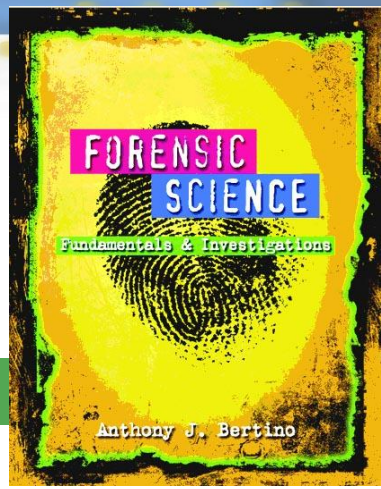
Chapter 7 *DNA Fingerprinting*

By the end of this chapter you will be able to:



- Explain how crime scene evidence is collected and processed to obtain DNA
- Describe how radioactive probes are used in DNA fingerprinting
- Explain how DNA evidence is compared for matching
- Explain how to use DNA fingerprinting to identify DNA from a parent, child, other relative, or a non-related individual

Introduction

- Except for identical twins, no 2 people have the same DNA.
- Since 1908s DNA has been used to investigate crimes, establish paternity, ID victims of war and large scale disasters.
- DNA is **individual** evidence
- Analysis of chromosomes of a sample of cells is **karyotyping**

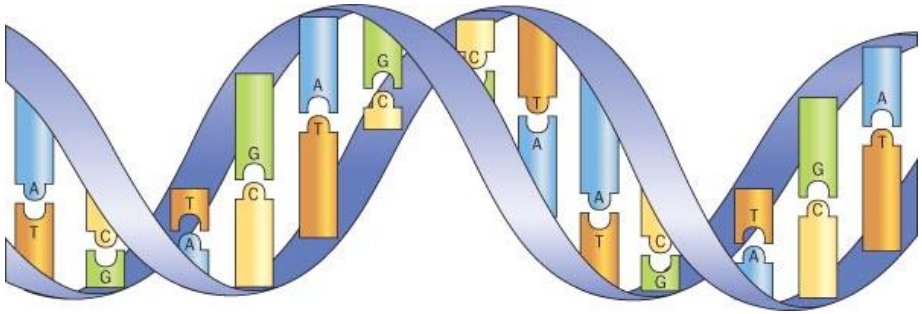
History of Biological Evidence in Forensics

- 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.

History of Biological Evidence in Forensics

- ***DNA profiling***
  - Also known as DNA fingerprinting
  - Used with a high degree of accuracy
- DNA can be extracted from small amounts of biological evidence
- Biological evidence is examined for the presence of inherited traits
- Examples of Biological evidence
  - Skin, blood, saliva, urine, semen, and hair

The Function and Structure of DNA



- DNA contains the genetic material of a cell; holds all of the instructions needed for a cell to make proteins and to replicate.
- Chromosomes are located in the cell nucleus
- Chromosomes contain long DNA strands wrapped around proteins

What is a chromosome?

- In the nucleus of each cell, the DNA molecule is packaged into thread-like structures called chromosomes.
- Each chromosome is made up of DNA tightly coiled many times around proteins called histones that support its structure.
- Chromosomes are not visible in the cell's nucleus—not even under a microscope—when the cell is not dividing.

What is a chromosome?

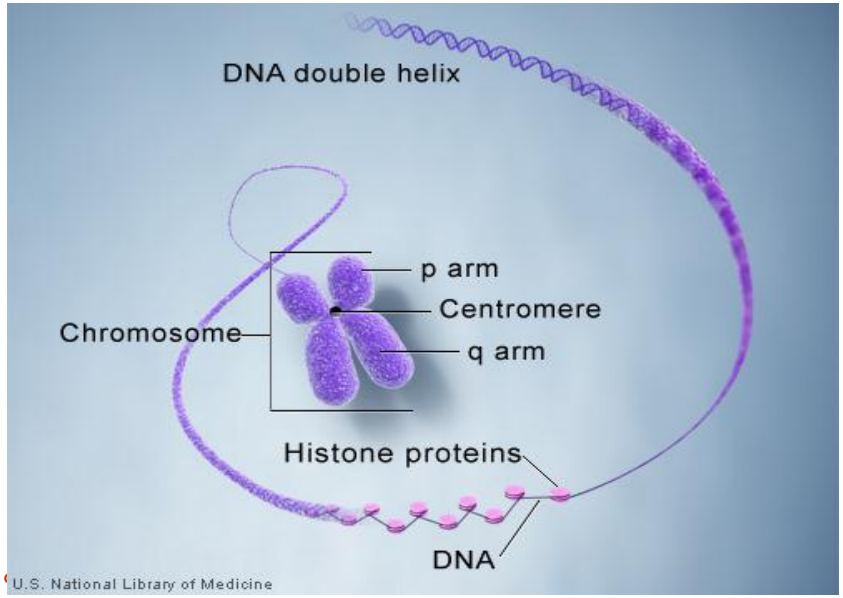
- However, the DNA that makes up chromosomes becomes more tightly packed during cell division and is then visible under a microscope.
- Most of what researchers know about chromosomes was learned by observing chromosomes during cell division.

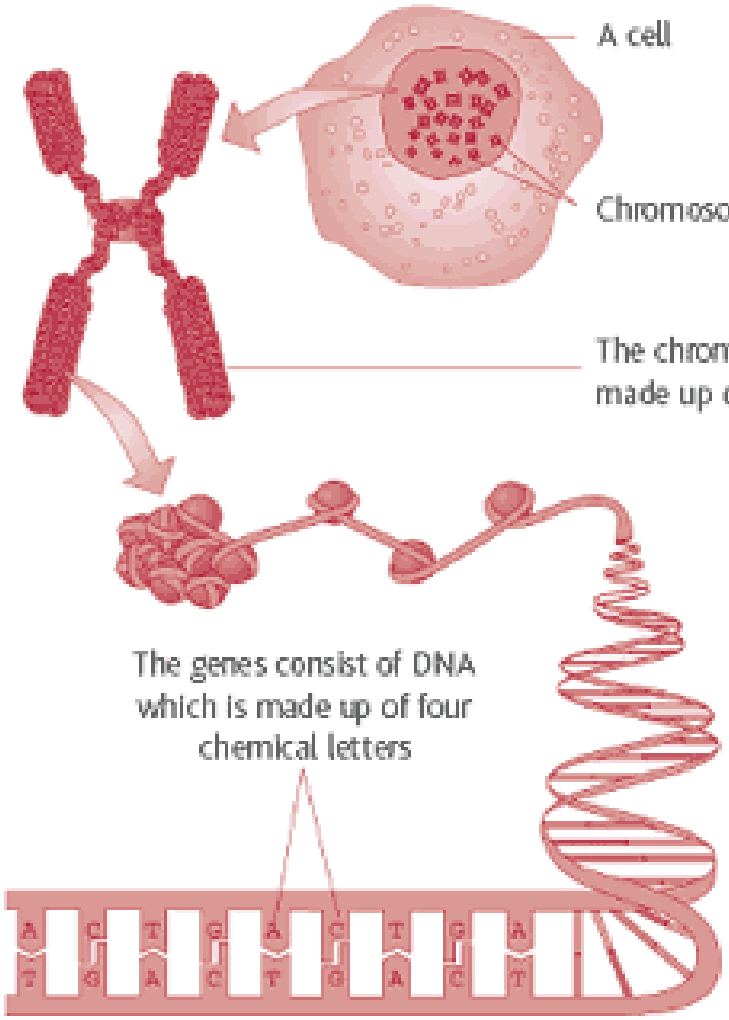
What is a chromosome?

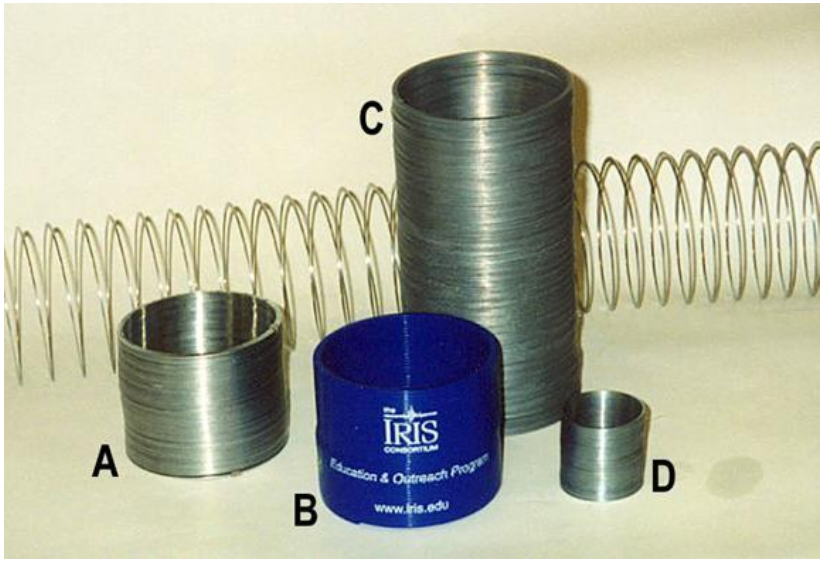
- Each chromosome has a constriction point called the centromere, which divides the chromosome into two sections, or “arms.”
- The short arm of the chromosome is labeled the “p arm.”
- The long arm of the chromosome is labeled the “q arm.”

What is a chromosome?

- The location of the centromere on each chromosome gives the chromosome its characteristic shape, and can be used to help describe the location of specific genes.




A cell
Chromosomes – 23 pairs
The chromosome is made up of genes
The genes consist of DNA which is made up of four chemical letters


C
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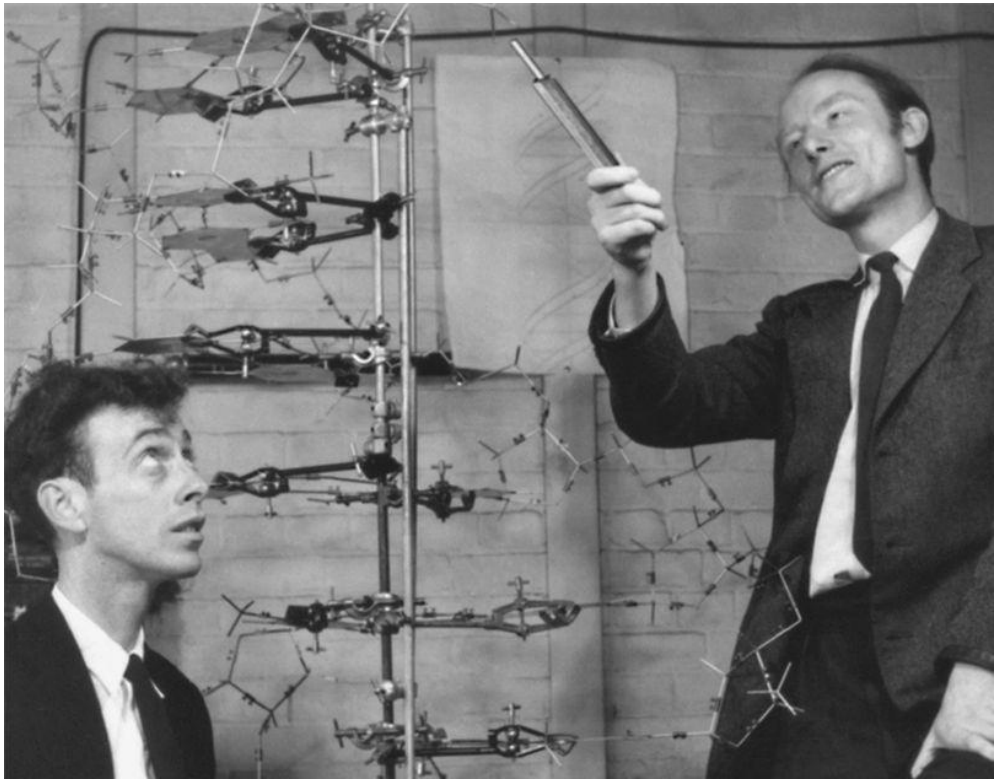
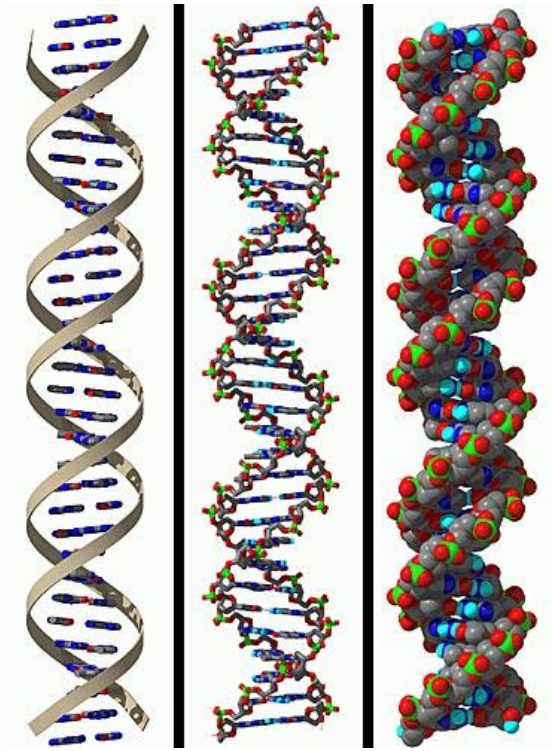
IRIS
Education & Outreach Program
www.iris.edu

The Function and Structure of DNA

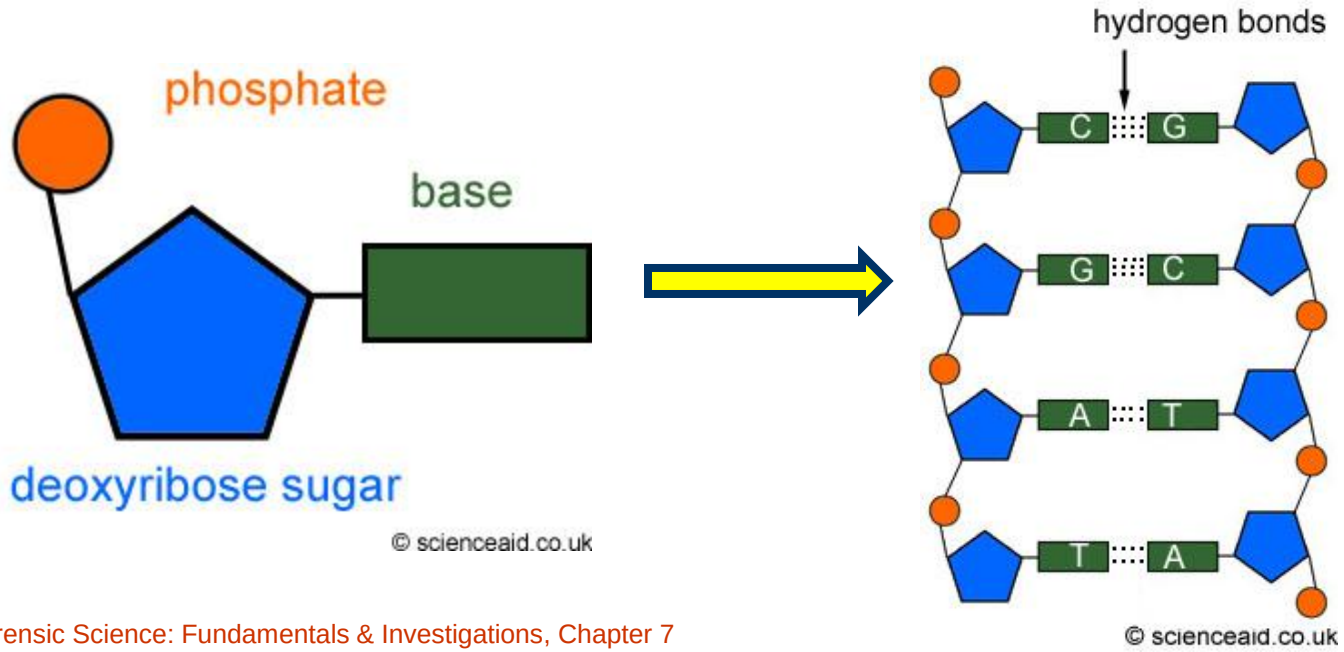
- ***Double helix***--two coiled DNA strands





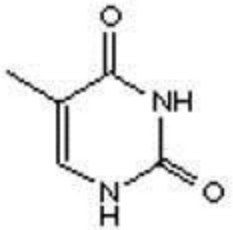
The Function and Structure of DNA

- Composed of nucleotides--unit containing a sugar molecule (deoxyribose), phosphate group and a nitrogen-containing base

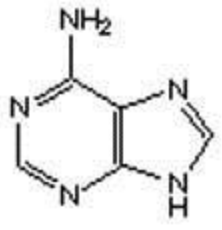


The Function and Structure of DNA

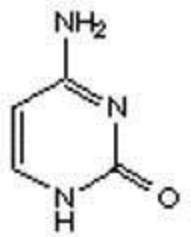
- *Nitrogenous Bases*—pairs of molecules that form the rungs of the DNA "ladder"
- Four types of Bases
  - A (adenine)
  - C (cytosine)
  - G (guanine)
  - T (thymine)
- Base-Pairing Rules
  - adenine (A) binds only with thymine(T)
  - Cytosine (C) binds only with guanine (G)



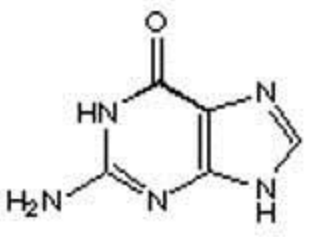
Thymine (T)



Adenine (A)



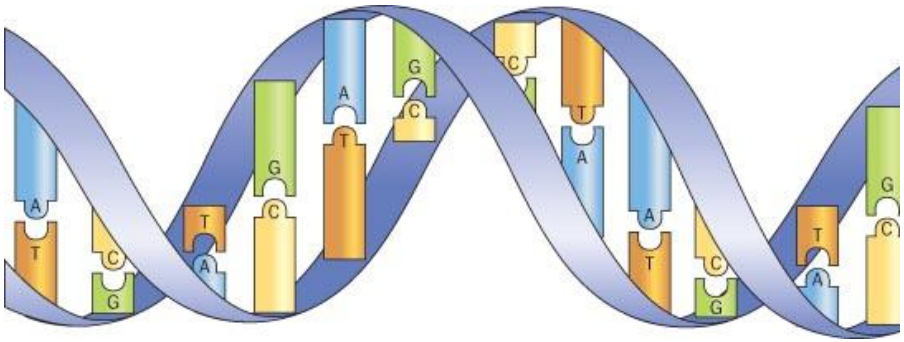
Cytosine (C)

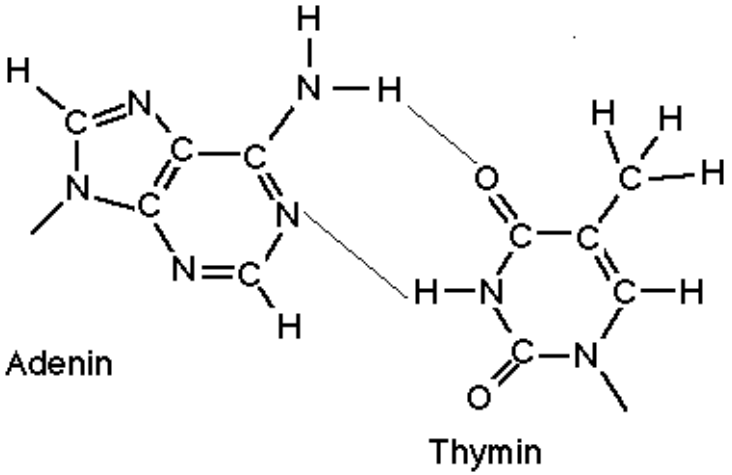


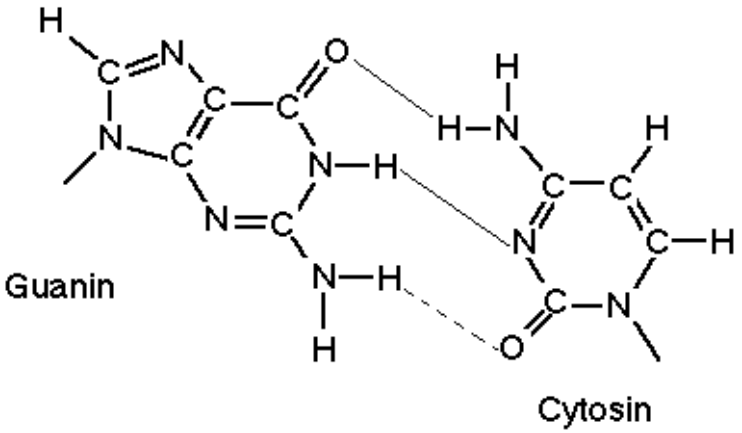
Guanine (G)

The Function and Structure of DNA

- Base-Pairing Rules
  - adenine (A) binds only with thymine(T)
  - Cytosine (C) binds only with guanine (G)







The Function and Structure of DNA

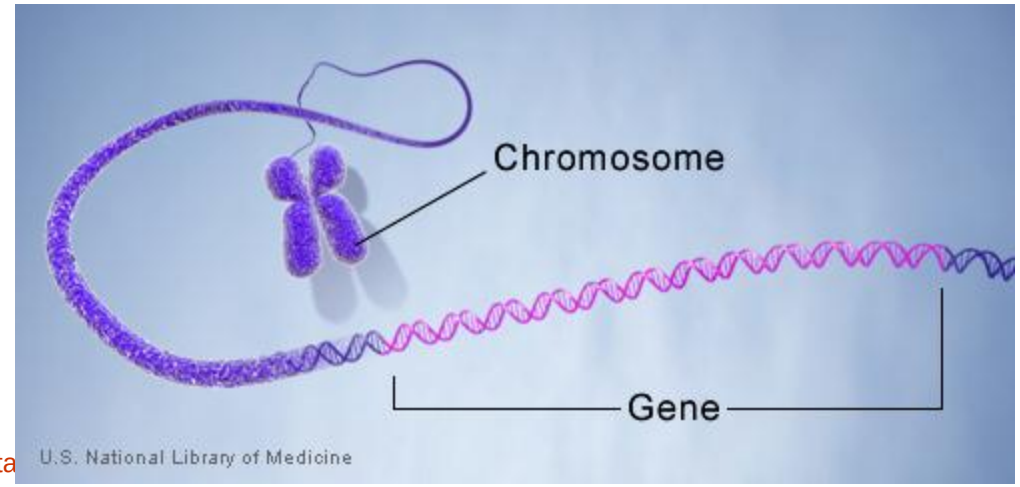
- Human DNA consists of about 3 billion bases, and more than 99 percent of those bases are the same in all people.
- The order, or sequence, of these bases determines the information available for building and maintaining an organism, similar to the way in which letters of the alphabet appear in a certain order to form words and sentences.

What is a gene?

- A gene is the basic physical and functional unit of heredity.
- Genes, which are made up of DNA, act as instructions to make molecules called proteins.
- In humans, genes vary in size from a few hundred DNA bases to more than 2 million bases.

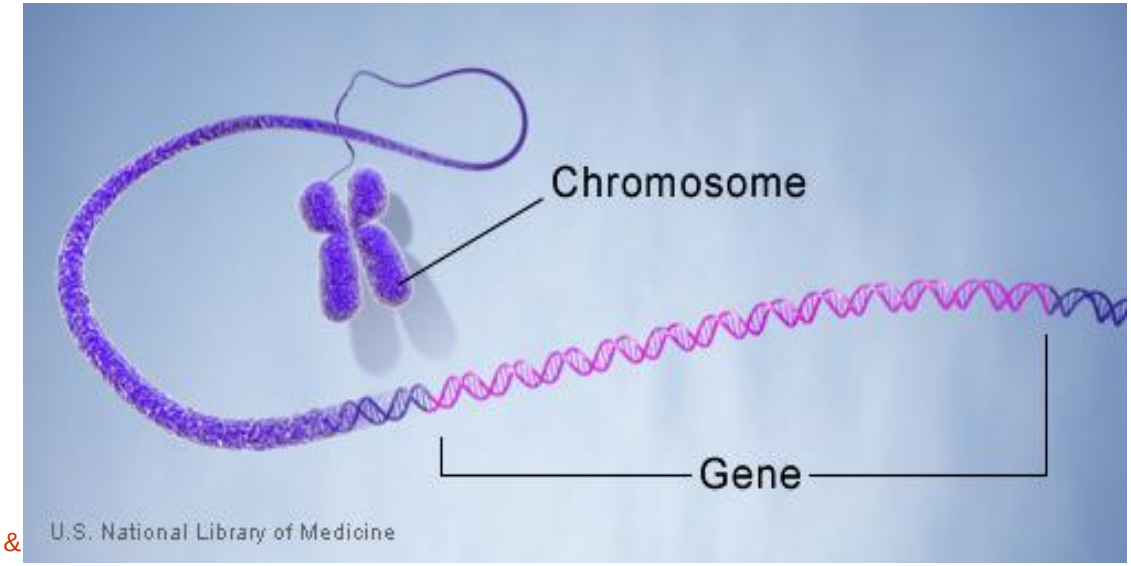
What is a gene?

- Every person has two copies of each gene, one inherited from each parent.
- Most genes are the same in all people, but a small number of genes (less than 1 percent of the total) are slightly different between people.



What is a gene?

- Alleles are forms of the same gene with small differences in their sequence of DNA bases.
- These small differences contribute to each person's unique physical features.



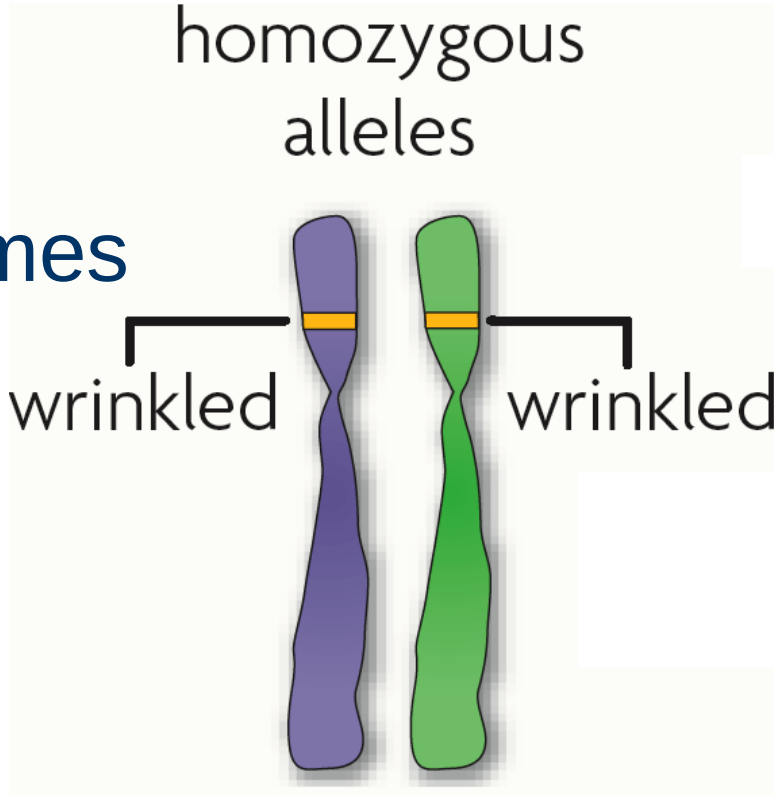
Genes

Each gene has a **locus**,

a specific position

on a pair of

homologous chromosomes



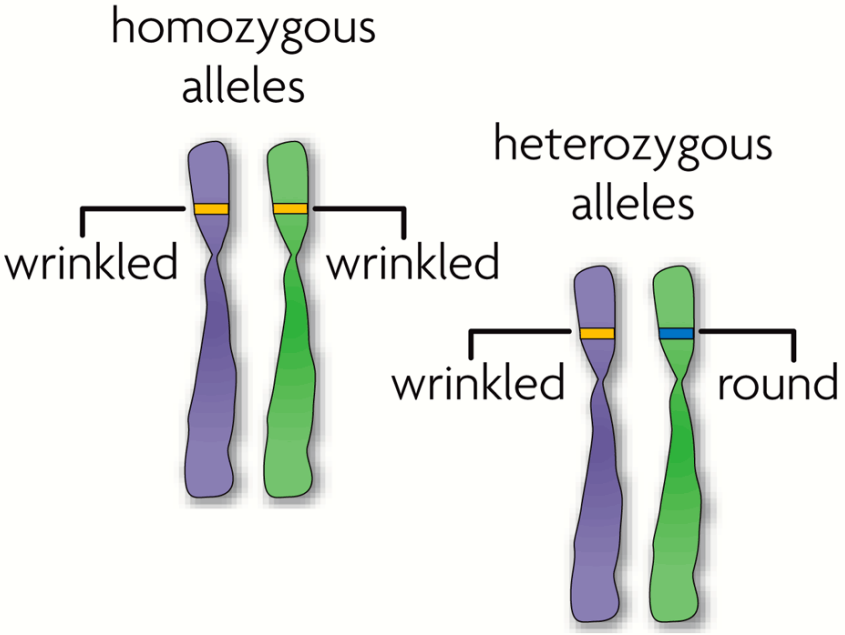
Alleles

An **allele** is an alternative form of a gene

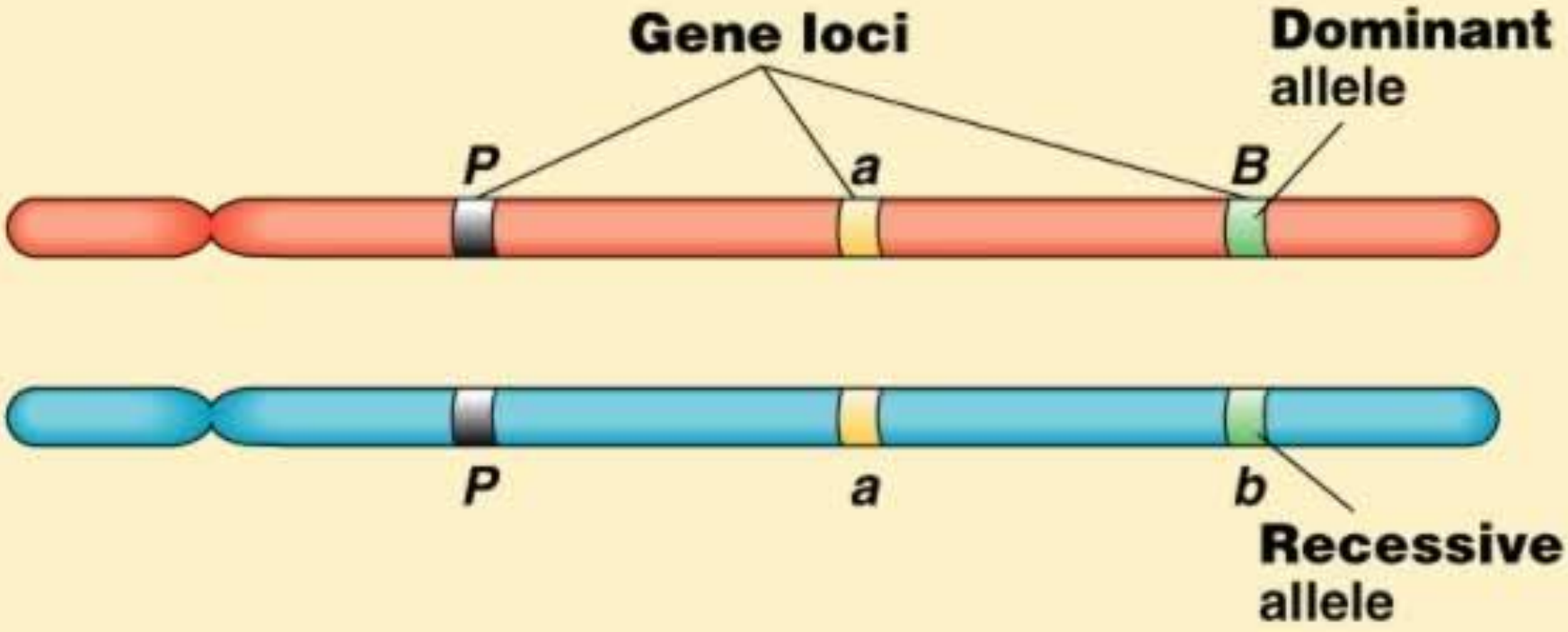
-Each parent donates one allele for every gene

- Pea plants have 2 alleles for shape - they receive one from each parent
- For shape, there is a wrinkled allele and a round allele

Homozygous alleles are identical to each other.



Heterozygous alleles are different from each other.



Genotype: *PP* *aa* *Bb*

Homozygous for the dominant allele

Homozygous for the recessive allele

Heterozygous

Genes influence the development of traits

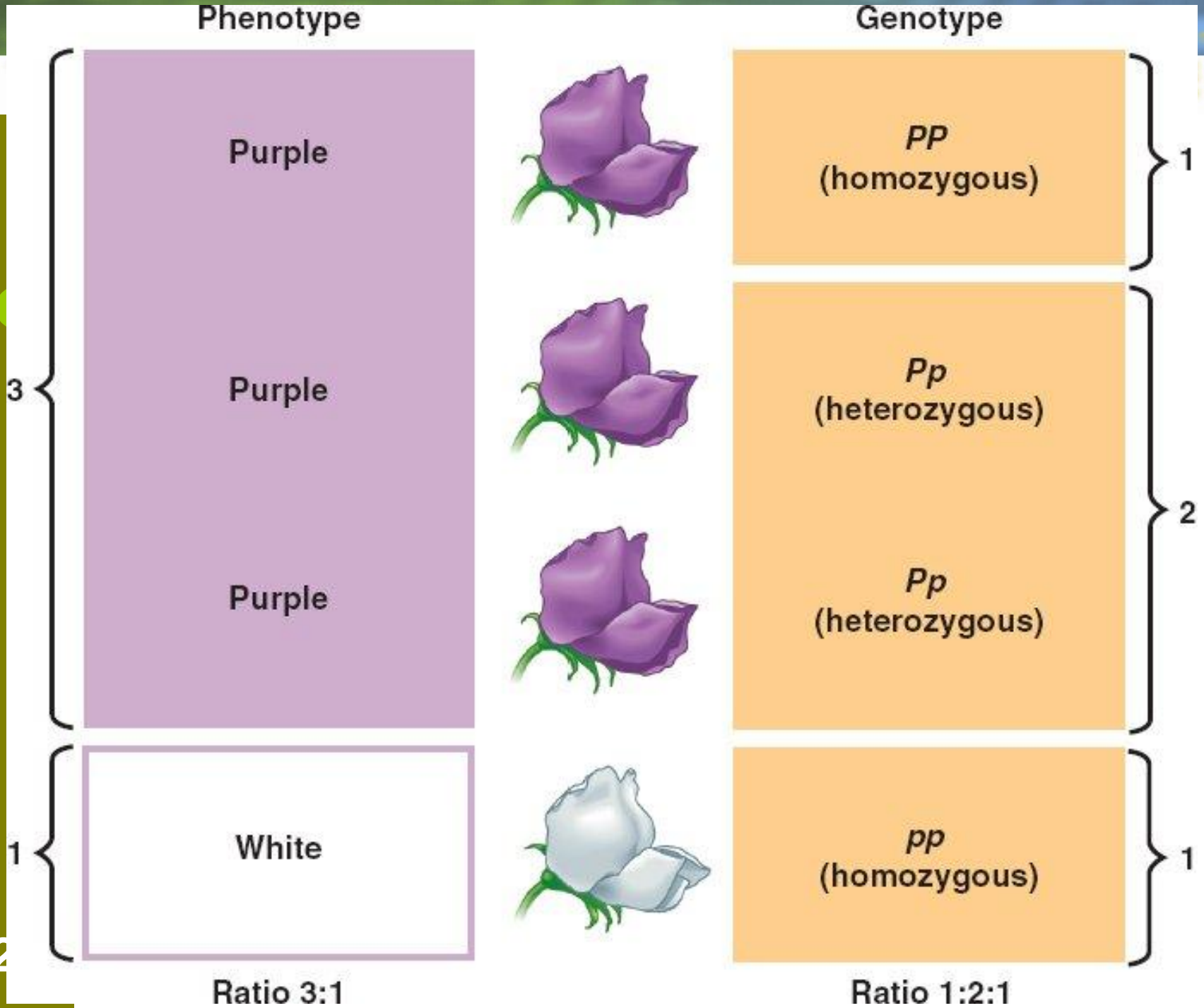
- All of an organism's genetic material is called the **genome**
- A **genotype** A **phenotype** is the physical expression of a trait

Genotype: refers to the actual genes

1. The gene combination of an organism
   - It consists of 2 alleles
   - For example:
     - Pure dominant, 2 dominant genes
     - Pure recessive, 2 recessive genes
     - Hybrid, 1 dominant and 1 recessive gene

Phenotype: physical expression of a trait

- The way an organism looks
  - No matter what genes are present, phenotype of a tall pea plant is tall and a short pea plant is short


Phenotype
Genotype
Purple
PP
(homozygous)
1
3
Purple
Pp
(heterozygous)
Purple
Pp
(heterozygous)
2
1
White
pp
(homozygous)
1
Ratio 3:1
Ratio 1:2:1

Collection and Preservation of DNA

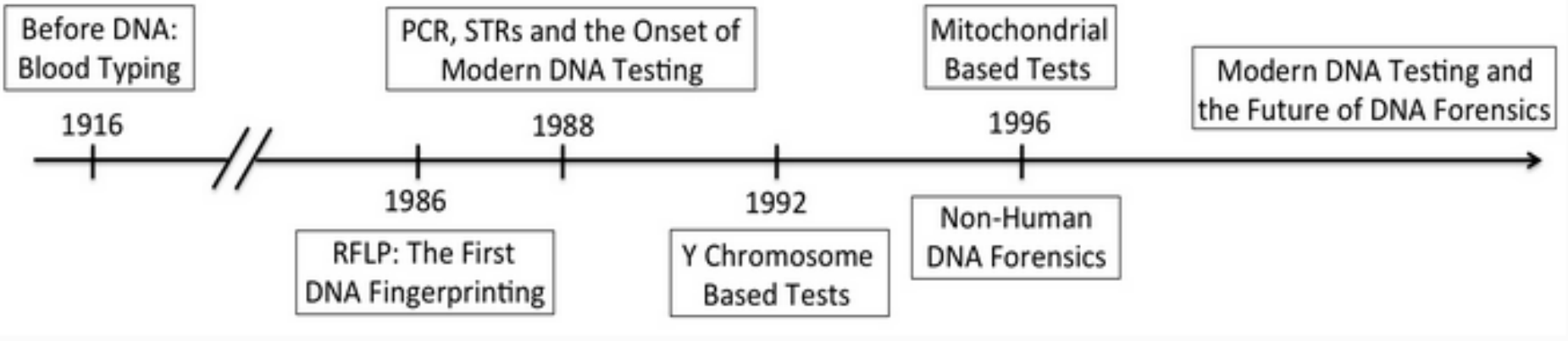
1. Use disposable gloves and collection instruments
2. Avoid physical contact, talking, sneezing, and coughing in the evidence area
3. Air-dry evidence and put it into new paper bags or envelopes
4. Dry or freeze the evidence
5. Keep evidence cool and dry during transportation and storage

DNA Identification

- *Polymorphisms:* Non-coded (junk) DNA that contain unique patterns of repeated base sequences that that are unique to individuals
- RFLP - Restriction Fragment Length Polymorphism

Non-Coding DNA (junk)

- 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


Before DNA: Blood Typing
1916
PCR, STRs and the Onset of Modern DNA Testing
1988
Mitochondrial Based Tests
1996
Modern DNA Testing and the Future of DNA Forensics
1986
RFLP: The First DNA Fingerprinting
1992
Y Chromosome Based Tests
Non-Human DNA Forensics

DNA Profile

Repeating DNA sequences

- Variable Numbers of Tandem Repeats **(VNTR)**
  - The number of repeats varies from person to person
  - 9 to 80 bases in length
- Short Tandem Repeats **(STR)**
  - 2 to 5 bases in length
  - Shorter lengths make STRs easier to use than VNTRs
- VNTR and STR data are analyzed for
  - tissue matching
  - inheritance matching

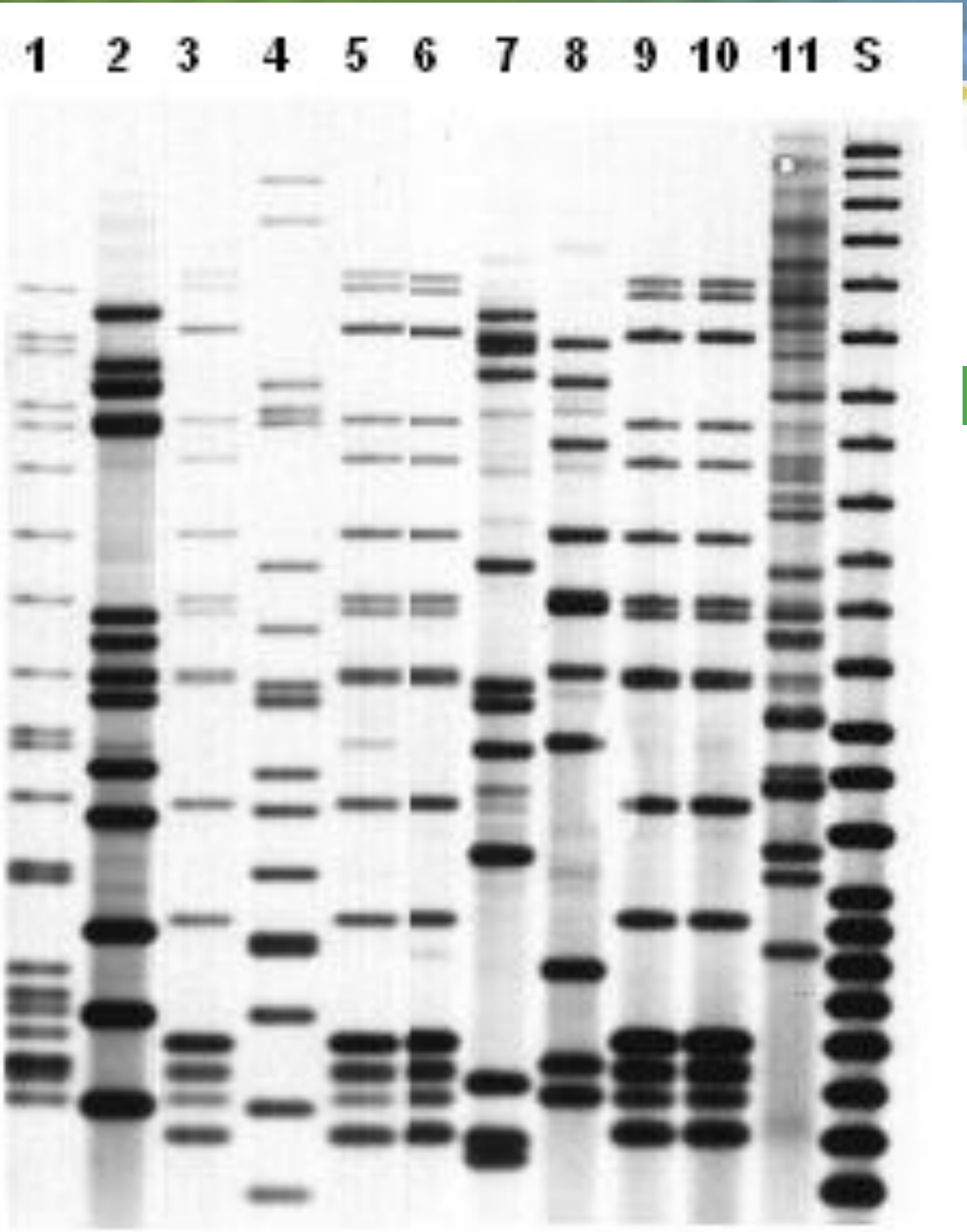
RFLP--Restriction Fragment Length Polymorphisms

- Fragments are cut from the sequence of bases by a restriction enzyme.
- The enzyme find its combination, bonds at one end and dissolves through the DNA at the other.
- Fragments are loaded into a gel and run by electrophoresis.
- DNA is extracted from the gel by blotting it into a nylon membrane.

RFLP--Restriction Fragment Length Polymorphisms

- Radioactive phosphorus-32 probes are added to the membrane which bond to the precise DNA fragments making them radioactive.
- Then the membrane is placed over standard X-ray film where the radiation emitted from the P-32 gradually exposes the film and shows the DNA bands.

This process takes about 10 weeks to complete.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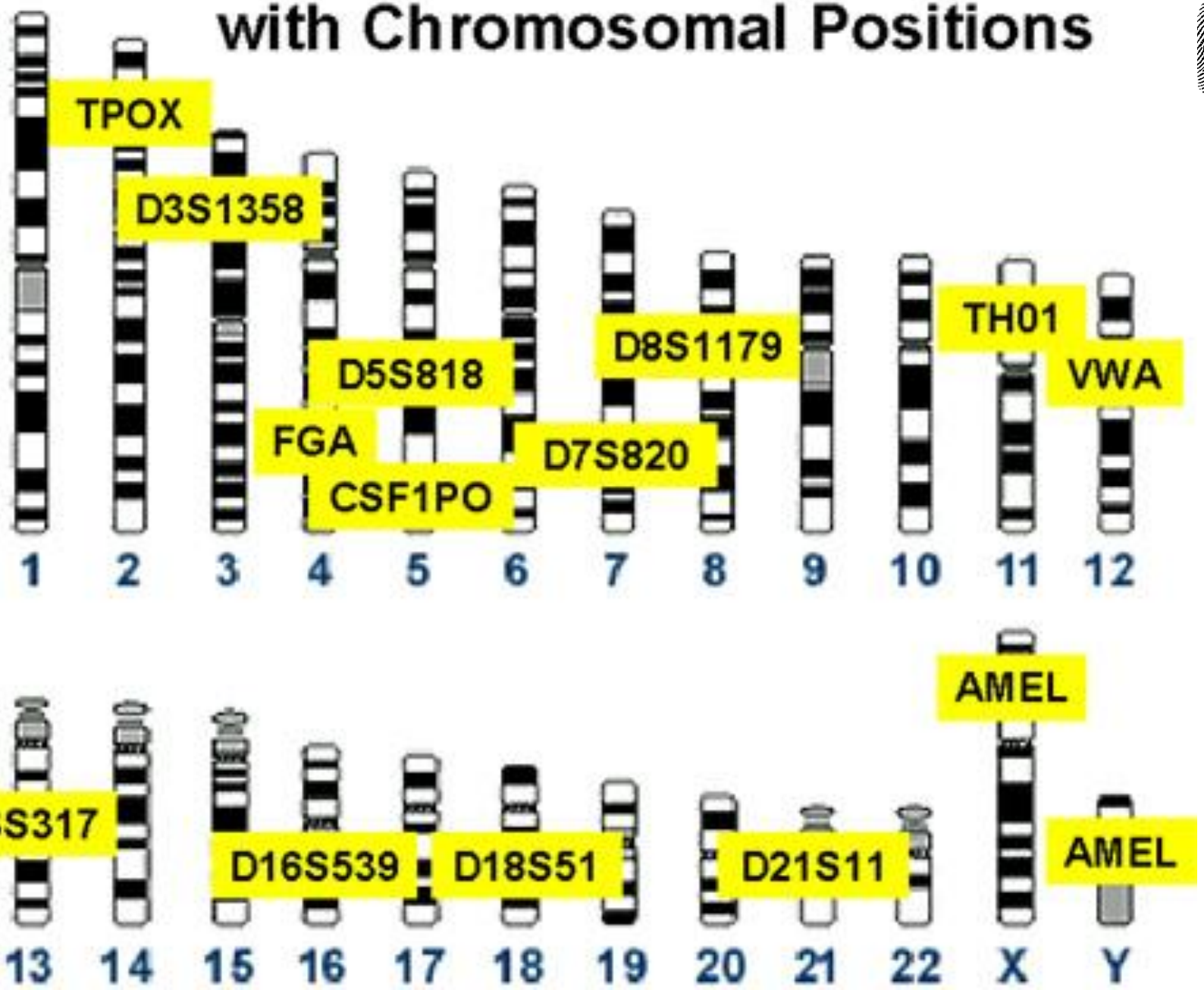
9
10
11
S

Short Tandem Repeats (STR)

- STR is the latest method of DNA typing.
- STR's are locations (loci) on the chromosome
- contain short sequences of 3 to 7 bases that repeat themselves with the DNA molecule.
- advantages include a higher discrimination than RFLP, less time, smaller sample size, and less susceptible to degradation.

13 CODIS Core STR Loci with Chromosomal Positions

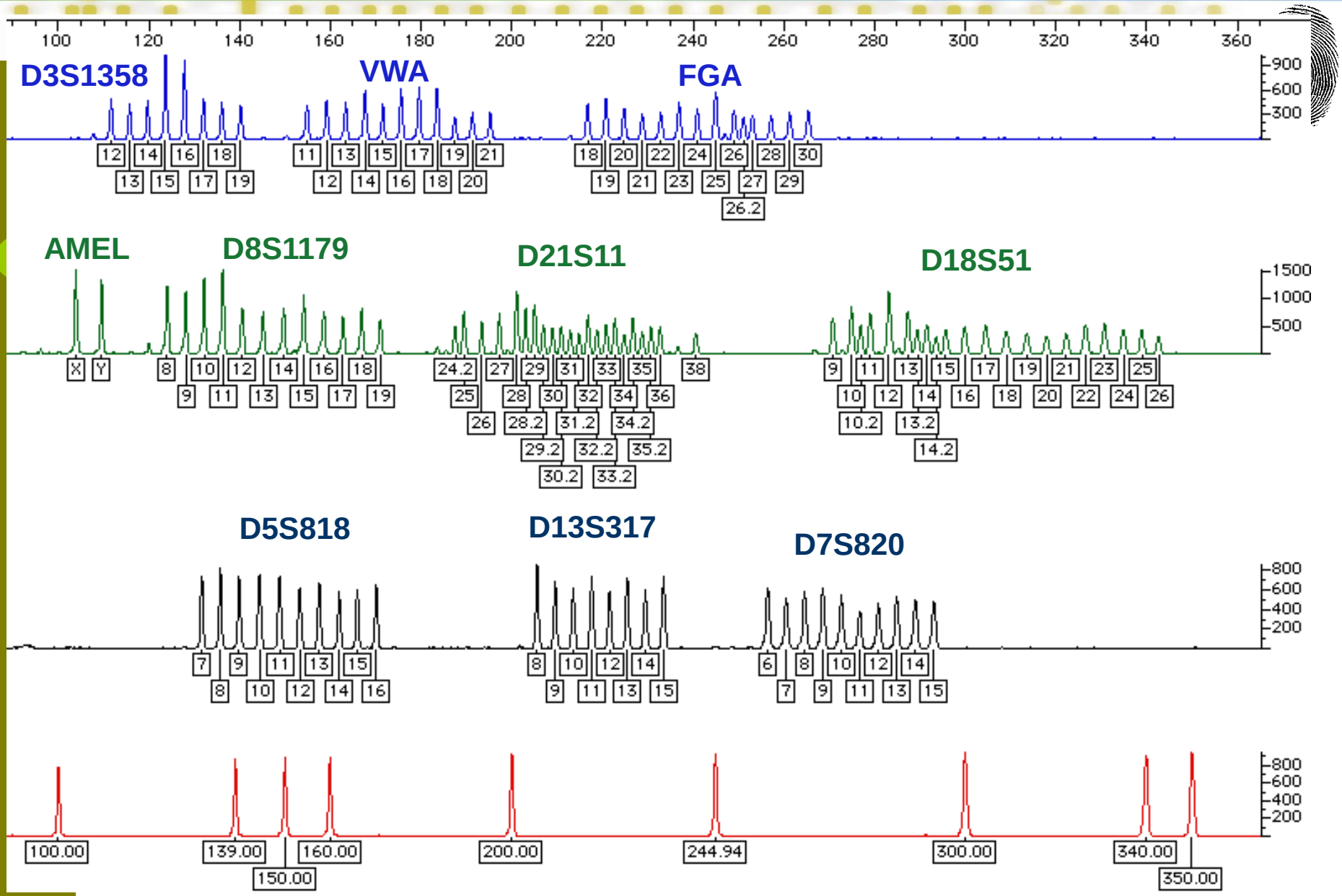


STR

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.

Profiler Plus Allelic Ladders



Population Genetics

- Study of variation in genes among a group of individuals; proportion of people with a specific trait is determined by the proportion of alleles for those traits.

Ex.) among Asian populations, blue eyes are rare, so few people in that population have the allele that codes for blue eyes.

Population Genetics

Ex.) Blood groups

	Caucasians	African American	Hispanic	Asian
O +	37%	47%	53%	39%
O -	8%	4%	4%	1%
A +	33%	24%	29%	27%
A -	7%	2%	2%	0.5%
B +	9%	18%	9%	25%
B -	2%	1%	1%	0.4%
AB +	3%	4%	2%	7%
AB -	1%	0.3%	0.2%	0.1%

American RedCross: Blood Type and the American Population

Almost 100% of South Americans have type O

Preparing DNA Samples for Fingerprinting

1. DNA is mixed with special enzymes
2. Enzymes cut apart the DNA in specific places forming different sized fragments
3. DNA is separated within an agarose gel
4. An electric current is passed through the gel separating the fragments by size

Preparing DNA Samples for Fingerprinting

Extraction

1. Cells are isolated from biological evidence such as blood, saliva, urine, semen, and hair
2. The cells are disrupted to release the DNA from proteins and other cell components
3. The DNA can be extracted from the cell nucleus

Preparing DNA Samples for Fingerprinting

Amplification

- VNTR analyses—polymerase chain reaction (PCR) can be used to amplify the DNA that contains the VNTRs
- STR profiles—restriction enzymes are unnecessary; PCR allows the amplification of the strands with STR sequences

PCR--Polymerase Chain Reaction

PCR is a technique for making many copies of a defined segment of a DNA molecule. It looks at six different inherited traits, each controlled by a specific gene. Every gene has at least two alternative forms called alleles. An individual receives one allele from mother and one from father. If the alleles are the same, the individual is said to be homozygous for the trait; if the two alleles are different, the individual is heterozygous.

PCR--Polymerase Chain Reaction

- Heat the DNA strands which causes the strands to separate (unzip).
- Cool the mixture and add primers, 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 degrees C for the completion.

PCR--Polymerase Chain Reaction

The outcome is a doubling of the number 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.

Preparing DNA Samples for Fingerprinting

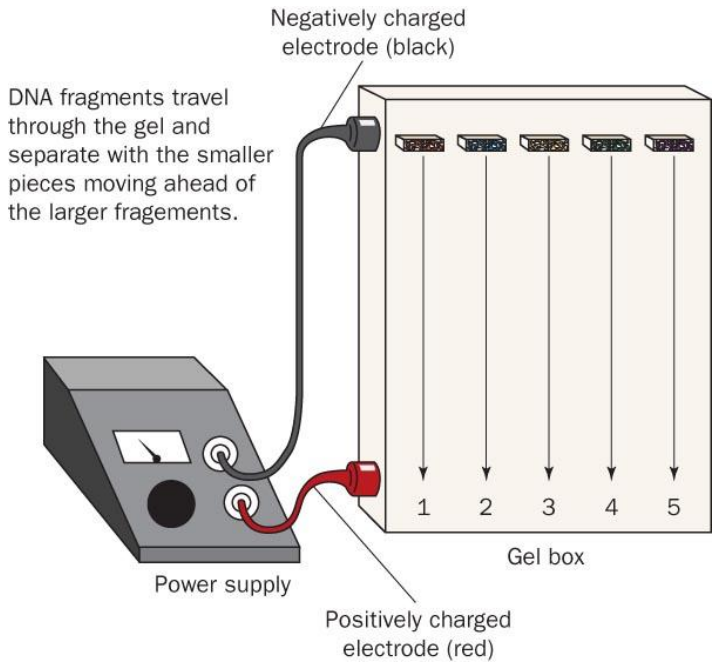
Loading the gels

Electrophoresis

- DNA samples are placed in gels through which electronic currents are passed
- DNA fragments line up in bands along the length of each gel

Electrophoresis

- An electrophoresis apparatus running five sample of DNA
- Arrows show the movement of the negatively charged DNA fragments through the gel matrix

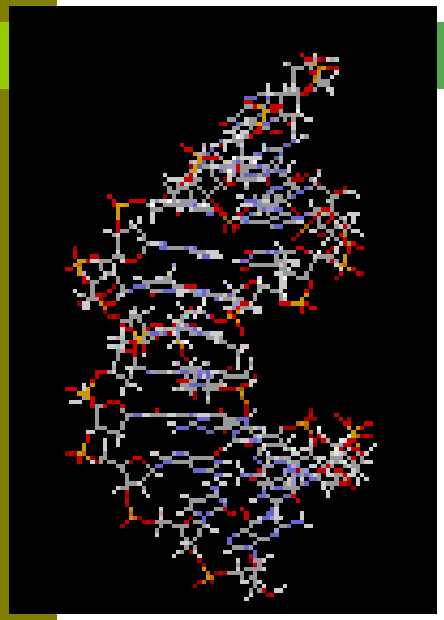


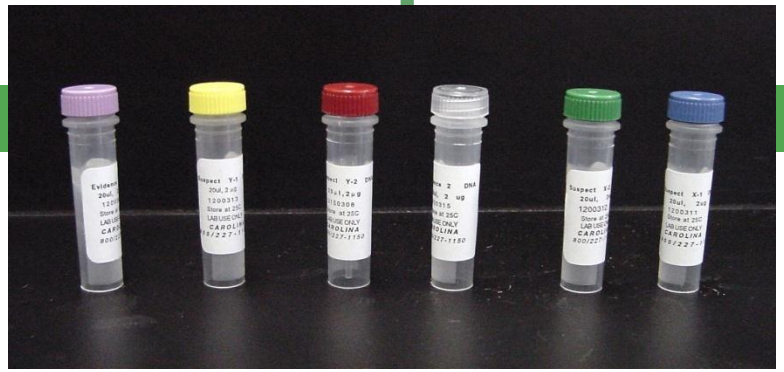
Electrophoresis

An electrical current moves through a substance causing molecules to sort by size.

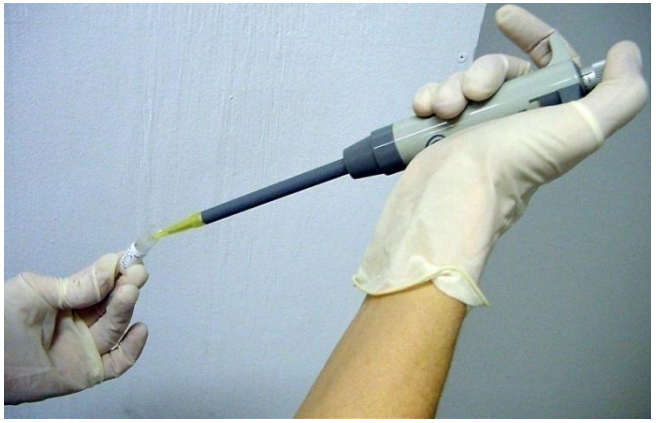
Smaller, lighter molecules will move the furthest on the gel.

Electrophoresis

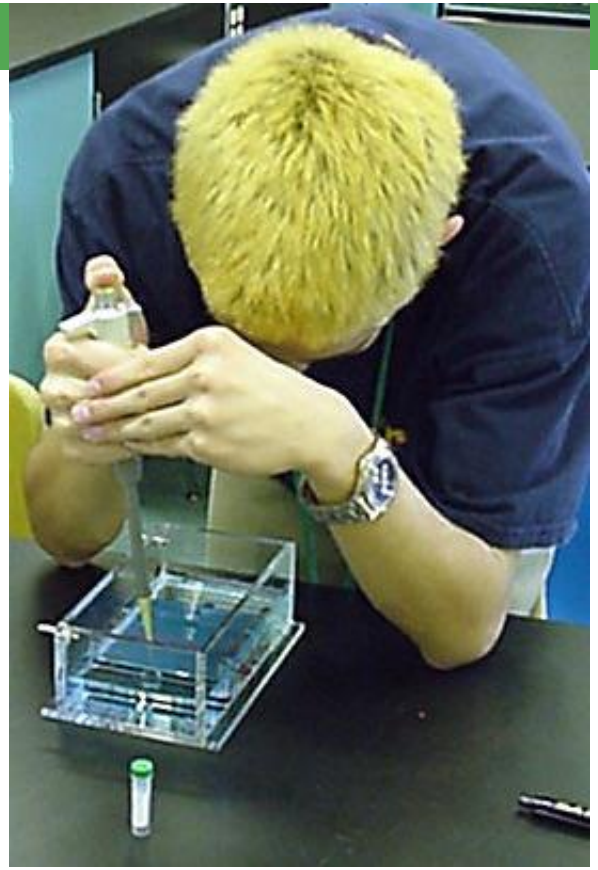




Pipette the DNA.



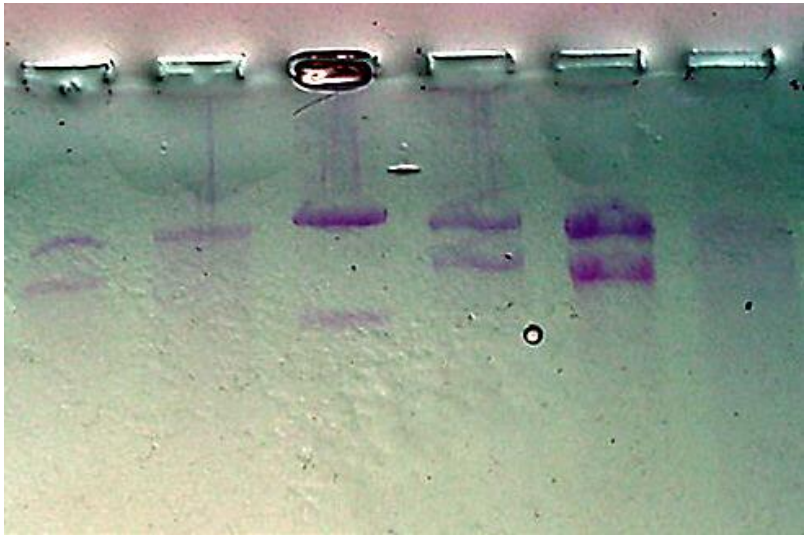
Electrophoresis (cont.)



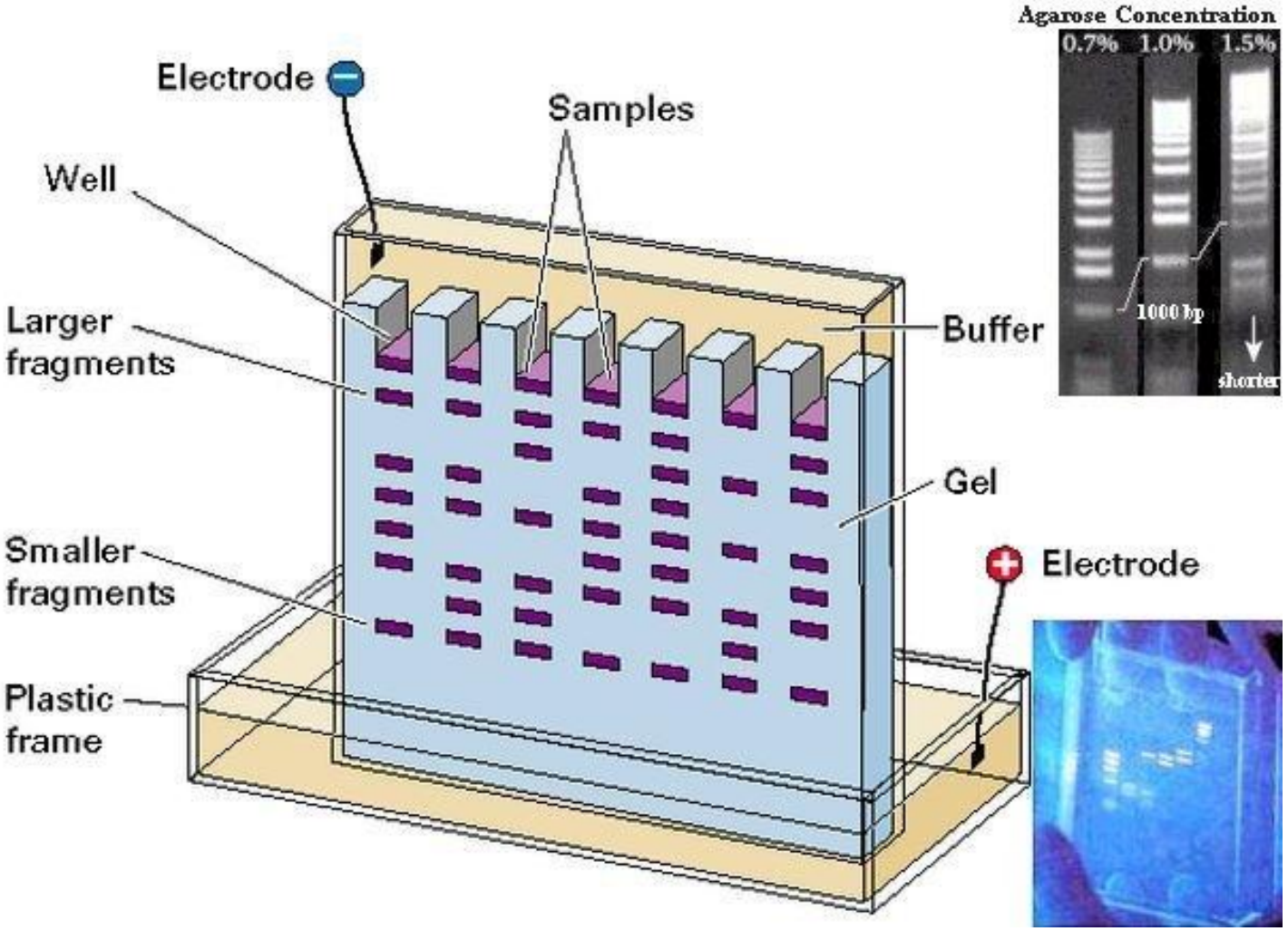
Load DNA into the gel wells.

Electrophoresis (cont.)

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



Gel Analysis



Analysis of DNA Fingerprints and Applications

Bands and widths are significant in matching samples of DNA

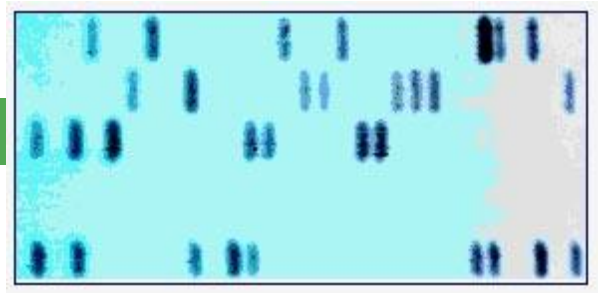
Three Possible Outcomes

- **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.

DNA Profile Matching

- Tissue Matching
  - Two samples that have the same band pattern are from the same person
- Inheritance Matching
  - Each band in a child's DNA fingerprint must be present in at least one parent

Probes



- DNA probes
  - identify the unique sequences in a person's DNA
  - are made up of different synthetic sequences of DNA bases complimentary to the DNA strand
  - bind to complimentary bases in the strand (see the fragmentary DNA bands above)
- In most criminal cases, 6 to 8 probes are used

Analysis of DNA Fingerprints and Applications

- DNA fingerprinting can
  - match crime scene DNA with a suspect
  - determine maternity, paternity, or match to another relative
  - eliminate a suspect
  - free a falsely imprisoned individual
  - identify human remains

. . . . . Summary . . . .

- DNA contains the information needed for replication in a sequence of nitrogenous bases.
- DNA analysis allows even a small sample of tissue to be identified with a single individual.
- DNA contains, in non-coding regions called *junk DNA,* many repeated sequences that vary in number between individuals.
- These differences between individuals can be used to produce a DNA fingerprint for an individual.

Summary

- Polymerase chain reaction (PCR) for DNA amplification has largely eliminated the problem resulting from the tiny samples usually available.
- DNA evidence must be collected carefully to avoid contamination with other DNA.
- DNA analysis involves extraction, electrophoresis, and visualization.
- DNA profiles are kept by police agencies in electronic databases.