What is today's objective?

Day 2: The DNA FP

1. EXTRACTION

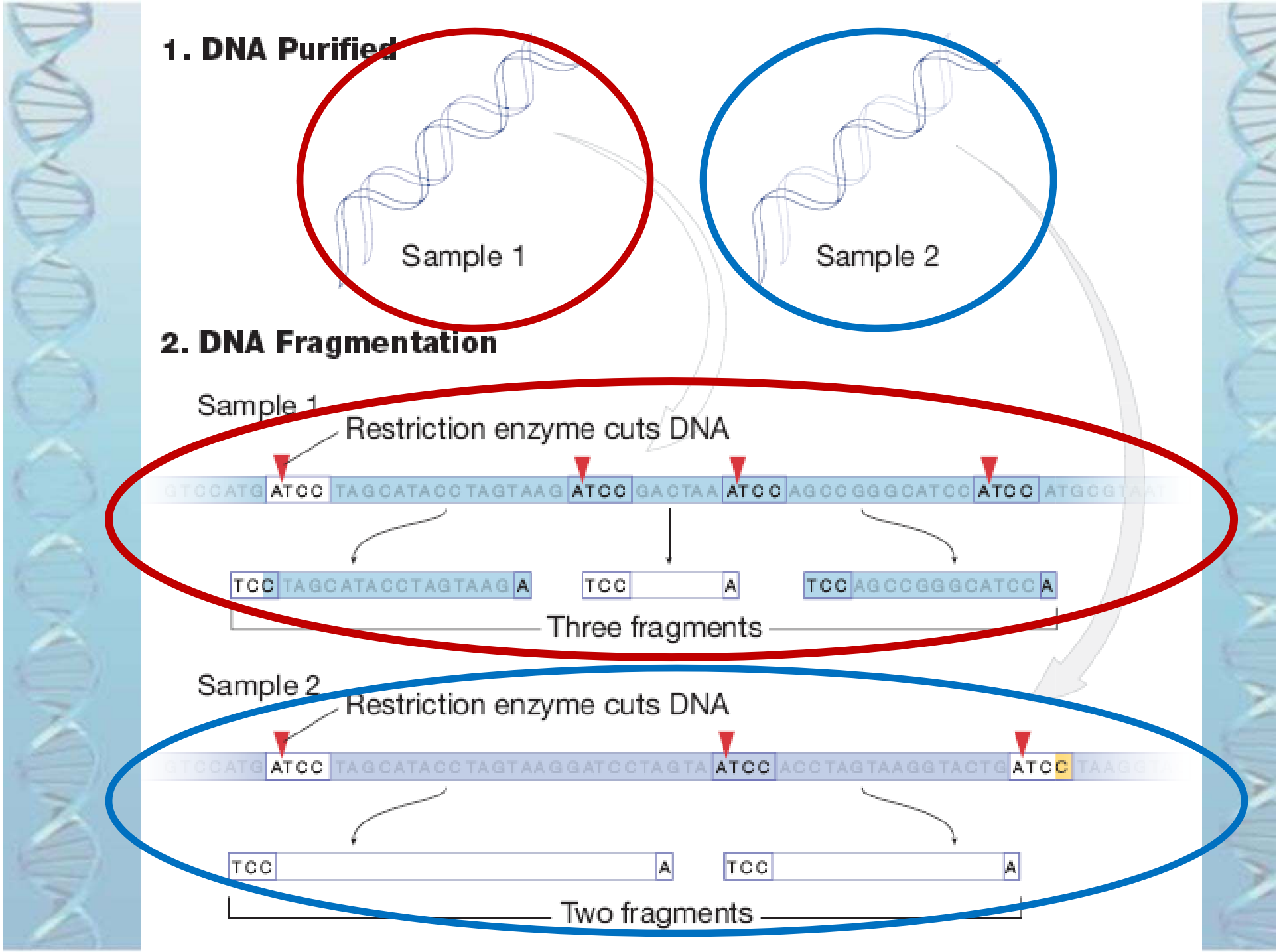
Cells are isolated from tissue

Disrupt cells to release the DNA

http://learn.genetics.utah.edu/content/labs/extraction/

2. Restriction Fragments

- **Restriction Enzymes (REs)** are 'molecular scissors' that cut DNA @ **SPECIFIC** base sequences
  - *There are many different types of REs*
  - *ex. HindIII looks for the sequence* AACGTT *and cuts between the 2 As --* A / ACGTT
- When REs cut DNA into pieces, it makes **fragments** of **many different lengths**
  - *VNTRs may be in these fragments*
- You can use ≥ 1 type of RE on a sample


1. DNA Purified
Sample 1
Sample 2
2. DNA Fragmentation
Sample 1
Restriction enzyme cuts DNA
ATCC
TAGCATACCTAGTAAG
ATCC
GACTAA
ATCC
AGCCGGGCATCC
ATCC
TCC
TAGCATACCTAGTAAG
A
TCC
A
TCC
AGCCGGGCATCC
A
Three fragments
Sample 2
Restriction enzyme cuts DNA
ATCC
TAGCATACCTAGTAAGGATCCTAGTA
ATCC
ACCTAGTAAGGTACTG
ATCC
TCC
A
TCC
A
Two fragments

1. Section of victim's DNA:

There are three regions of repetitive DNA.

2. Section of suspect's DNA:

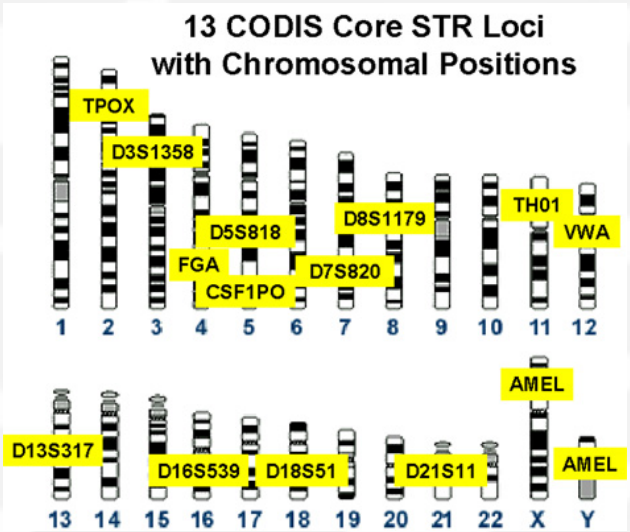
The same three regions of repetitive DNA are present here, but some include different numbers of repeats. Now let's compare this sample to...

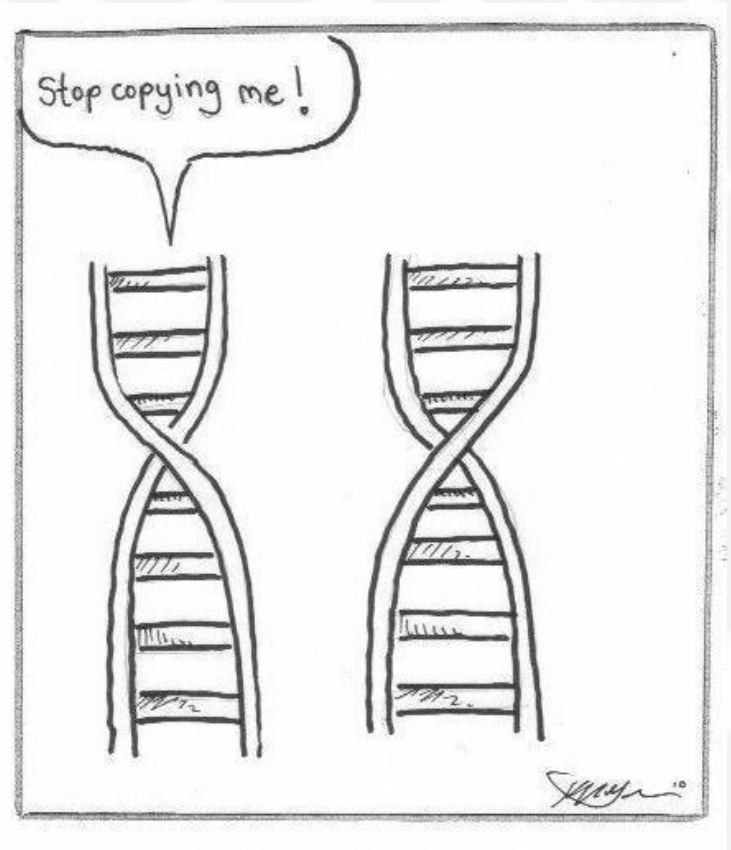
3. Section of DNA from crime scene hair:

The lengths of the repetitive sequences match the lengths in the suspect's DNA — so the DNA found at the crime scene belongs to the suspect.

3. AMPLIFICATION

- Use **PCR** to amplify certain pieces of DNA that contain VNTRs or STRs



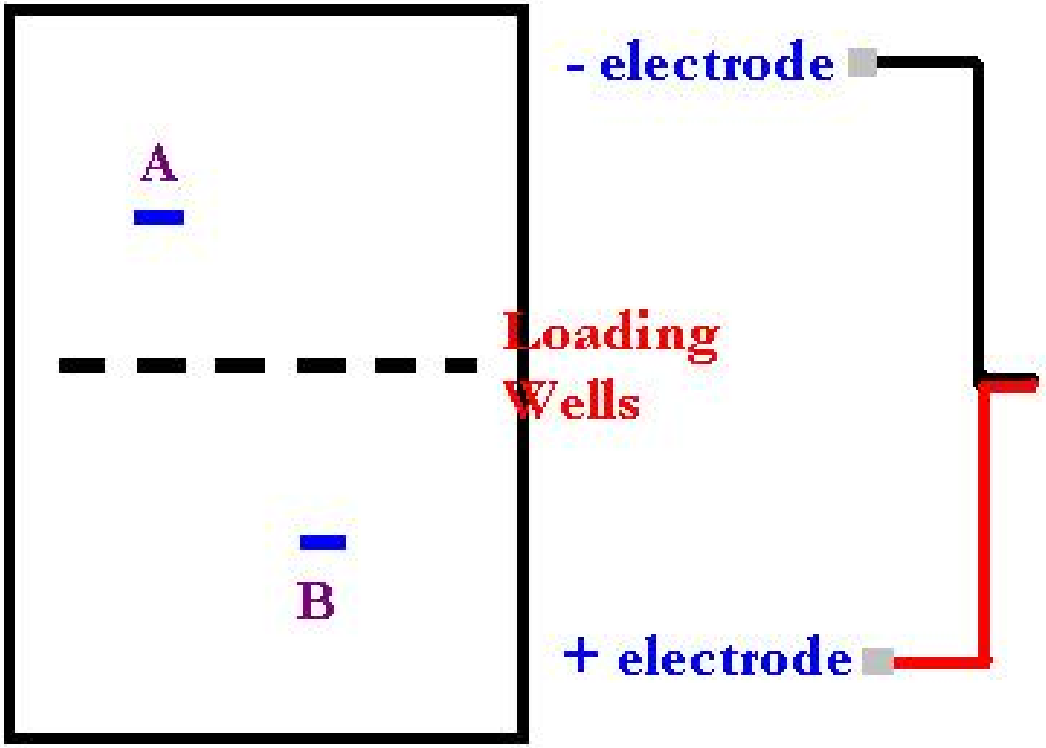


4. ELECTROPHORESIS

- Separates molecules electrically by

  charge, size, and shape

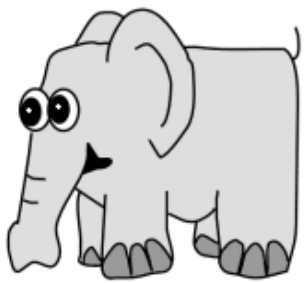
- DNA has a **negative charge**

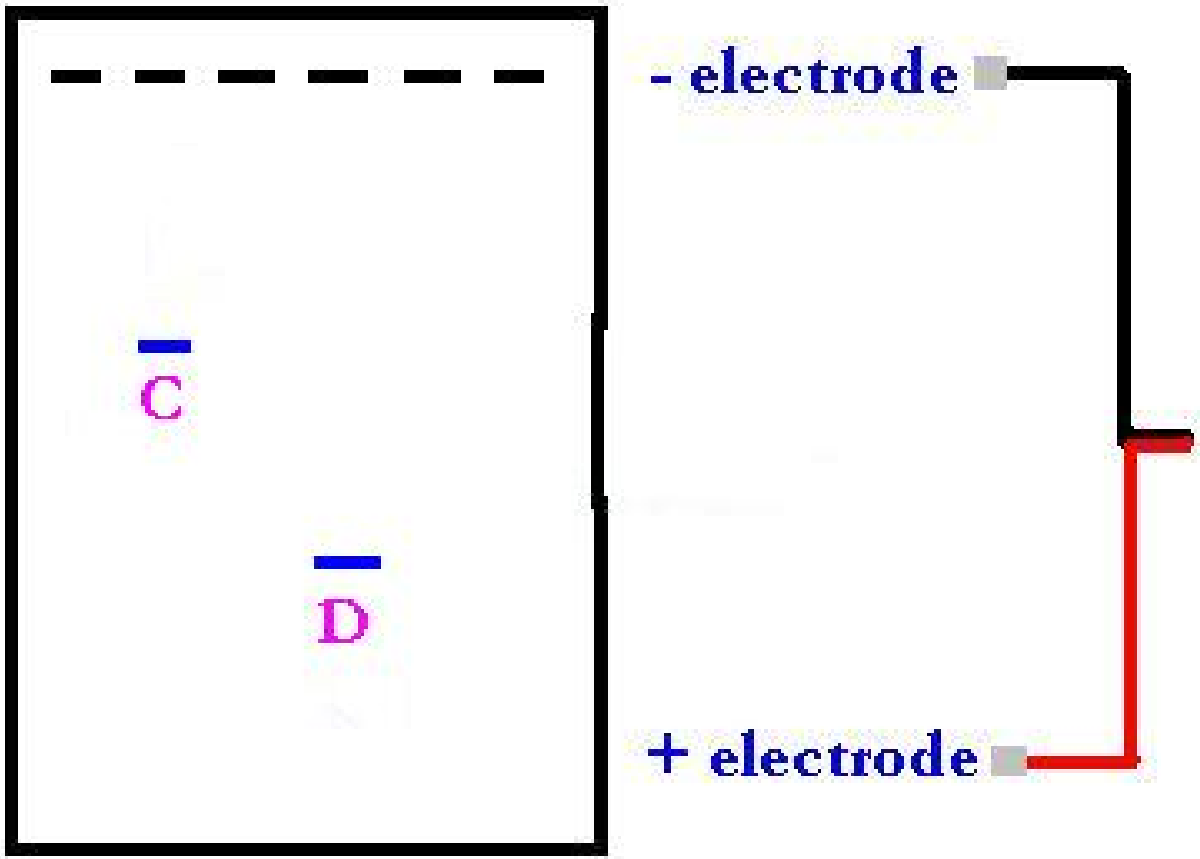


Size of molecule is next factor

SMALLER moves fastest

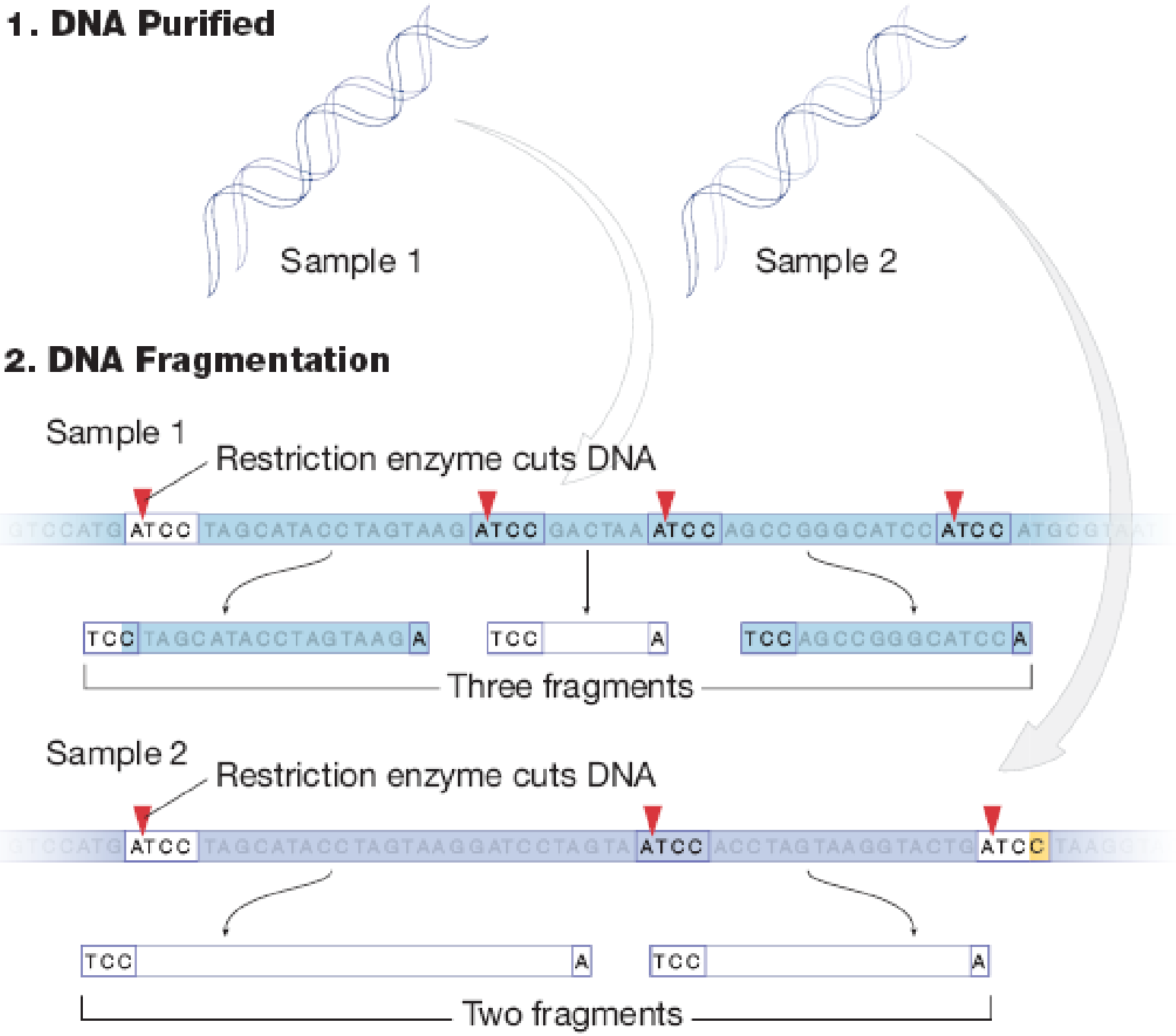
BIGGER moves slower

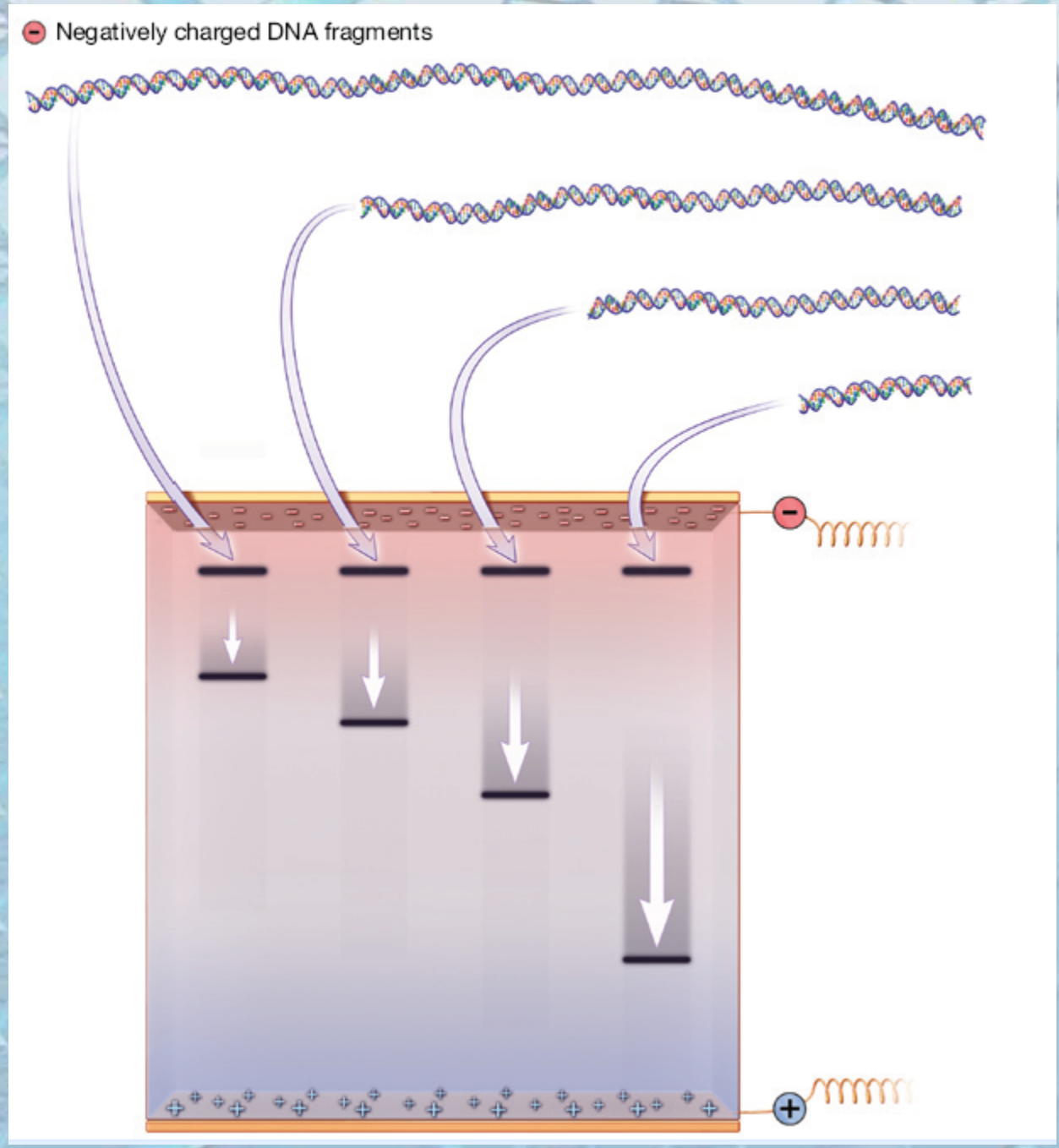


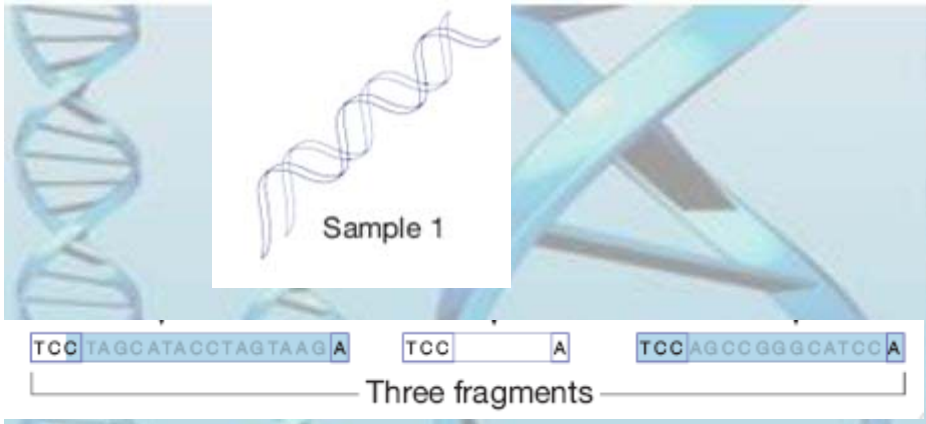


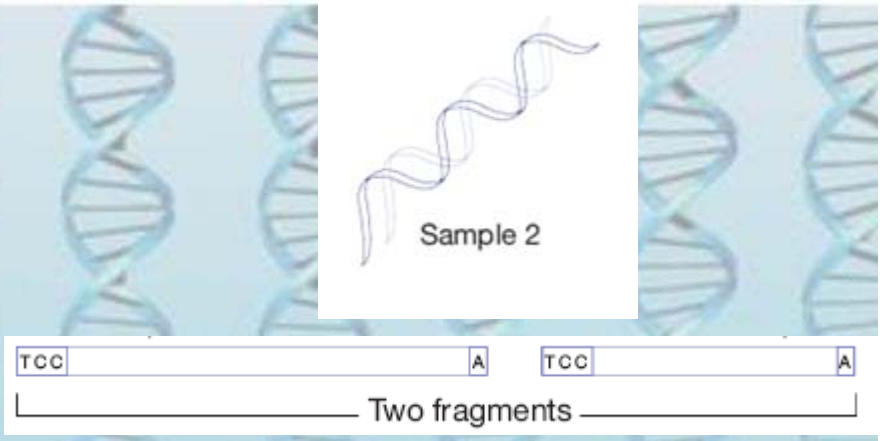
STEPS OF DNA FPING

1. **Extraction**: [illegible]s extracted from the cell
2. **Restriction Fragments**: C[illegible] with **restriction enzymes**
3. **Amplification**: us[illegible]R to make copies
4. **Electrophoresis**: DNA [illegible]ed onto a gel and separated by size
5. **Making it Permanent**

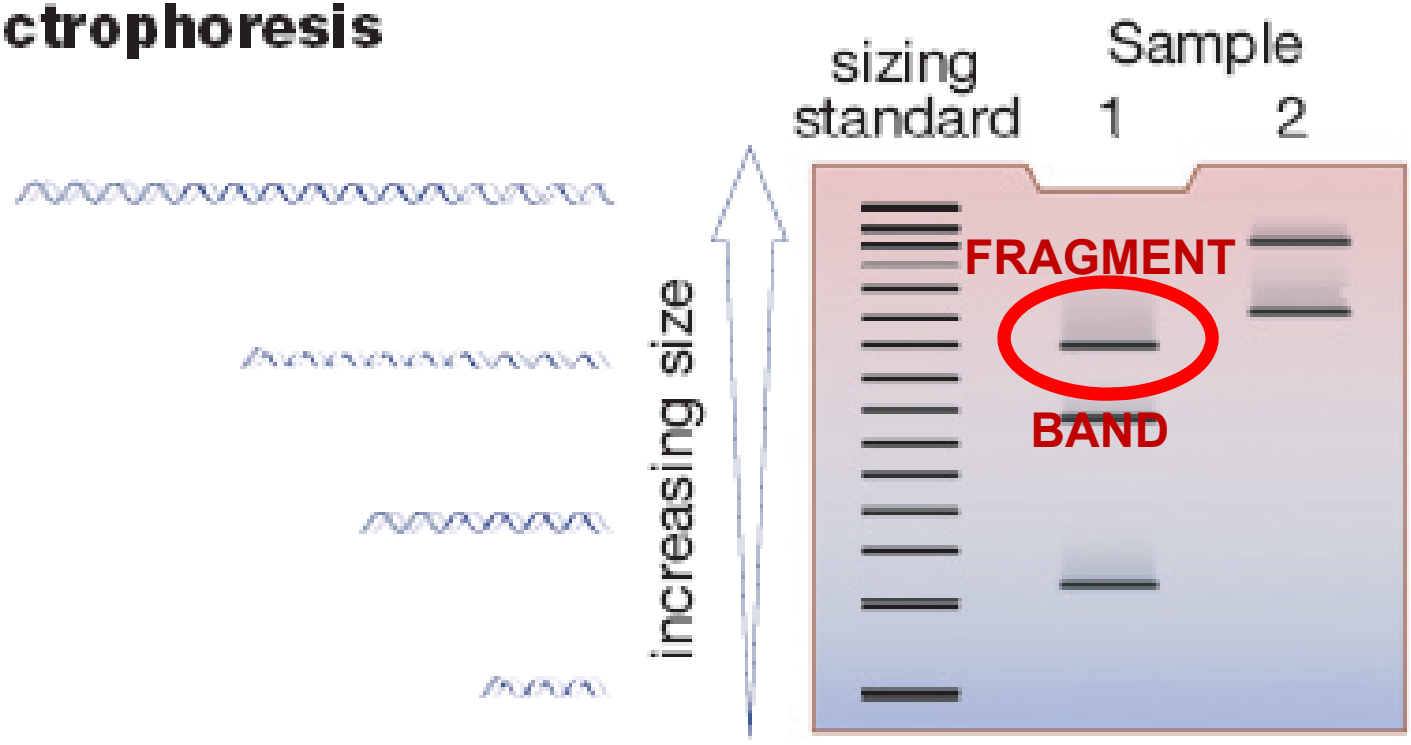

1. DNA Purified
Sample 1
Sample 2
2. DNA Fragmentation
Sample 1
Restriction enzyme cuts DNA
ATCC
ATCC
ATCC
ATCC
TCC
A
TCC
A
TCC
A
Three fragments
Sample 2
Restriction enzyme cuts DNA
ATCC
ATCC
ATCC
TCC
A
TCC
A
Two fragments

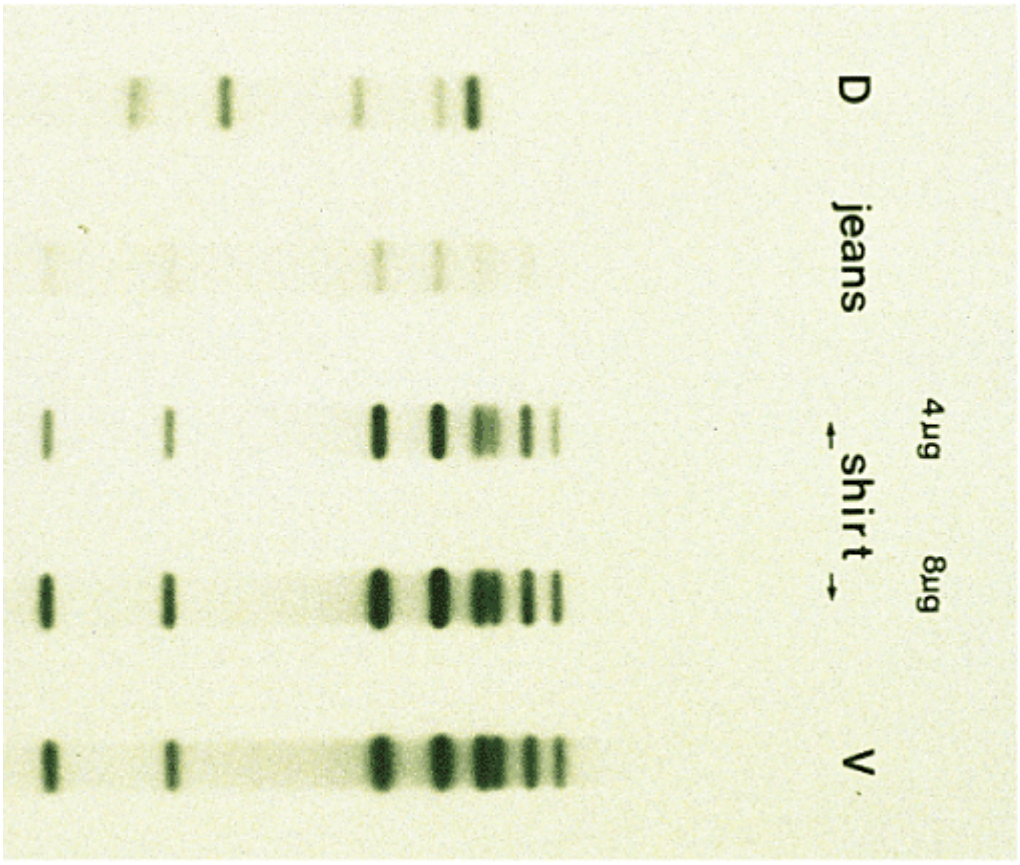

Negatively charged DNA fragments





Gel Electrophoresis





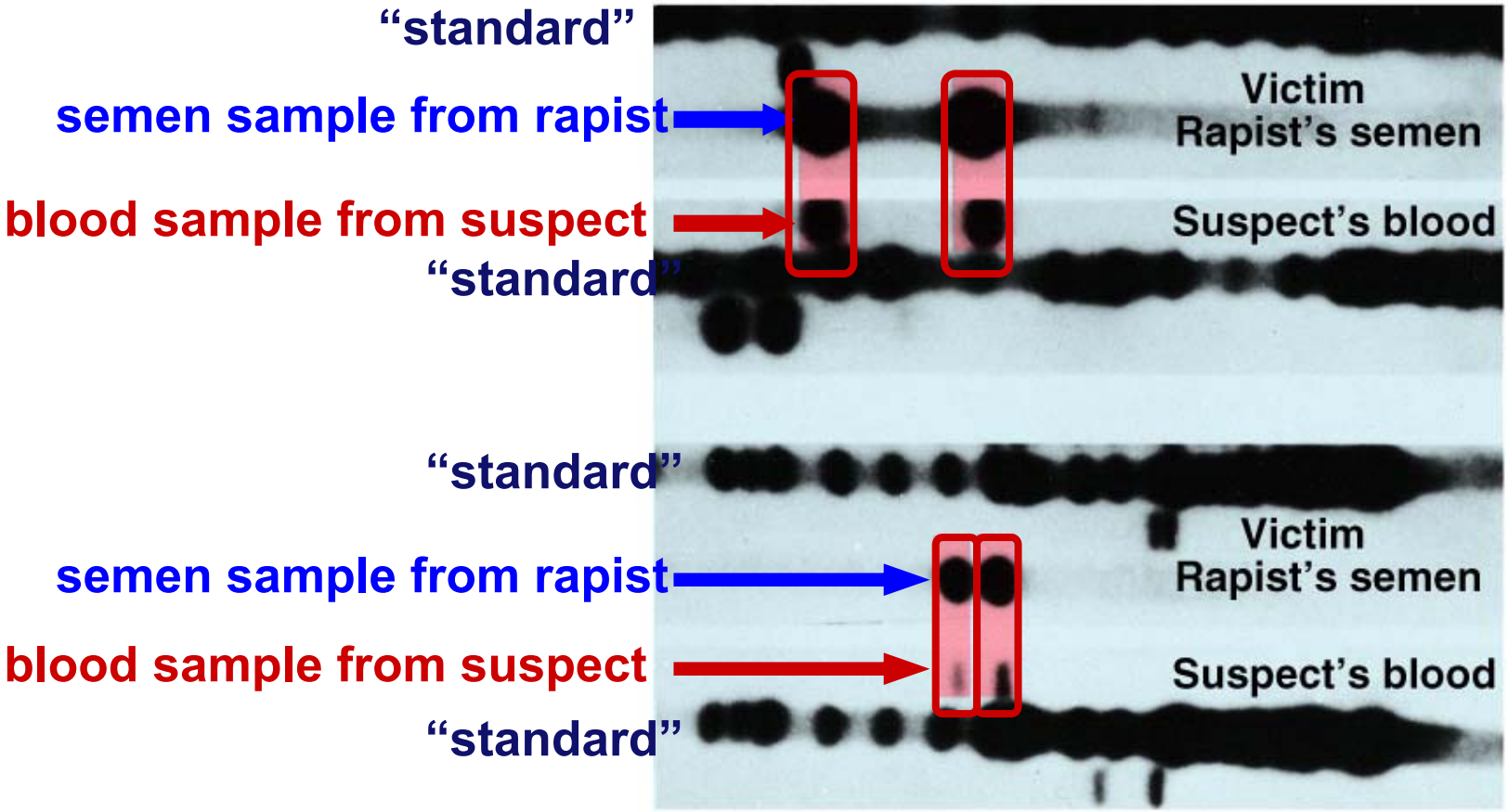
Defendant's blood

Blood from defendant's clothes

Victim's blood

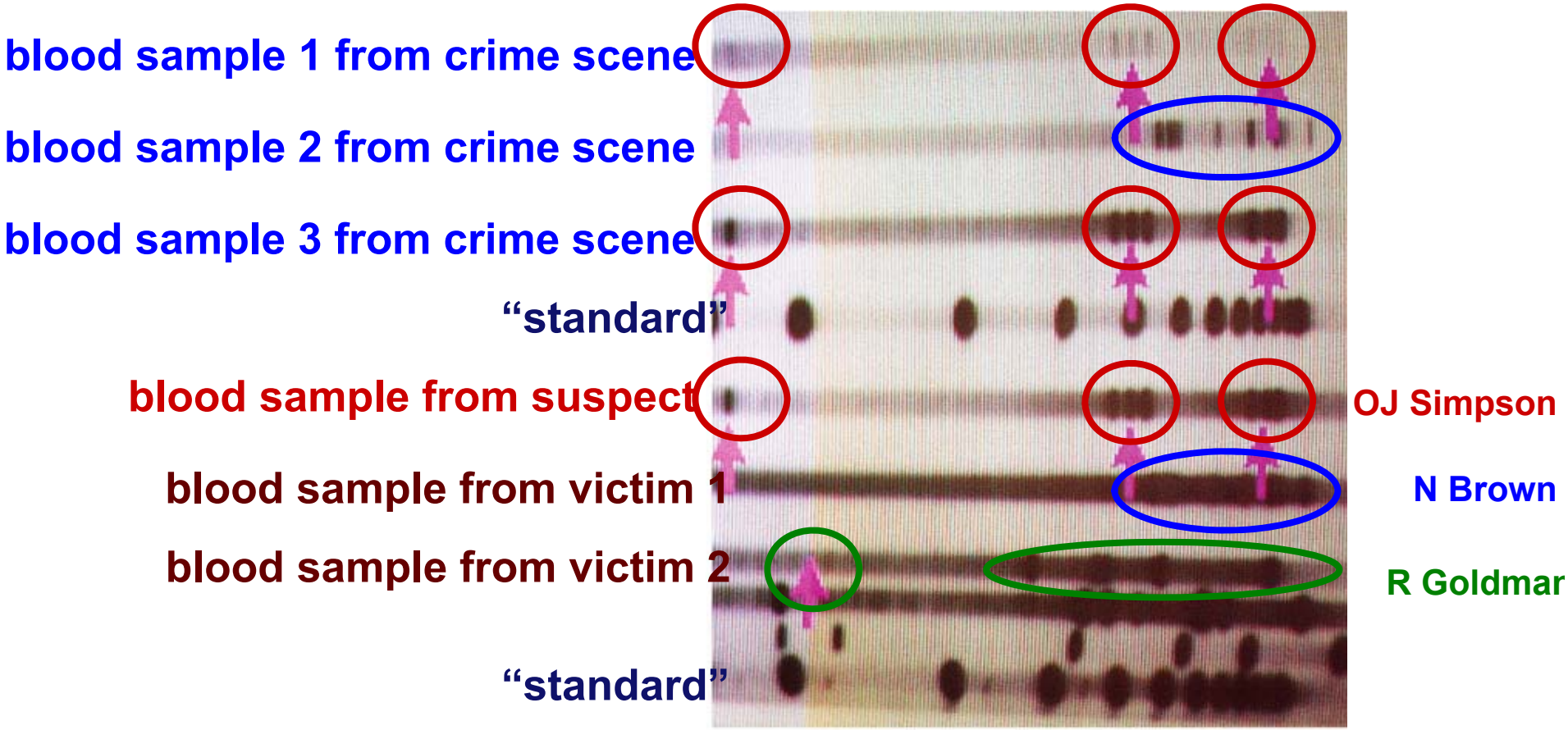
RFLP / electrophoresis use in forensics

- 1st case successfully using DNA evidence
  - 1987 rape case convicting Tommie Lee Andrews
  - Showed that DNA is DNA… no matter where it comes from



Evidence from murder trial

—Do you think suspect is guilty?



From OJ Simpson to Rapid DNA

1995 ➲ NOW

Statistics from DNA could only be calculated in the millions, which is less than the population of the earth, and could not be considered a definitive match.

DNA statistics can be cast in high trillions or septillions or beyond, making a match with 100% certainty.

OJ Simpson tried on the infamous leather gloves in an attempt to prove they were not his.

Evidence would not be tried on for fit, ever. Instead, it would be swabbed for DNA to prove the suspect had worn it.

Technology could not rule out speculation that the DNA belonged to a relative of OJ Simpson, including his son.

Autosomal and Mitochondrial DNA methods can determine if DNA belongs to a suspect or a relative with a definitive answer.

DNA mixtures could not be properly separated into the individual contributors.

Mixture deconvolution programs can decipher profiles of three, four or possibly five individuals.

The average juror did not know anything about DNA, much less the analysis process, leaving them confused by the testimony.

The use of DNA for criminal cases is widely known and almost expected by a majority of jurors who have an understanding and trust in DNA results.

DNA processing typically took weeks or even months to complete, depending on the workload of the laboratory.

Rapid DNA instruments can process up to seven samples in less than 2 hours without the need for traditional laboratory-based analysis.

5. MAKING IT PERMANENT

A) TRANSFERRING THE FRAGMENTS TO A NYLON MEMBRANE

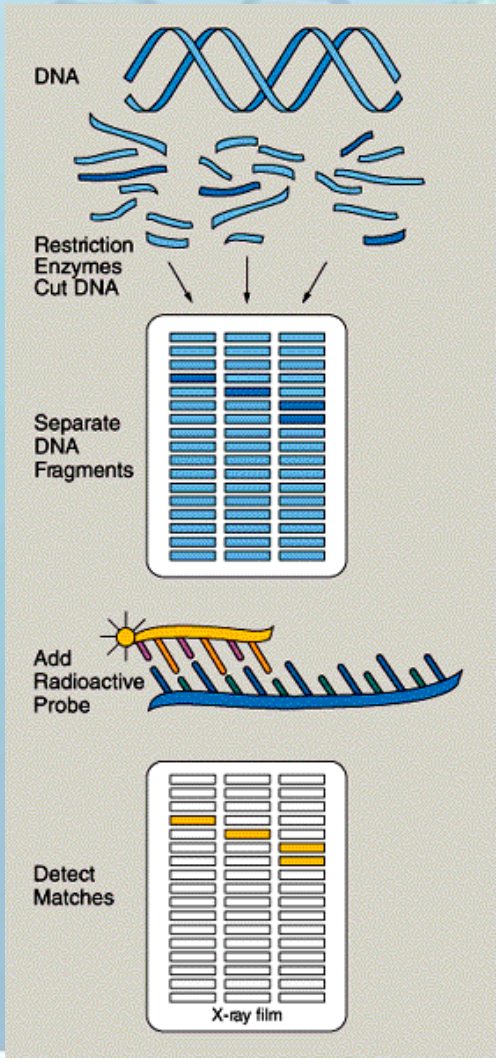
Southern Blot: tech puts a sturdy nylon membrane on top of gel to make it easier to handle.

The DNA bands on gel transfer to nylon

5. MAKING IT PERMANENT

B) TAGGING THE FRAGMENTS WITH A DNA PROBE

The bands of DNA are made visible using probes (aka radioisotopes), **radioactive element chemicals** that attach to the **specific** segment w/in each band of DNA on the nylon membrane

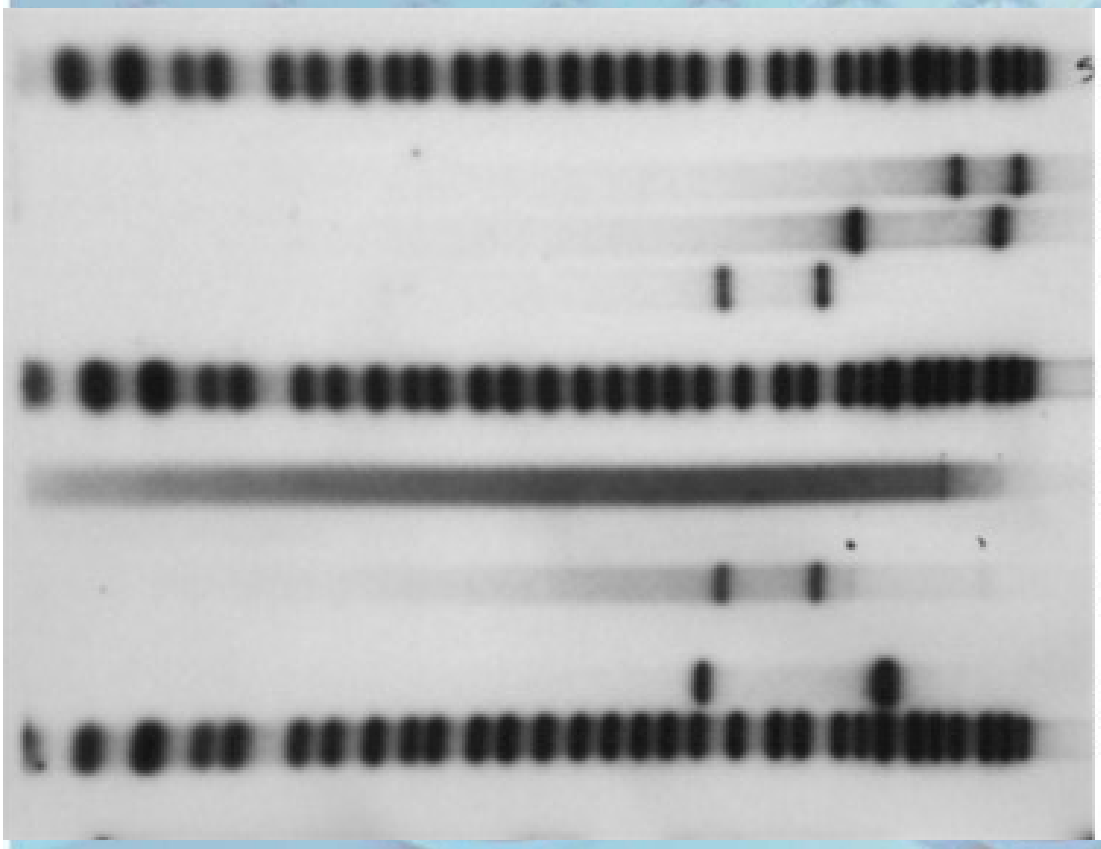


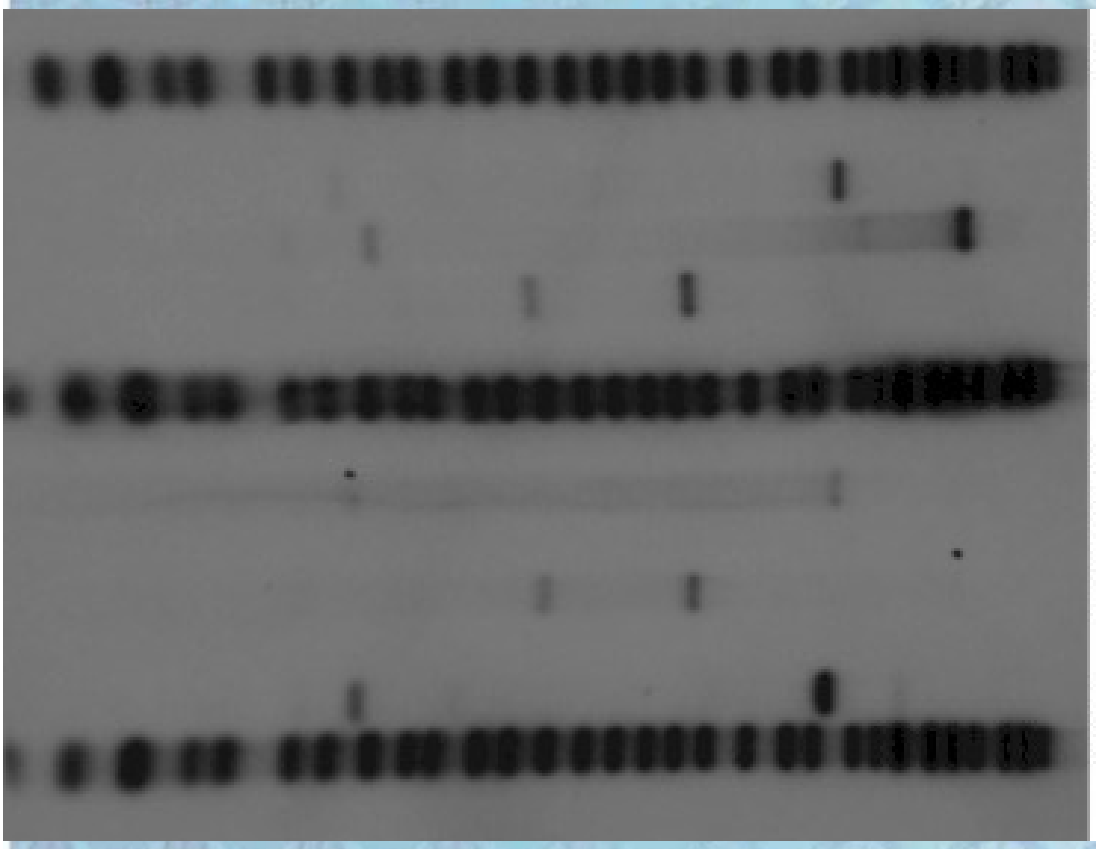
5. MAKING IT PERMANENT

C) VISUALIZING THE FRAGMENTS THROUGH AUTORADIOGRAPHY

- Tech places nylon between 2 sheets of x-ray film in order to make an **autoradiograph** (***autorad***).

The radioisotope-tagged DNA bands on nylon are exposed to the film, making a pattern on the film.


SIZE MARKERS
VICTIM
SUSPECT 1
SUSPECT 2
SIZE MARKERS
FEMALE VAGINAL EXTRACT
MALE VAGINAL EXTRACT
CONTROL SAMPLE
SIZE MARKERS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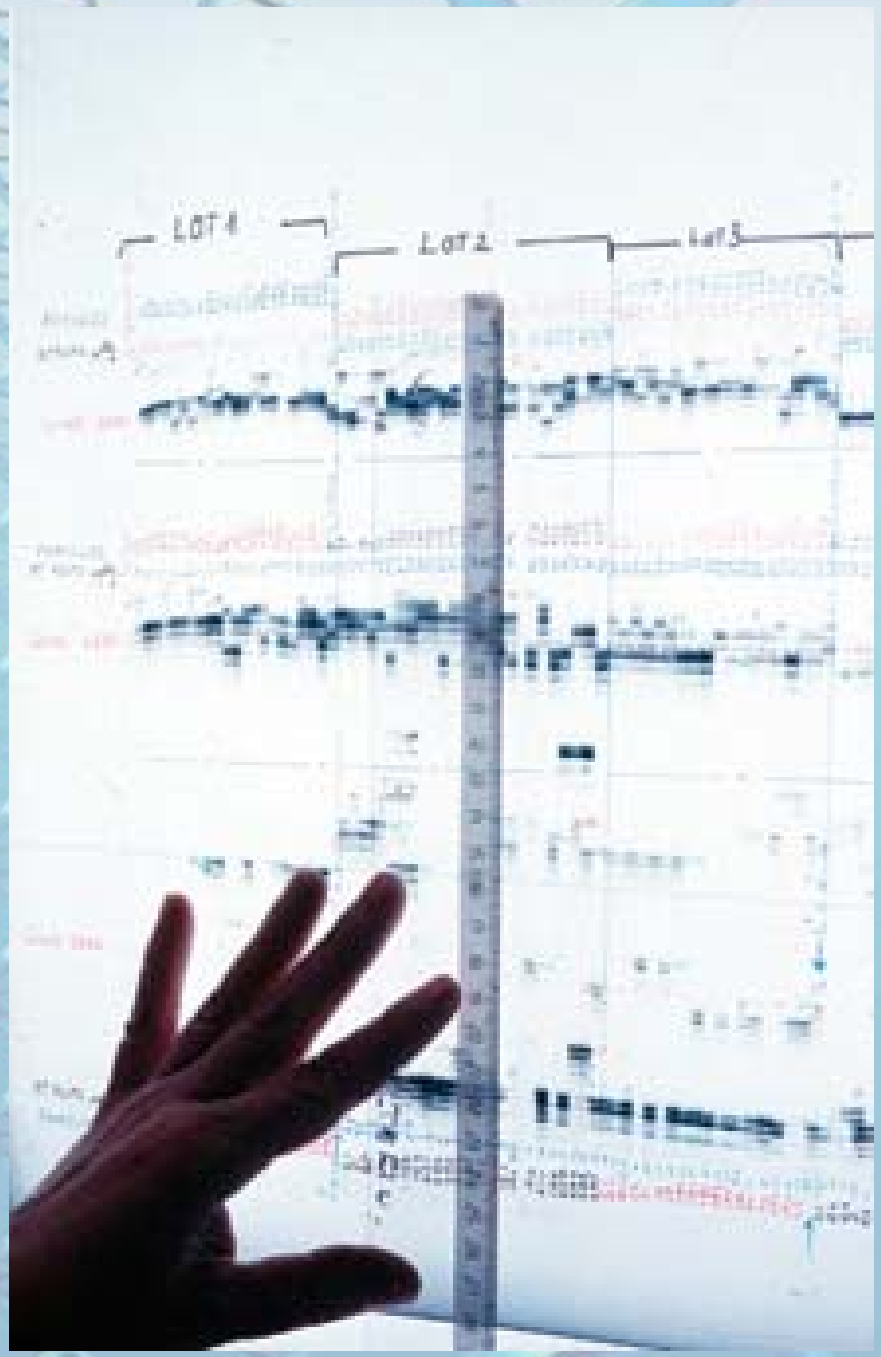

LOT 1
LOT 2
LOT 3

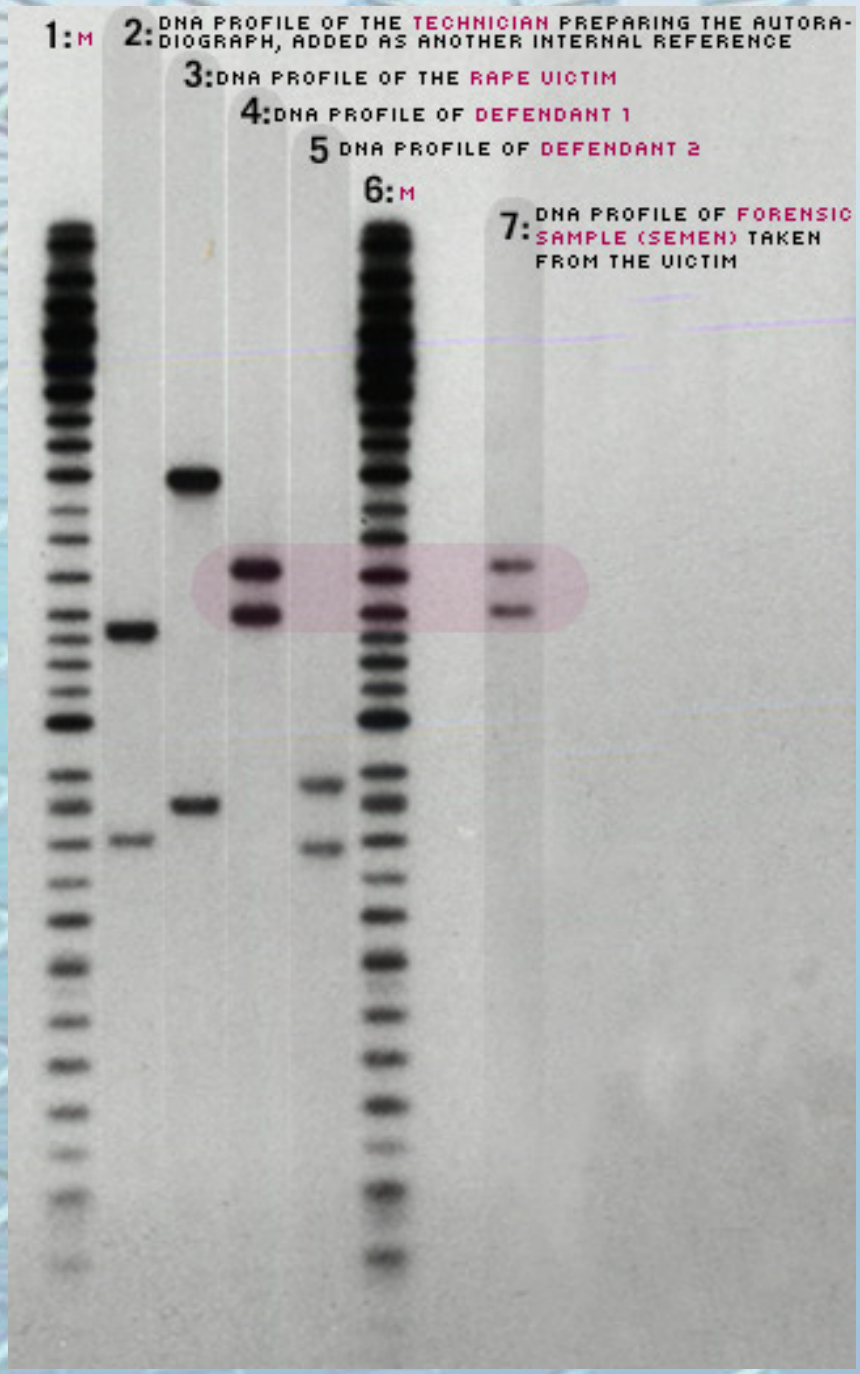
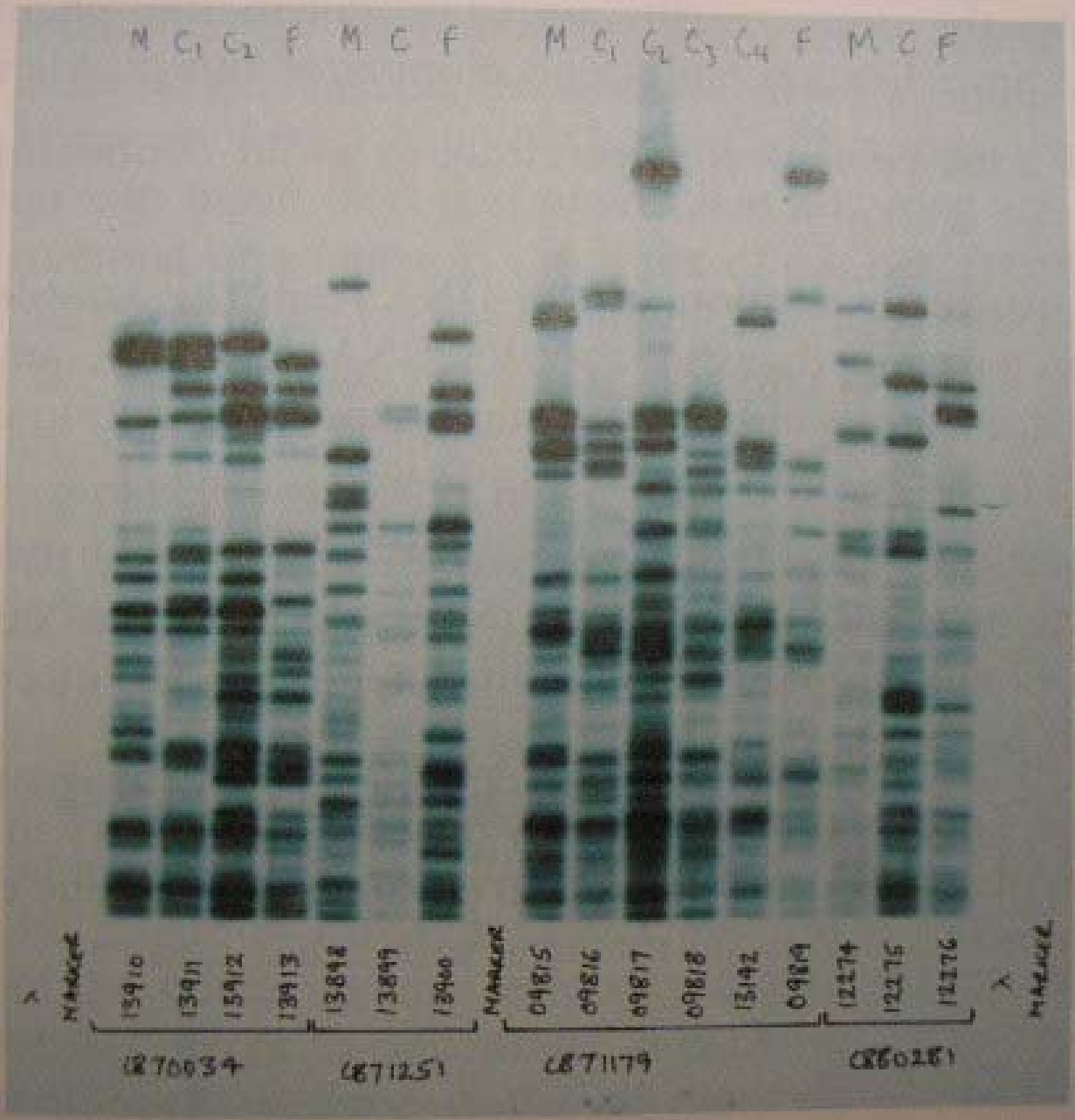

1: M
2: DNA PROFILE OF THE TECHNICIAN PREPARING THE AUTORADIOGRAPH, ADDED AS ANOTHER INTERNAL REFERENCE
3: DNA PROFILE OF THE RAPE VICTIM
4: DNA PROFILE OF DEFENDANT 1
5 DNA PROFILE OF DEFENDANT 2
6: M
7: DNA PROFILE OF FORENSIC SAMPLE (SEMEN) TAKEN FROM THE VICTIM

Figure 7-8. *The results of a DNA fingerprint analysis.*



NDIS: National DNA Index System

- National DNA database done by the FBI
- Developed to enable public forensic labs to create searchable DNA databases of authorized DNA profiles.
- Provides a central database of the DNA profiles from all user labs
- CODIS uses computer programs to search across all databases for a potential match

Since the FBI's National DNA Index System, or NDIS, came online in 1998, forensic labs in the United States have been generating profiles by analyzing a specific set of 13 genetic markers.

Starting January 1, 2017, that number rose to 20, an advance made possible by close collaboration between scientists at the FBI and the National Institute of Standards and Technology (NIST). The additional markers vastly increase the statistical certainty of DNA identifications and allow investigators to identify suspects that could otherwise slip through the cracks.

To meet the new year's deadline, all labs that submit profiles to NDIS had to upgrade their protocols and meet a series of quality assurance standards set by the FBI.
This upgrade was necessary in part due to the rapid growth of the system, which has expanded to include nearly 16 million profiles related to criminal investigations and 30,000 related to missing persons. NDIS now has to add more markers for the same reason a growing city might have to add a new area code. It ensures that everyone can have their own number.

In addition, this upgrade makes international DNA searches more effective by increasing the number of markers that the U.S. system has in common with those of other nations. The number of markers used in both the United States and Europe, for example, will rise from eight to 15.

The new markers will also help solve a problem that often comes up in cases where the DNA has started to break down. In those cases, forensic analysts can't always get a read on all 13 markers, and they end up with a partial profile.

"If you've got a case where seven markers drop out, the statistics may be too weak to establish an identity," said Mike Coble, a research geneticist at NIST. When that happens, a perpetrator might escape the notice of investigators and remain free to commit more crimes.

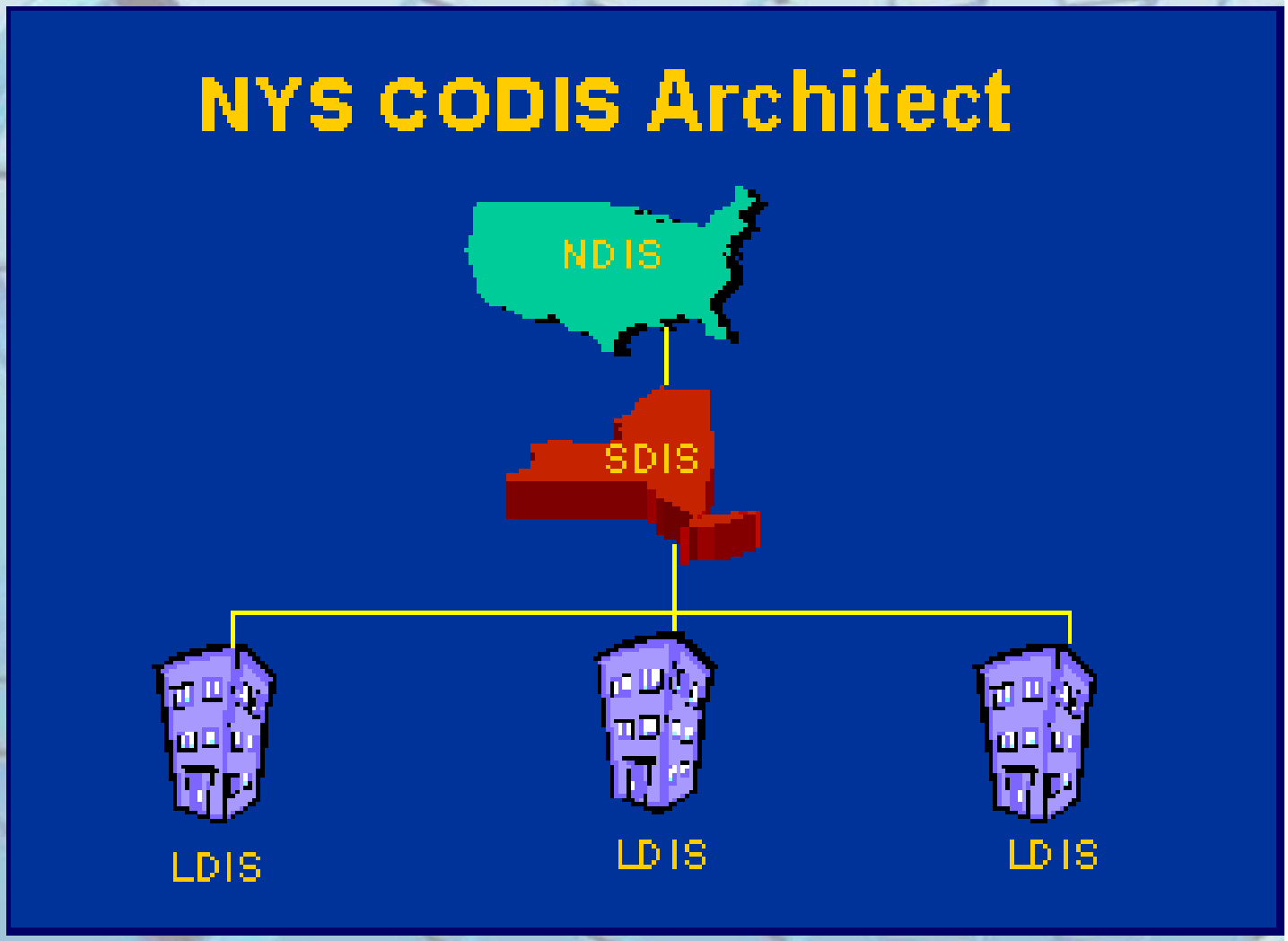
"But if you start with 20 markers, seven can drop out and you'll still have what's considered a full profile today," Coble said.

NATIONAL

DNA Index System

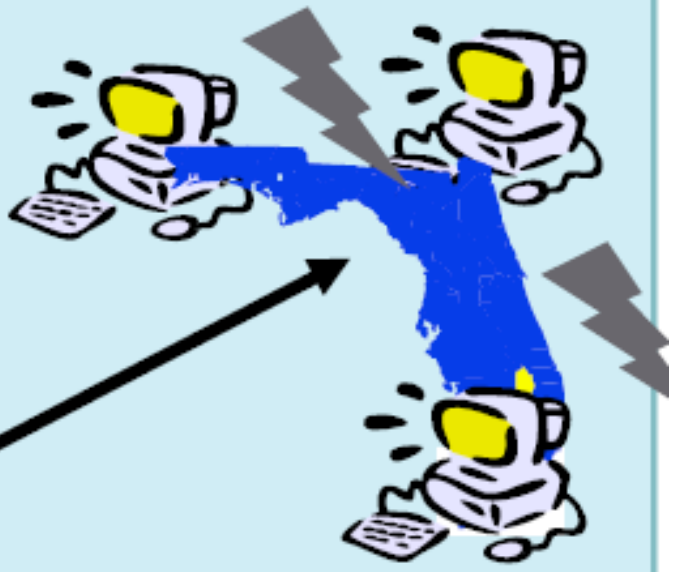
STATE

LOCAL

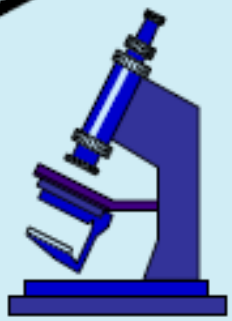


CODIS: How Does It Work?

Convicted Offender
DNA Profiles



Statewide DNA
Database



Crime Lab

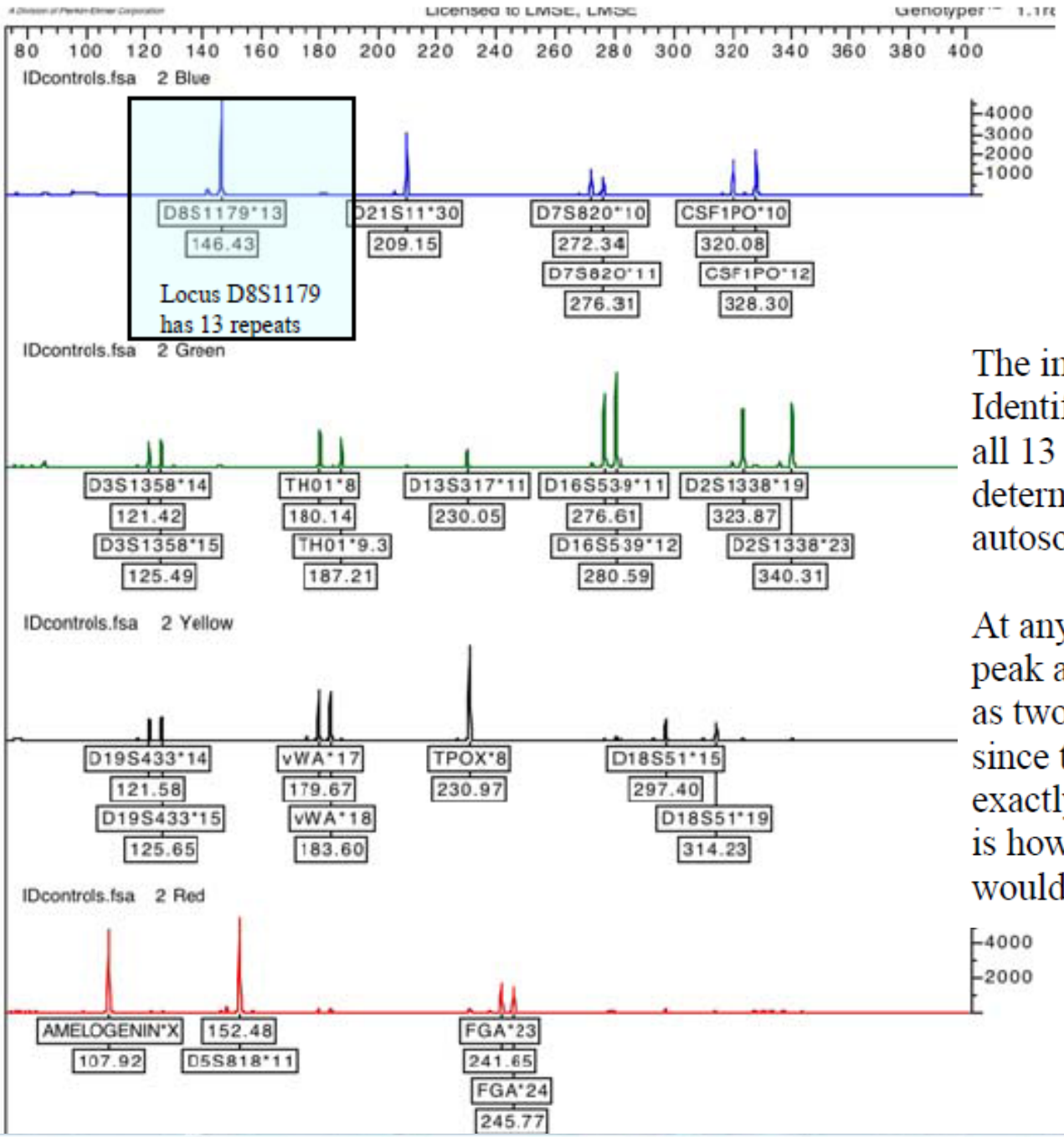
Case! Solved

Biological Screening
Workshop

nfstc

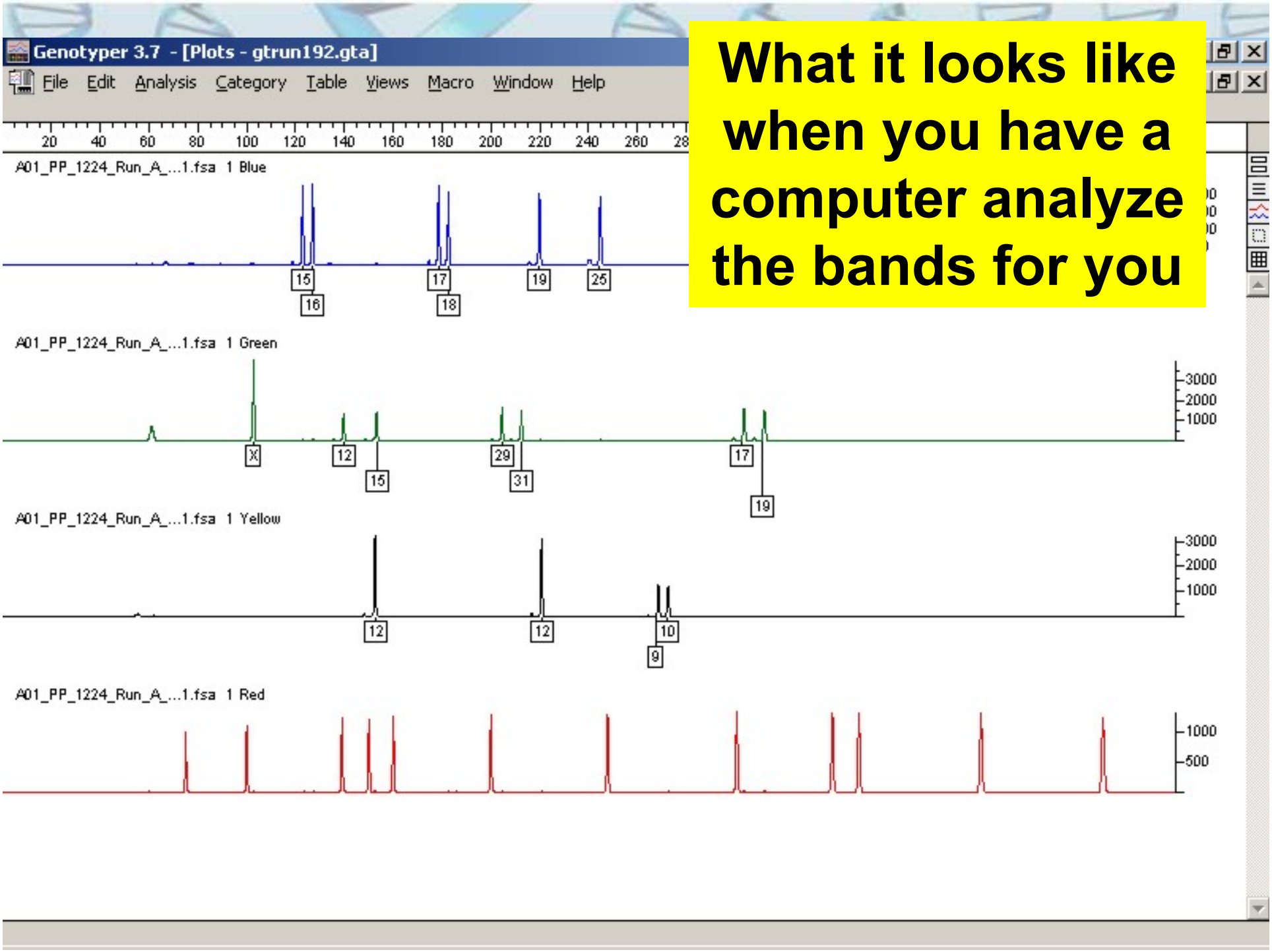
- A match made b/t profiles can link CS to each other or ID serial offenders
- Matches made b/t the forensic evid and CODIS can provide investigators w/ the ID of a suspect
- If an "**offender hit**" is obtained, that info can be used as **probable cause** to obtain a new DNA sample from the suspect so the match can be confirmed before an arrest is made

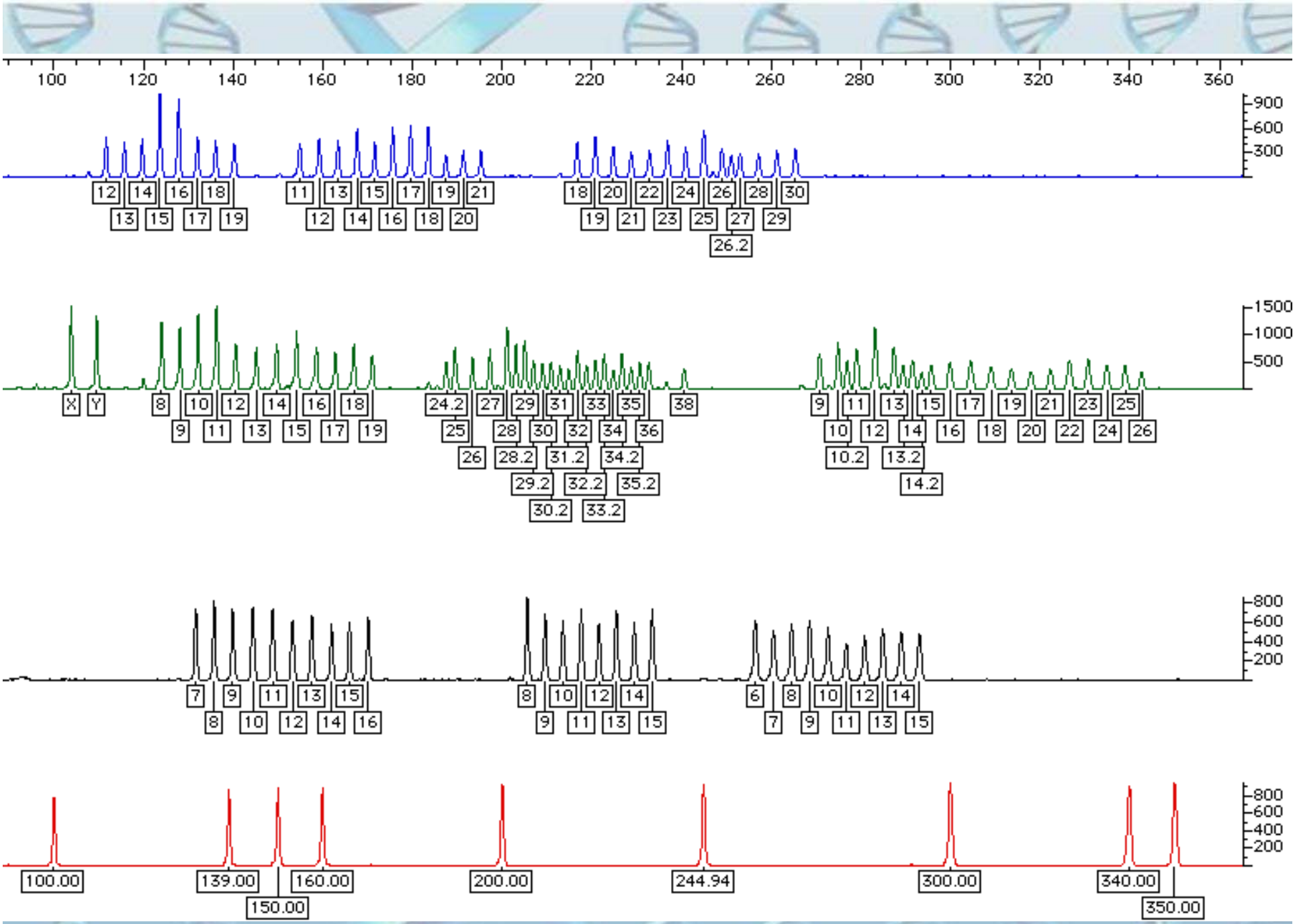
What does real forensic data look like?



The image to the left shows the Identifiler reaction, which includes all 13 CODIS loci, Amelogenin to determine gender, and 2 additional autosomal markers.

At any given locus where only one peak appears we interpret that score as two of the same allelic values, since the peaks are actually sitting exactly on top of one another. This is how a homozygous allelic score would appear.


Genotyper 3.7 - [Plots - gtrun192.gta]
File Edit Analysis Category Table Views Macro Window Help
20 40 60 80 100 120 140 160 180 200 220 240 260
A01_PP_1224_Run_A_...1.fsa 1 Blue
15 16 17 18 19 25
A01_PP_1224_Run_A_...1.fsa 1 Green
3000 2000 1000
X 12 15 29 31 17 19
A01_PP_1224_Run_A_...1.fsa 1 Yellow
3000 2000 1000
12 12 10 9
A01_PP_1224_Run_A_...1.fsa 1 Red
1000 500
What it looks like when you have a computer analyze the bands for you


100 120 140 160 180 200 220 240 260 280 300 320 340 360
900 600 300
12 14 16 18
13 15 17 19
11 13 15 17 19 21
12 14 16 18 20
18 20 22 24 26 28 30
19 21 23 25 27 29
26.2
1500 1000 500
X Y
8 10 12 14 16 18
9 11 13 15 17 19
24.2 27 29 31 33 35 38
25 28 30 32 34 36
26 28.2 31.2 34.2
29.2 32.2 35.2
30.2 33.2
9 11 13 15 17 19 21 23 25
10 12 14 16 18 20 22 24 26
10.2 13.2
14.2
800 600 400 200
7 9 11 13 15
8 10 12 14 16
8 10 12 14
9 11 13 15
6 8 10 12 14
7 9 11 13 15
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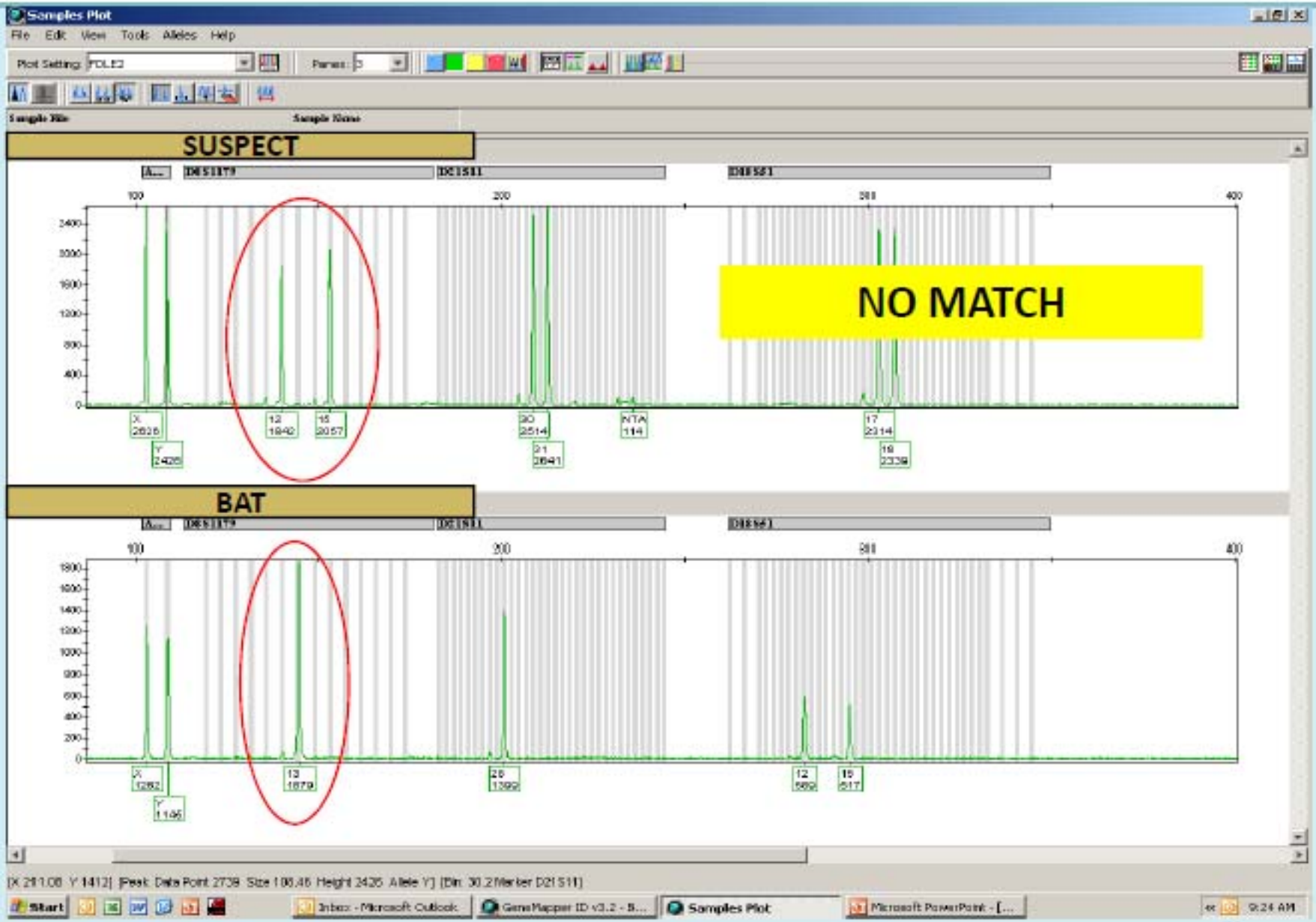
160.00
200.00
244.94
300.00
340.00
350.00



Biological Screening Workshop

nfstc

Image courtesy of Beth Ordeman

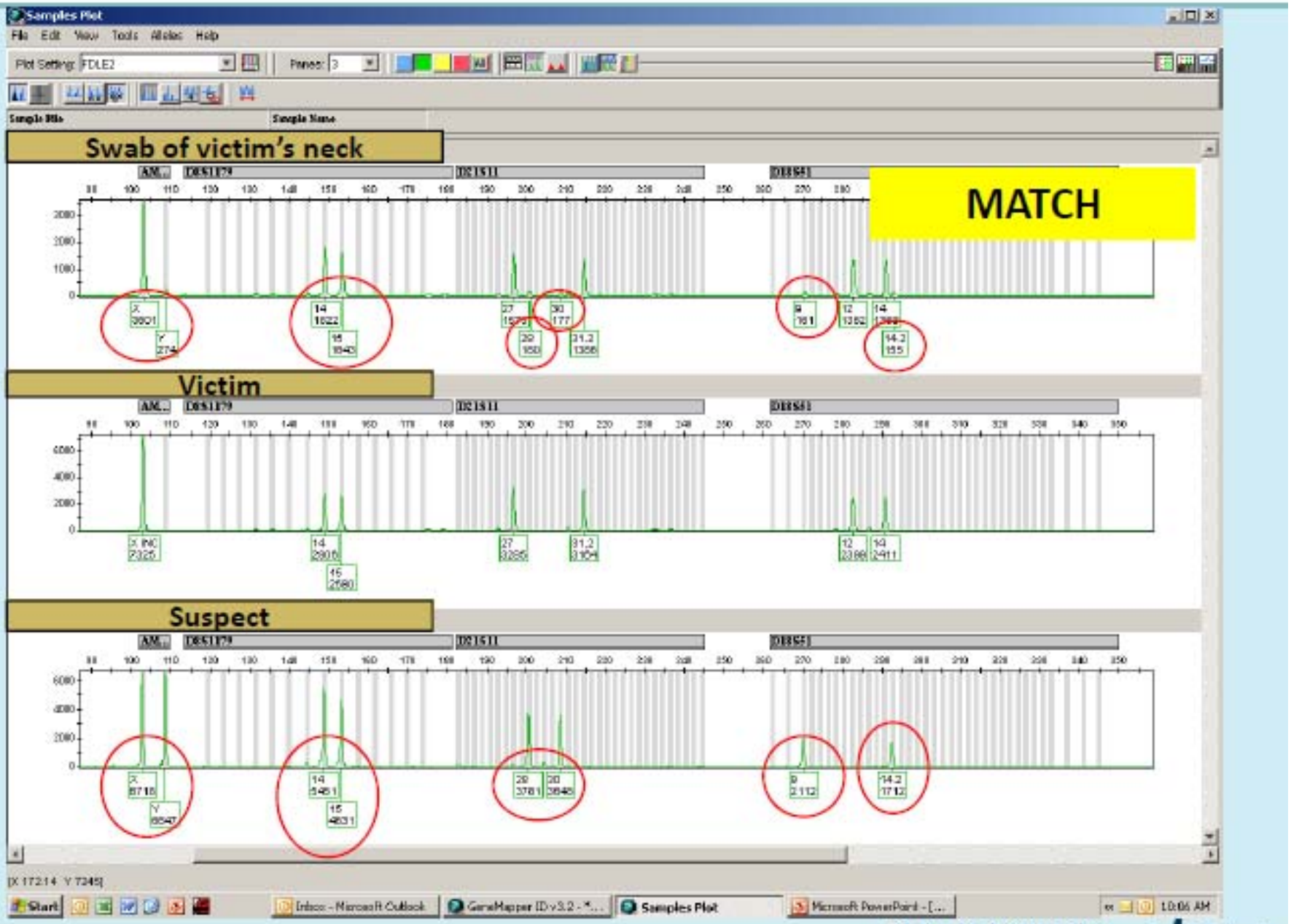
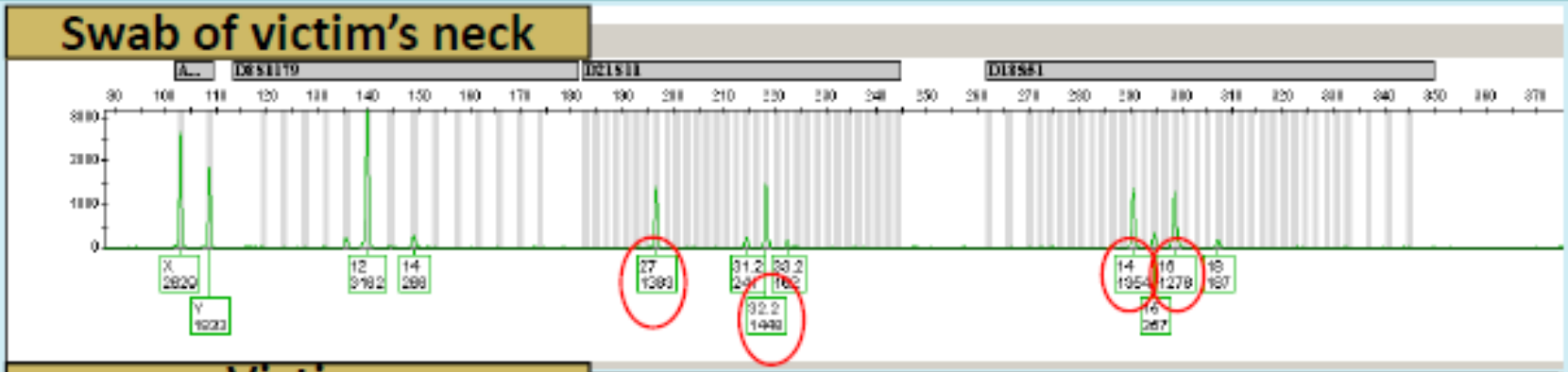
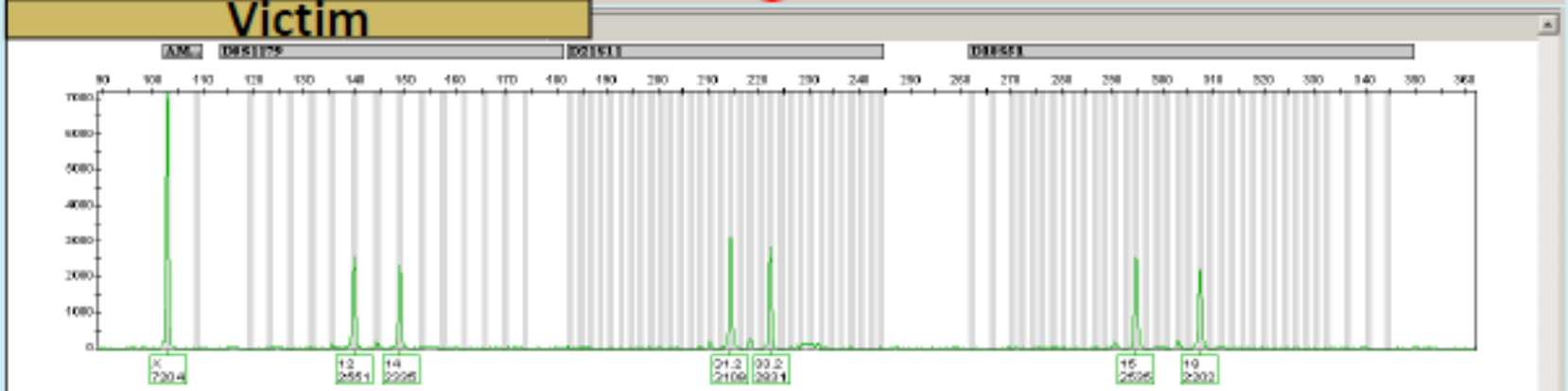


Image courtesy of Beth Ordeman

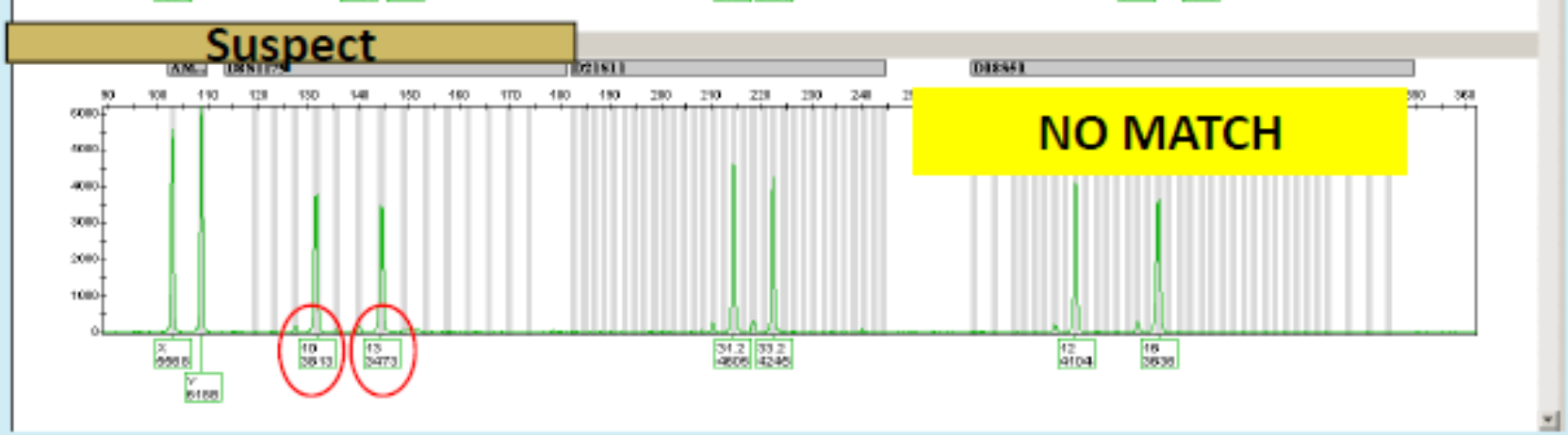
Swab of victim's neck



Victim



Suspect



NO MATCH

Image courtesy of Beth Ordeman