DNA Analysis

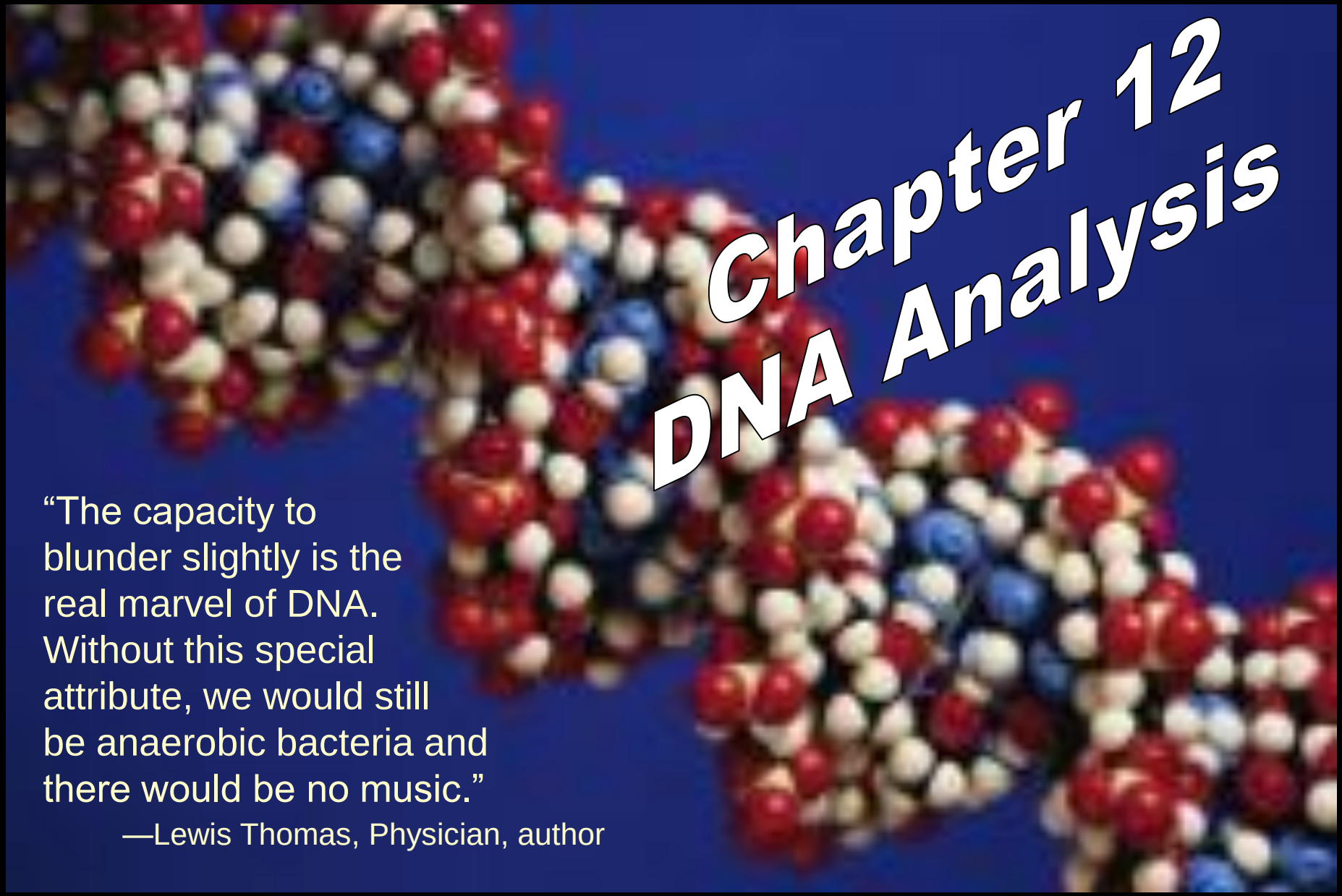
Students will learn:

- That DNA is a long-chain polymer found in nucleated cells, which contain genetic information.
- That DNA can be used to identify or clear potential suspects in crimes.
- How DNA is extracted and characterized.
- How to apply the concepts of RFLP, PCR, and STRs to characterize DNA.
- The role that statistics plays in determining the probability that two people would have the same sequence in a fragment of DNA.

DNA Analysis

Students will be able to:

- Explain that DNA is a long molecule, tightly packed in the form of a chromosome with genetic material wrapped around it.
- Isolate and extract DNA from cells.
- Describe the function and purpose of a restriction enzyme.
- Calculate probabilities of identity using STR.



Chapter 12
DNA Analysis

"The capacity to blunder slightly is the real marvel of DNA. Without this special attribute, we would still be anaerobic bacteria and there would be no music."

—Lewis Thomas, Physician, author

Historical Information

- **James Watson and Francis Crick**—1953 discovered the configuration of the DNA molecule
- **Ray White**—1980 describes first polymorphic RFLP marker
- **Alec Jeffreys**—1985 isolated DNA markers and called them DNA fingerprints
- **Kary Mullis**—1985 developed PCR testing
- **1988**—FBI starts DNA casework
- **1991**—first STR paper
- **1998**—FBI launches CODIS database

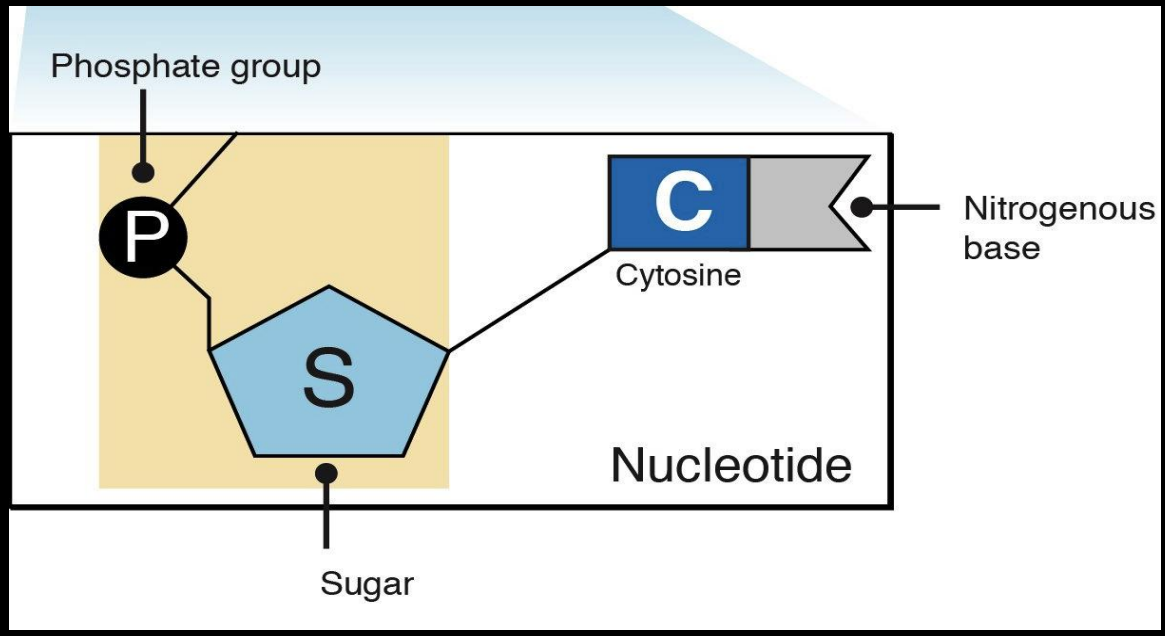
People of Historical Significance

James Watson, Francis Crick, and Maurice Wilkins jointly received the Nobel Prize in 1962 for their determination of the structure of DNA. What is interesting about this fact is that Rosalind Franklin had as much to do with the discovery as the other three gentlemen with her work with X-ray crystallography. She died of cancer and could not be honored for her work. Find out more at Chemical Achievers:

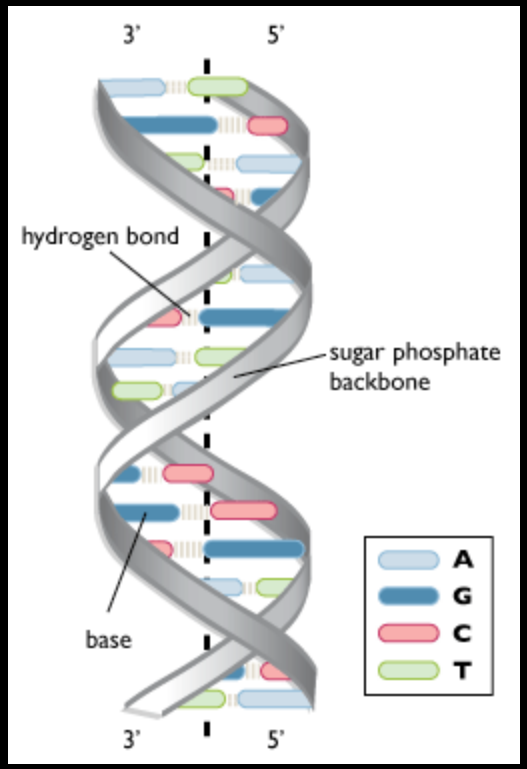
www.chemheritage.org/EducationalServices/chemach/ppb/cwwf.html

General DNA Information

- Double helix—two coiled DNA strands
- Composed of nucleotides—units containing a sugar molecule (deoxyribose), phosphate group and a nitrogen-containing base



Double helix – with Phosphates linked along the sides and N-bases bonded to its compliment base (steps of the ladder)



General DNA Information

Four bases

- Adenine
- Cytosine
- Guanine
- Thymine

Bases always pair A to T and G to C

In humans, the order of these bases is 99.9% the same.

From N – base to Cell

- N base on Nucleotide
- Nucleotides make DNA strands
- DNA in genes
- Genes on chromosomes
- Chromosomes in Nucleus
- Nucleus in the cell

Where Is DNA Found?

- Genes are portions of DNA that code for specific proteins
- DNA is found in all nucleated body cells—white blood cells, semen, saliva, urine, hair root, teeth, bone, tissue
- Most abundant in buccal (cheek) cells
- Red blood cells have no nuclei; and therefore, no nuclear DNA
- DNA obtained from blood comes from white blood cells

DNA Typing

DNA typing is a method in which DNA is converted into a series of bands that ultimately distinguish each individual. Only one-tenth of a single percent of DNA (about 3 million bases) differs from one person to the next. Scientists use these regions to generate a DNA profile of an individual.

Non-Coding Regions

- 3 percent of the human DNA sequences code for proteins
- 97 percent is non-coding and is repetitive; repeating the same sequence over and over
- 50 percent of the human genome has interspersed repetitive sequences

Uses of DNA Profiling

- To identify potential suspects
- To exonerate individuals
- To identify crime and casualty victims
- To establish paternity
- To match organ donors

DNA TYPING "Fingerprinting"

- **RFLP**—Restriction Fragment Length Polymorphism
- **PCR**—Polymerase Chain Reaction
- **STR**—Short Tandem Repeats

RFLP—Restriction Fragment Length Polymorphisms

Restriction enzymes are used to cut DNA into smaller fragments that can then be separated and characterized for identification

- **Isolate**—separate DNA from the cell
- **Cut**—using restriction enzymes to make shorter base strands
- **Sort**—by size using electrophoresis
- **Analyze**—the specific alleles for identification

RFLP – EXAMPLE: this restriction enzyme cuts between A and G ; notice row C, the fragments are now shorter than what you started with on row A

PCR—Polymerase Chain Reaction

PCR is a technique used for making copies of a defined segment of a DNA molecule. This can be valuable when the amount of evidence is minimal. Millions of copies of DNA can be made from a single speck of blood.

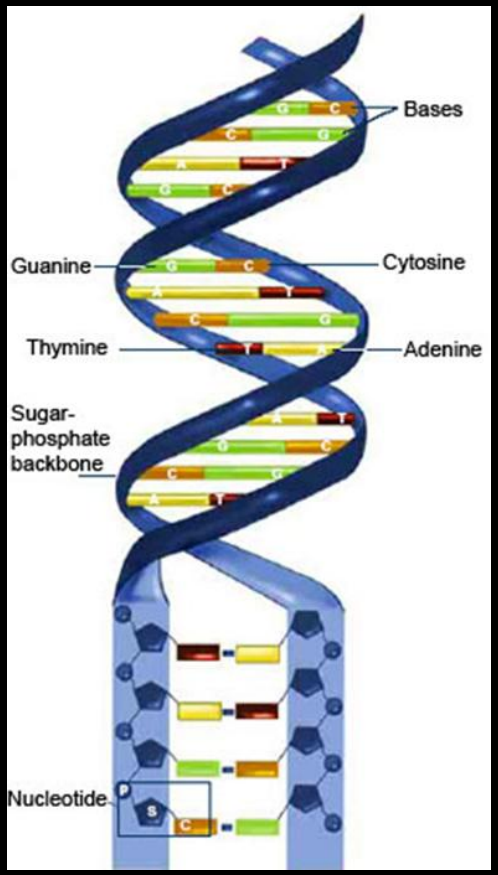
PCR—Polymerase Chain Reaction Procedure

- Heat the DNA strands, causing the strands to separate (unzip).
- Cool the mixture and add a primer, a short sequence of base pairs that will add to its complementary sequence on the DNA strand.
- Finally, add a DNA polymerase and a mixture of free nucleotides to the separated strands. Heat again to around 75° C for the completion.

PCR—Polymerase Chain Reaction

Heat separates ("unzips") the strand of DNA

The H-bond that keeps the N-bases together is broken →

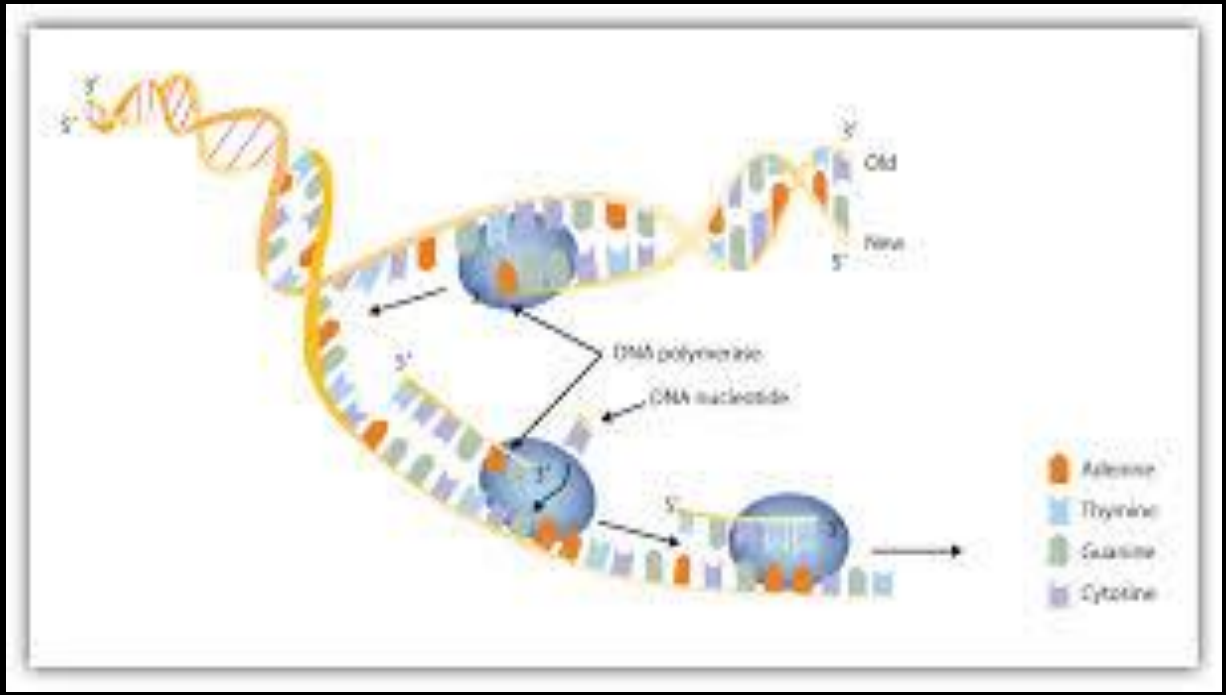


PCR—Polymerase Chain Reaction

The outcome is a doubling of the number of DNA strands. Heating, cooling, and strand rebuilding is repeated typically 25 to 30 times, yielding more than one million copies of the original DNA molecule. Each cycle takes less than two minutes from start to finish.

PCR—Polymerase Chain Reaction

New complimentary bases find their match, then the original strand of DNA becomes 2 strands, then 2 becomes 4, 4 becomes ___, and so on…



Advantages of PCR

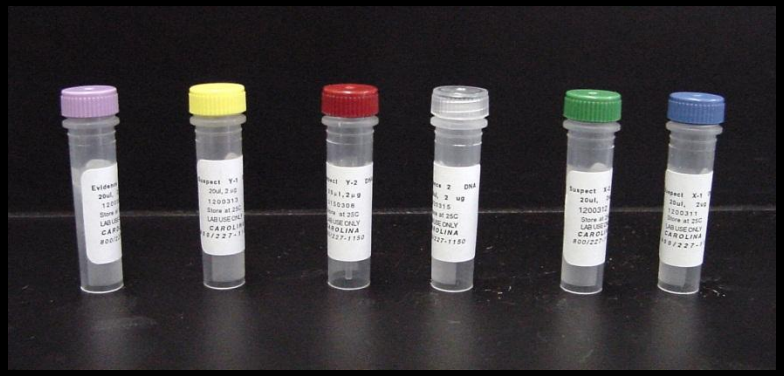
- Minute amounts of DNA may be used for amplification.
- DNA degraded to fragments only a few hundred base pairs in length can serve as effective templates for amplification.
- Large numbers of copies of specific DNA sequences can be amplified simultaneously with multiplex PCR reactions.
- Commercial kits are now available for easy PCR reaction setup and amplification.

Contaminant DNA, such as fungal and bacterial sources, will not amplify because human-specific primers are used. However, human contamination can be a problem.

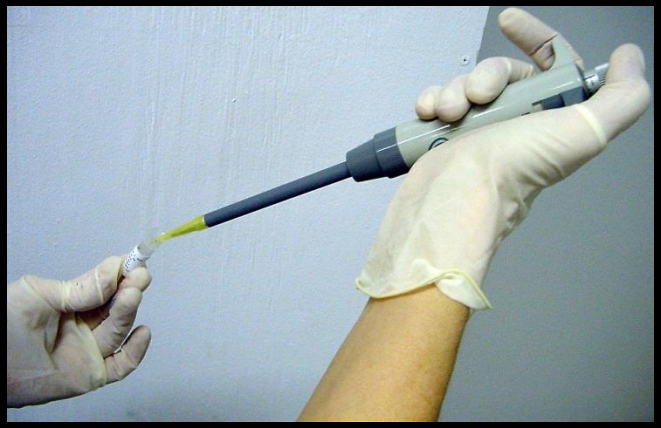
Electrophoresis

- A technique used to sort DNA fragments.
- An electrical current is moved through a gel substance causing molecules to sort by size.
- The smaller, lighter molecules will move the furthest on the gel.
- After developing, the fragments can be visualized for characterization.

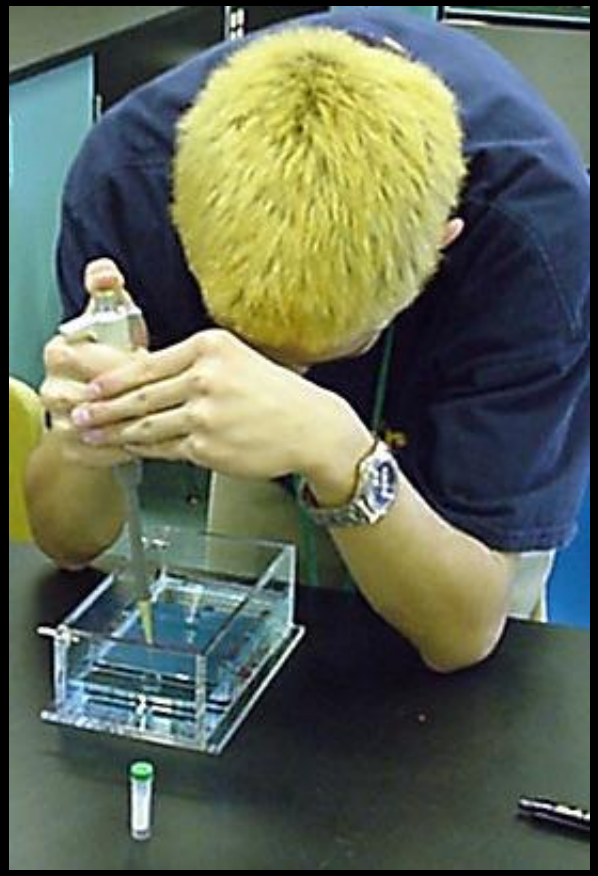
Electrophoresis



Pipette the DNA.



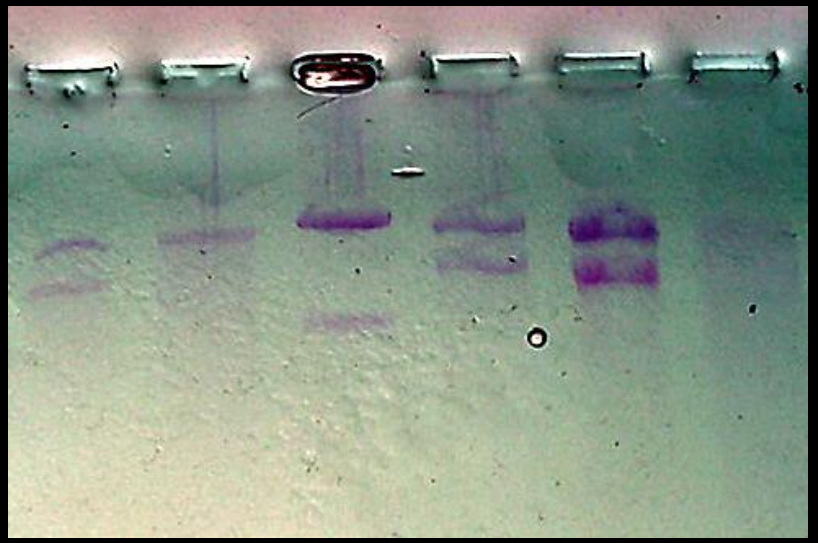
Electrophoresis



Load DNA into the gel wells.

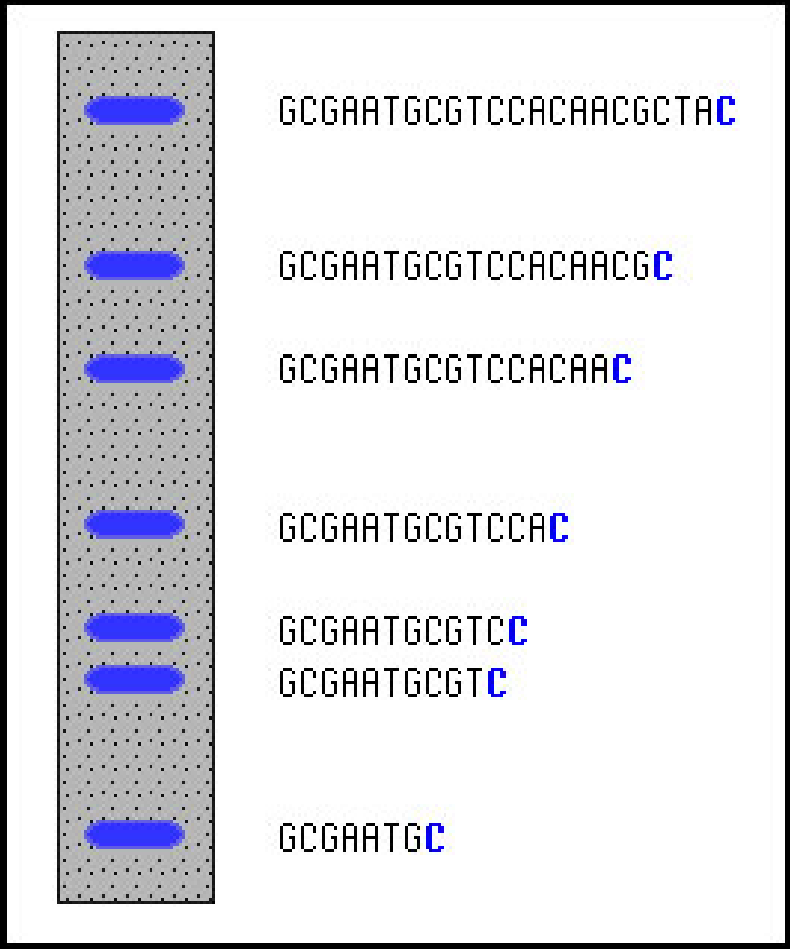
Electrophoresis

- Run the gel.
- Observe and compare bands of DNA.



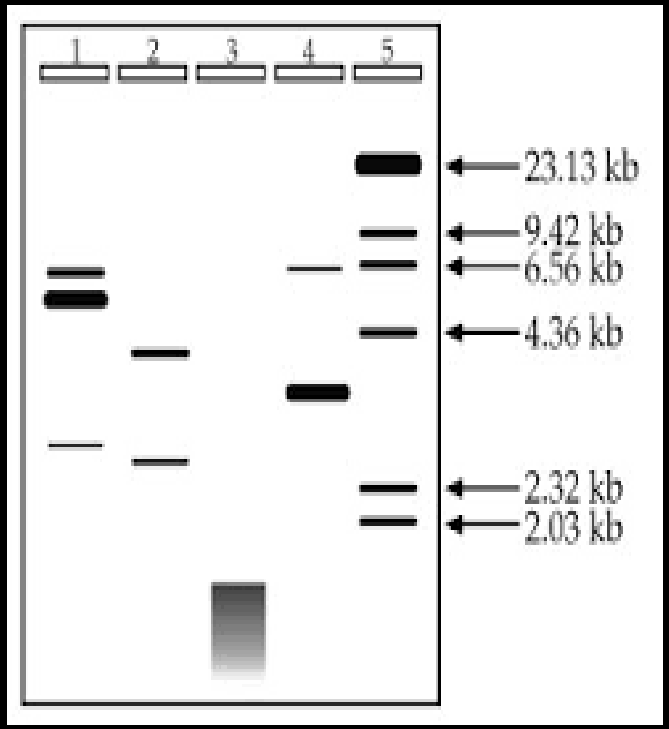
Gel Drawing:

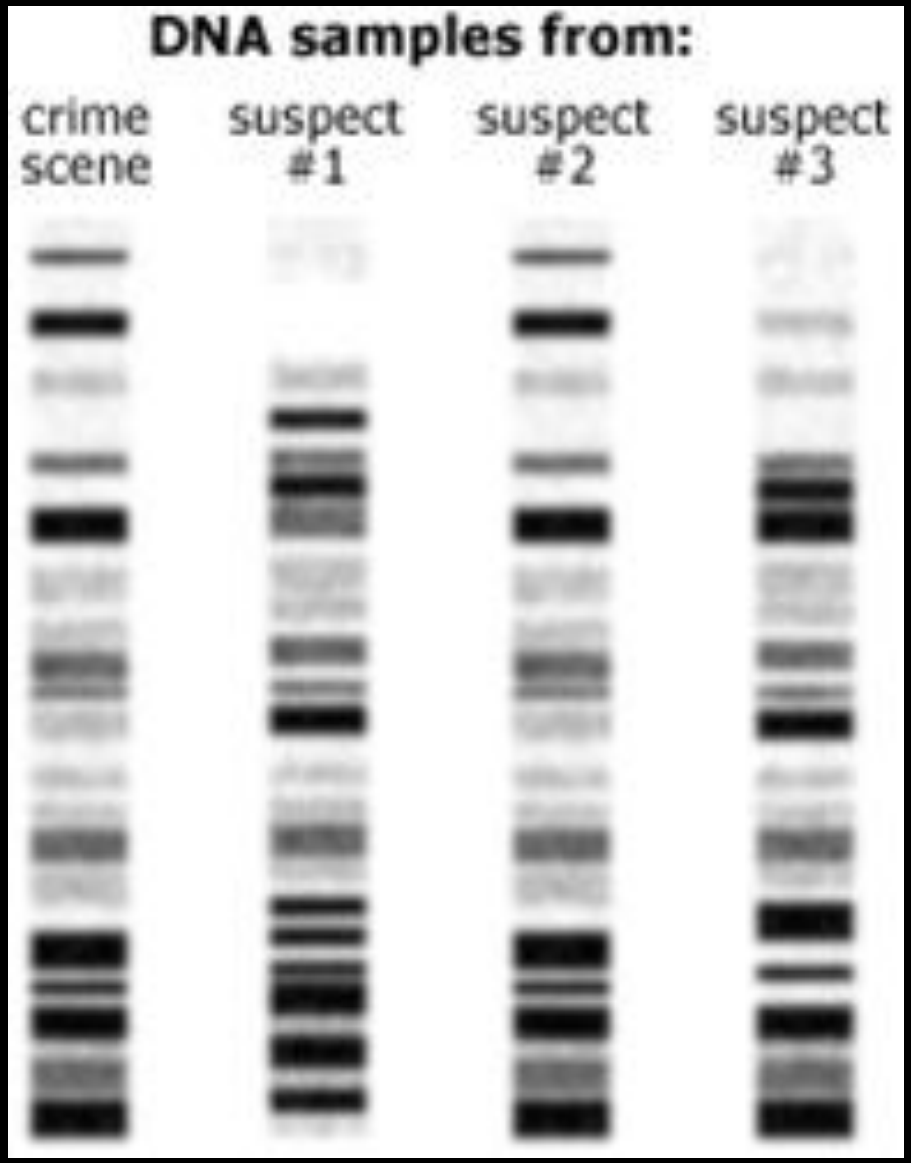
- DNA Fragment sizes -



DRAWING:

notice the smaller one travelled furthest down the gel

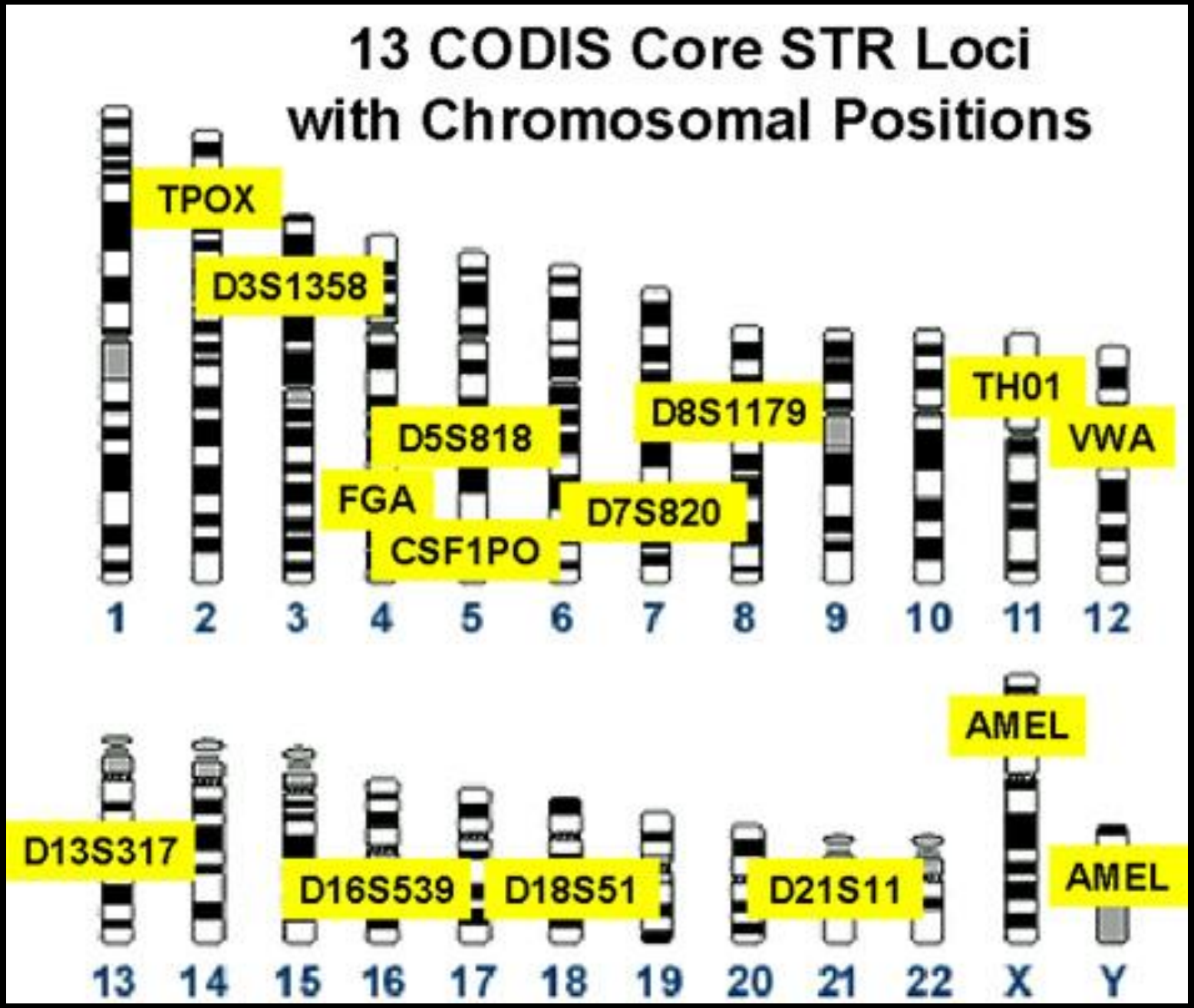



DNA samples from:
crime scene
suspect #1
suspect #2
suspect #3

Bands of DNA

Short Tandem Repeats (STR)

STR is another method of DNA typing. STR's are locations (loci) on the chromosome that contain short sequences of 2 to 5 bases that repeat themselves in the DNA molecule. The advantages of this method are that it provides greater discrimination, requires less time, a smaller sample size, and the DNA is less susceptible to degradation.


13 CODIS Core STR Loci
with Chromosomal Positions
TPOX
D3S1358
D5S818
FGA
CSF1PO
D7S820
D8S1179
TH01
VWA
AMEL
D13S317
D16S539
D18S51
D21S11
AMEL
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

Short Tandem Repeats (STR) Procedure

- Extract the gene TH01 from the sample. (TH01 has seven human variants with a repeating sequence of A-A-T-G)
- Amplify the sample by means of PCR
- Separate by electrophoresis
- Examine the distance the STR migrates to determine the number of times TH01 repeats

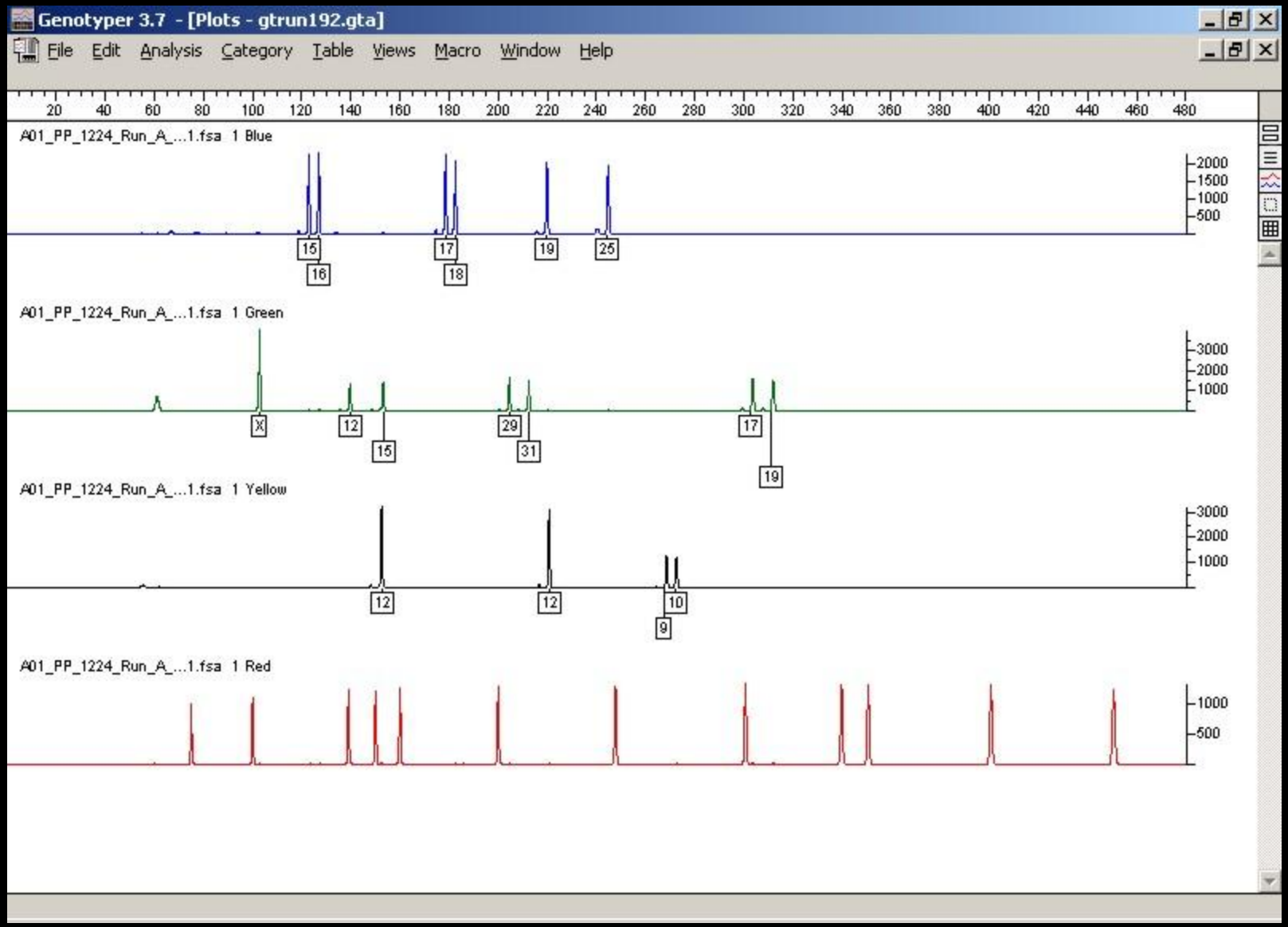
Short Tandem Repeats (STR)

- Each person has two STR types for TH01—one inherited from each parent.
- By continuing the process with additional STRs from other genes, you can narrow down the probability of DNA belonging to only one person.

Short Tandem Repeats (STR)

- STR typing is visualized by peaks shown on a graph. Each represents the size of the DNA fragment.
- The possible alleles are numbered for each loci.

STR Example


Genotyper 3.7 - [Plots - gtrun192.gta]
File Edit Analysis Category Table Views Macro Window Help
20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480
A01_PP_1224_Run_A_...1.fsa 1 Blue
2000
1500
1000
500
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

Determining Probability

Databases have been established that determine how often a particular allele on a loci appears in a given population. By increasing the number of alleles on different loci the probability of having two people with the exact combination becomes miniscule.

Three Possible Outcomes

- **Match**—The DNA profile appears the same. Lab will determine the frequency.
- **Exclusion**—The genotype comparison shows profile differences that can only be explained by the two samples originating from different sources.
- **Inconclusive**—The data does not support a conclusion as to whether the profiles match.

Types of DNA

Nuclear

- found in the nucleus
- constitutes 23 pairs of chromosomes inherited from both parents
- each cell contains only one nuclei

Mitochondrial

- found in the cytoplasm
- is inherited only from mother
- each cell contains hundreds to thousands of mitochondria
- can be found in skeletal remains
- Advantage: can use old, degraded samples

Nuclear DNA is present in the head of the sperm. Mitochondrial DNA is present in the tail. At conception, the head of the sperm enters the egg and unites with the nucleus. The tail falls off, losing the father's mitochondrial DNA.

Mitochondrial DNA

- Analysis of mDNA is more:
  - rigorous
  - time consuming
  - costly than nucleic testing of DNA
- mDNA is constructed in a circular or loop
- 37 genes are involved in mitochondrial energy generation
- Is used when nuclear DNA typing is not possible

FBI's CODIS DNA Database

***C**ombined **D**NA **I**ndex **S**ystem*

Launched October 1998
Links all 50 states
Requires >4 RFLP markers and/or 13 core STR markers
Used for linking serial crimes and unsolved cases with repeat offenders