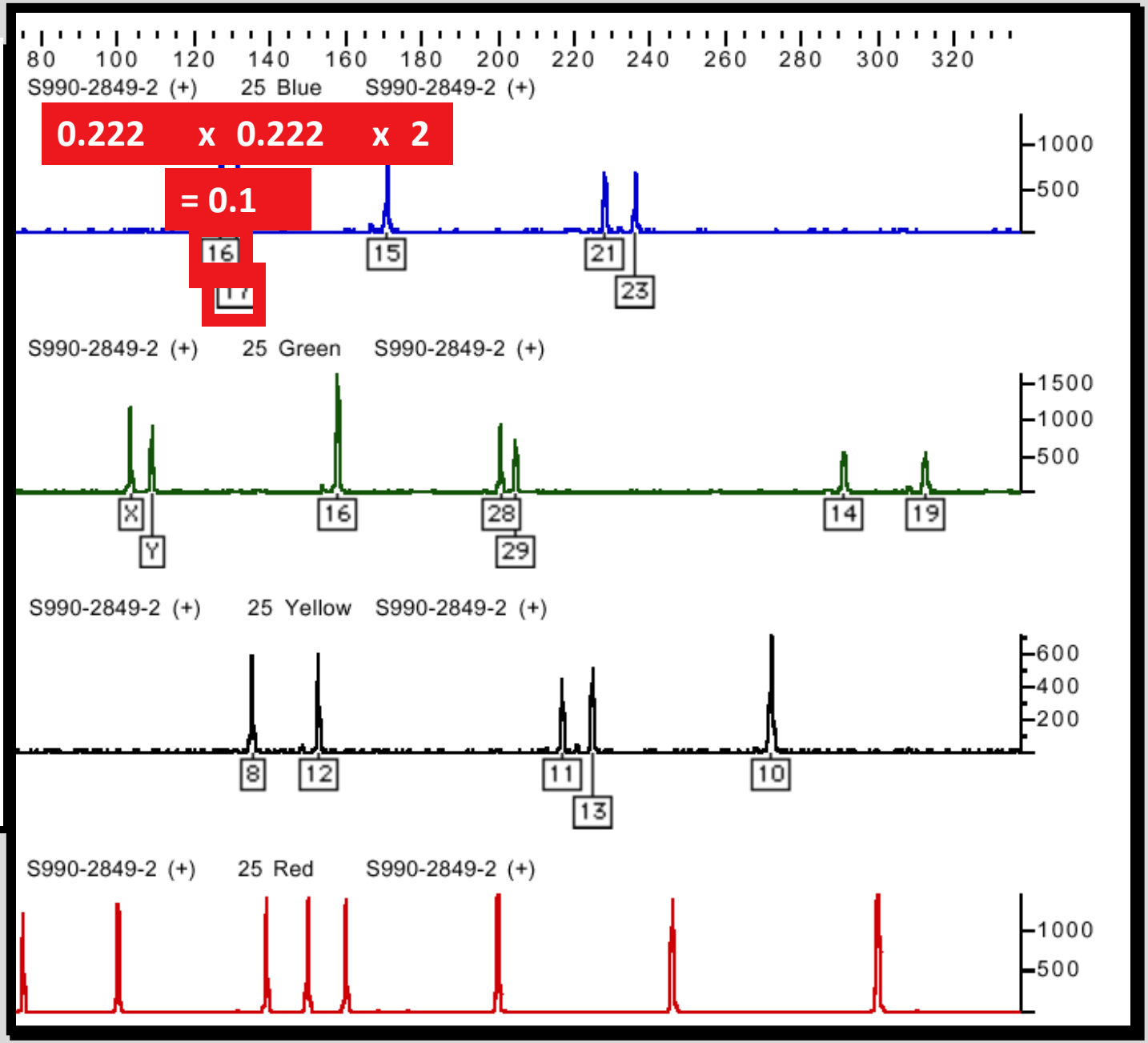
**Allele Frequencies**

Locus D3S1358
Race Caucasian
(N = 203)

| Allele | Frequency |
|---|---|
| 12 | 0.012 |
| 13 | 0.012 |
| 14 | 0.140 |
| [redacted] | |
| 19 | 0.012 |

Locus vWA
Race Caucasian
(N = 196)

| Allele | Frequency |
|---|---|
| 11 | 0.012 |
| 12 | 0.012 |
| 13 | 0.012 |
| 14 | 0.102 |
| 15 | 0.082 |



# Statistical estimates: the product rule

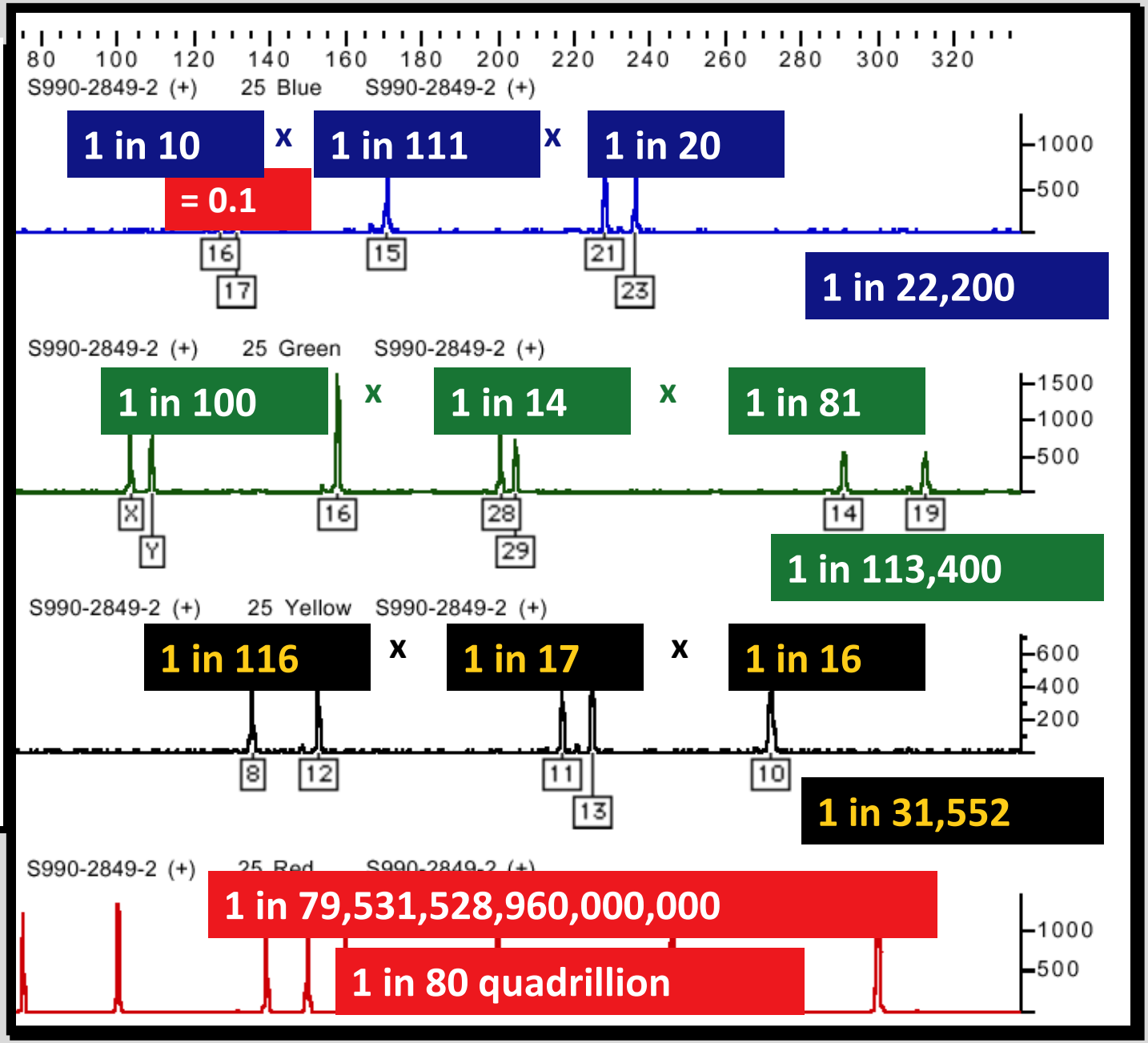
**Allele Frequencies**

Locus D3S1358
Race Caucasian
(N = 203)

| Allele | Frequency |
|---|---|
| 12 | 0.012 |
| 13 | 0.012 |
| 14 | 0.140 |
| 15 | 0.246 |
| 16 | 0.222 |
| 17 | 0.222 |
| 18 | 0.163 |
| 19 | 0.012 |

Locus vWA
Race Caucasian
(N = 196)

| Allele | Frequency |
|---|---|
| 11 | 0.012 |
| 12 | 0.012 |
| 13 | 0.012 |
| 14 | 0.102 |
| 15 | 0.082 |



What more is there to say after you have said: “The chance of a coincidental match is one in 80 quadrillion?”

What more is there to say after you have said: “The chance of a coincidental match is one in 80 quadrillion?”

- Two samples really do have the same source
- Samples match coincidentally
- An error has occurred

# DNA match probability

- Random Match Probability (RMP)

- What is the chance of finding a random, unrelated person in a given population that has a given DNA profile?

- **NOT** the probability that the defendant is guilty

- **NOT** the probability that someone other than the defendant committed the crime

# Quantities of DNA

- Optimum amount of template for PCR: 0.5 to 2.0 ng
- 6 to 7 pg of DNA in each diploid human cell
- Our bodies are made of many billions if not trillions of cells
- pg = picogram (milligram, microgram, nanogram, picogram)
- SGM+ and Profiler Plus test kits are *designed* to fail with less than 100 pg to minimize these problems BUT new kits (SE filer, NGM, etc..) have NO minimal tresholds

# DNA content in different biological samples

| Type of sample | Amount of DNA |
|---|---|
| Blood | 30,000 ng/mL |
| stain 1 $cm^2$ | 200 ng |
| stain 1 $mm^2$ | 2 ng |
| Semen | 250,000 ng/mL |
| Postcoital vaginal swab | 0 - 3,000 ng |
| Hair | |
| plucked | 1 - 750 ng/hair |
| shed | 1 - 12 ng/hair |
| Saliva | 5,000 ng/mL |
| Urine | 1 - 20 ng/mL |

# DNA sampling

Reference samples

Crime scene (unknown) samples

Lynda Mann †1983
Dawn Ashworth †1986

15-years old girls raped and murdered

1st mass screening in the field

Colin Pitchfork

# General requirements for reference DNA sampling

- Easy to use
- Sample well preserved during the transport
- Compatible with current DNA techniques
- Non-invasive
- Non-intimate

Buccal swabs

Leriche A., Vanek D., Schmitter H. at al. (1998) Final report of the INTERPOL European Working Party on DNA Profiling. Proceedings from the Second European Symposium on Human Identification 48-54, Promega Corporation

# Buccal swabs

Sufficient amount of DNA for down-stream DNA identification applications

# General requirements for crime-scene DNA sampling

- Find the stain, document the stain, collect the stain, describe the stain, protect the stain, transport the stain
- Strict counter cross-contamination procedures MUST be in place
  - Protecting both the sample AND the CS investigator

**What is wrong?**


BIOHAZARD


Eye protection
must be worn

# General requirements for crime-scene DNA sampling

- ISO 17020 accreditation (optional)
- Clear written guidelines: what to swab, how to swab, what to collect, how to protect – *„Sampling for Dummies“*
- Proper chain of custody in place
- Changing gloves between different stains is a MUST
- **STERILE ≠ Human DNA free**

# General requirements for crime-scene DNA sampling - SWABS

- Easy use, efficient sampling
- Sample well preserved during the transport
- Security during the transport
- Compatible with current forensic genetics techniques
- Maximum DNA recovery
- **Human DNA-free, PCR inhibitor-free, DNase-free**
- **ISO 18385**