**AL Safwa University College**

**Dept. of *Medical Lab. Technique***

# HUMAN GENETIC LABORATORY

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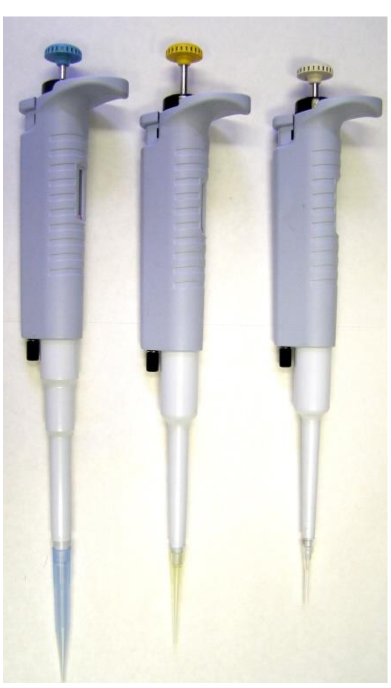
# Lab:1

## Instruments That used in Medical genetics field:

There are many devices used in the field of medical genetics, but we will address the main devices commonly used in this aspect.

**-Microliter pipettes:** A pipette is a laboratory tool commonly used in chemistry, biology and medicine to transport a measured volume of liquid.



**Microliter pipettes**

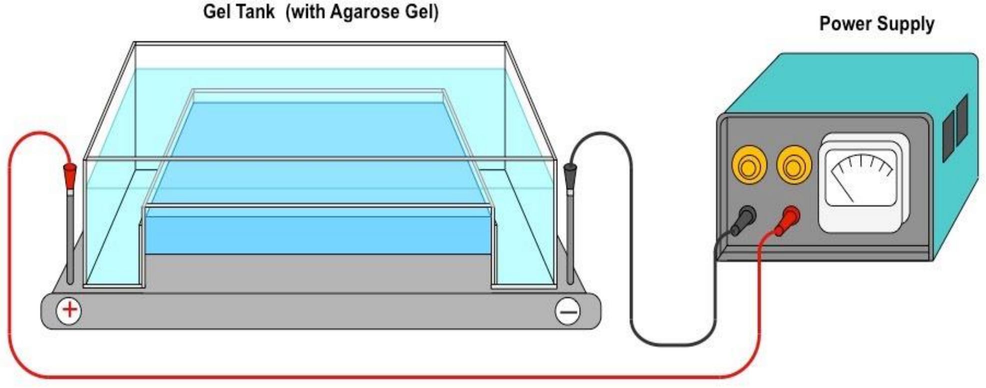
**Cooling centrifuge :**

Refrigeration constitutes an important added feature to any laboratory centrifuge. Refrigerated laboratory centrifuges temperature ranges as wide as -20C – 40C, making them perfect for DNA, RNA, PCR or antibody analysis.

**Cooling centrifuge**

## Gel Electrophoresis:

It used in laboratories to separate macromolecules based on size. The technique applies a negative charge so proteins move towards a positive charge. Electrophoresis is used for both DNA and RNA analysis.




**Gel Electrophoresis**

**Nanodrop device:**

This system allows scientists to quickly and easily quantify and assess purity of samples such as proteins and nucleic acids.

**Nanodrop device**

## PCR device:

technique used in genetics molecular biology to exponentially amplify a single copy or a few copies of a specific segment of DNA to generate thousands to millions of copies of a particular DNA sequence.

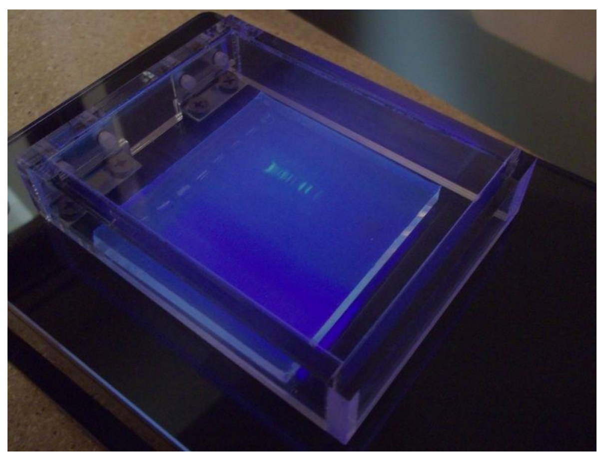
## PCR device

## RT-PCR:

A highly sensitive technique for the detection and quantitation of mRNA (messenger RNA). The technique consists of two parts: The synthesis of cDNA (complementary DNA) from RNA by reverse transcription (RT) and the amplification of a specific cDNA by the polymerase chain reaction (PCR).

## Ultra Violet Light Transiluminator:

An ultra-violet (UV) transilluminator is a standard piece of equipment used in life science laboratories for visualization of target DNAs and proteins. The UV transilluminator works by emitting high levels of UV radiation through the viewing surface.



## Water bath:

A water bath is laboratory equipment made from a container filled with heated water. It is used to incubate samples in water at a constant temperature over a long period of time.

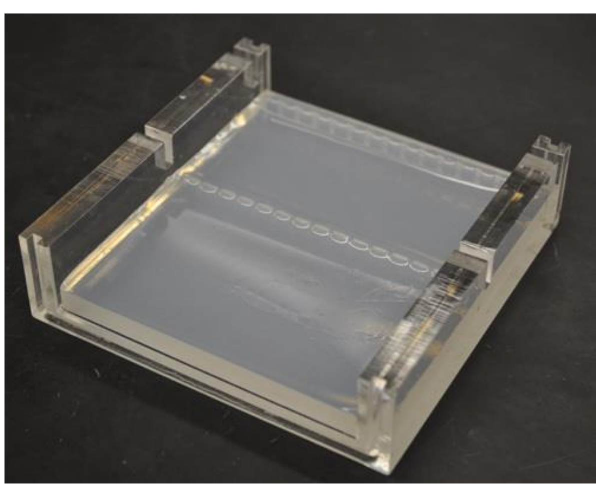
## Vortex:

A piece of laboratory equipment used to mix the contents of small tubes of liquids by means of rapid oscillation.

## -Material used in Medical genetics field:

### Agarose:

Agarose is a polysaccharide, generally extracted from certain red seaweed.. Agarose is frequently used for the separation of large molecules, especially DNA, by electrophoresis. Shorter DNA molecules can travel farther in an agarose gel in a given amount of time than longer counterparts.

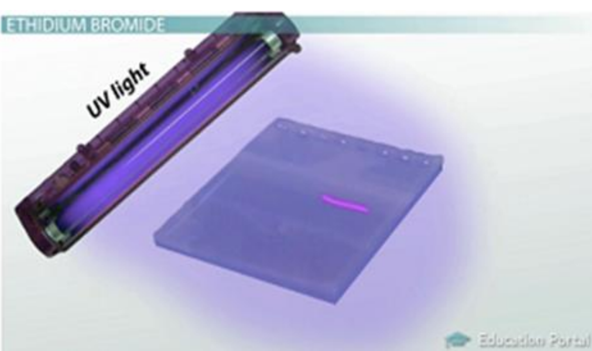


## Ethidium Bromide:

Ethidium bromide is a molecule commonly used to visualize DNA in agarose gel electrophoresis experiments. It both binds to DNA and fluoresces under the proper conditions. Ethidium bromide is known as an intercalating agent. The flat structure of ethidium bromide allows it to intercalate, or insert, between nitrogenous bases of a DNA molecule.When it is exposed to ultraviolet light, ethidium bromide fluoresces.

However, note that any molecule that binds to or in any way alters the molecular structure of DNA can be dangerous to a living organism. Although there is some debate about the exact nature of the health risk

posed by exposure to ethidium bromide, most organizations and agencies do consider it a risk. Because of the intercalating characteristic of ethidium bromide, it is believed by many to pose a mutagenic risk. However, with strong safety protocols in place, ethidium bromide continues to be one of the most common methods of visualizing nucleic acids in agarose gel electrophoresis experiments.




Ethidium bromide binds to DNA and fluoresces under an ultraviolet light

## Loading dye:

It contains two different dyes (bromophenol blue and xylene cyanol ) function of loading dye in electrophoresis is to allow the DNA sample to sink into the wells of the gel and to allow scientists to visually track the DNA sample as it runs through the gel.

## Ethylene diaminetetra acetic acid (EDTA):

EDTA is a chelator of divalent cations, particularly of magnesium ($Mg2^+$). As these ions are necessary co-factors for many enzymes, including contaminant nucleases, the role of the EDTA is to protect the nucleic acids against enzymatic degradation. But since $Mg2^+$ is also a co-factor for many useful DNA-modifying enzymes such as restriction enzymes and DNA polymerases.

## Tris Base Buffer:

Tris (hydroxymethyl) aminomethane, is a common biological buffer, It is an established basimetric standard and buffer used in biochemistry and molecular biology1. It is used in the formulation of buffer solutions in the pH range between 7.5 and 8.5. Tris buffer solutions are widely used in cell and molecular biology for processes such as protein and nucleic acid extraction and purification.

## TE buffer :

TE buffer is a commonly used buffer solution in molecular biology, especially in procedures involving DNA and RNA. "TE" is derived from its components: Tris, a common pH buffer, and EDTA, a molecule that chelates cations like $Mg2^+$. The purpose of TE buffer is to solubilize DNA or RNA, while protecting it from degradation.

# Lab:2

# Introduction to genetics & Cell Division

## Introduction to genetics:

Genetics : is the study of how genes bring about characteristics, or traits, in living things and how those characteristics are inherited , and explain how these features pass from generation to generation .

***In genetics**, a **feature** of a living thing is called a "**trait**". Some traits are part of an **organism's physical appearance**; such as **a person's eye-color**, **height** or **weight.**

**Other sorts** of traits are **not easily seen** ( **hidden** ) and include : **blood types** or **resistance to diseases**, the way our genes and environment interact to produce a trait can be complicated, for example, the chances of somebody **dying of cancer** or **heart disease** seems to depend on both their genes and their lifestyle.

**The language** used by DNA is called **the genetic code** , which allows the genetic machinery to read the information in the genes in **triplet sets** of codons.

The Austrian monk ***Gregor Mendel*** : **developed the science of genetics**. Mendel performed his experiments in the 1860s and 1870s, but the scientific community did not accept his work until early in the twentieth century. Because the principles established by Mendel form the basis for genetics, the science is often referred to as ***Mendelian genetics*.** It is also called ***classical genetics*** to distinguish it from another branch of biology known as **molecular genetics**.

Mendel believed that factors pass from parents to their offspring, **but he did not know of the existence of DNA**.

Modern scientists accept that **genes** are **composed of segments of DNA** molecules that control discrete hereditary characteristics.

**Nucleotides :** They form the rungs of the DNA ladder and are the repeating units in DNA. There are **four types of nucleotides (Adenine, Thymine, Guanine and Cytosine**) and it is the sequence of these nucleotides that carries information **in triplet sets called codon** .

**DNA:** A long molecule that looks like a twisted ladder. It is made of four types of simple units called Nucleotides, and this units that line up in a particular order within this large molecule. The order of these units carries genetic information, similar to how the order of letters on a page carries information.

**Gene:** Are portions of DNA molecules which is copied and inherited across generations , that determine characteristics of living things or providing information that an organism needs so it can (build or do something - like making an eye or a leg , or repairing a wound), through the processes of meiosis and reproduction, genes are transmitted from one generation to the next ; for example, children usually look like their parents because they have inherited their parents' genes .

***The genome for a human cell consists of about 100,000 genes.**

**Allele: The different forms of a given gene that an organism may possess**. For example, in humans, one allele of the eye-color gene produces green eyes and another allele of the eye-color gene produces brown eyes.

**Chromosome: A package for carrying DNA associated with especial protein in the nucleus cells**. that is bunched together into a compact structure called chromosome. Different species of organism have different numbers and sizes of chromosomes

**Genome:**The complete set of genes in a particular organism.

**Genetic engineering:** When people change an organism by adding new genes, or deleting genes from its genome.

**Mutation:** An event that changes the sequence of the DNA in a gene.

**phenotype:** A external individual form , or external appearance.

**Genotype:** A Genetic structure( composition) for individual.

**Diploid cell:** Cells have a double set of chromosomes, one from each parent. For example, human cells have a double set of chromosomes consisting of 23 pairs, or a total of 46 chromosomes, **(there are two genes for each characteristic).**

**Haploid cell OR Mono -ploid:** A cell have a single set of chromosomes. These haploid cells are gametes, or sex cells (sperm , ovum ) and they are

formed through meiosis. When gametes come together in sexual reproduction, the diploid condition is reestablished.

## Cell Division:

The cell cycle or cell-division cycle is the series of events that take place in a cell leading to its division and duplication (replication) that produces two daughter cells.

The cell cycle can be divided into three periods:

**1- interphase.**

**2- M– phase.**

a- mitosis

b- meiosis

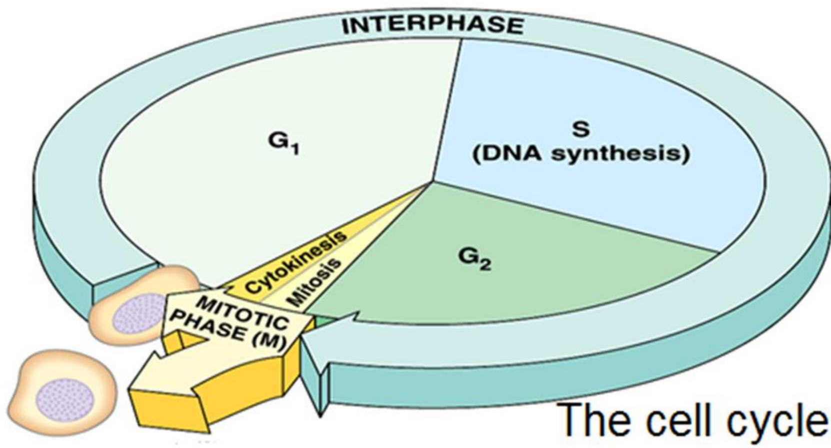
**3- cytokinesis.**

**1.Interphase** Aphase at which the cell contents replicate. It include 3 stages of cell cycle:

**1-G1 (Gap 1):-** It is the interval between mitosis and the DNA replication. During this phase the cell grows and a synthesis of RNA and protein takes place.

**2-S (Synthesis):-** During this phase,DNA replication occurs.

**3-G2 (Gap 2):-** During this phase, the cell prepares for mitosis. By the time of G2, the cell contains 2 identical copies of each 46 chromosomes, called **sister chromatids**. Sister chromatids exchange materials during or after S phase, a process known as **Sister chromatid exchange.**




**2.a.Mitosis** The process of cell division which results in the production of two daughter cells from a single parent cell. **Mitosis can be divided into stages**

- Prophase
- Metaphase
- Anaphase
- Telophase

***Prophase** 1- Chromatin **condense** into well defined chromosomes.
2- Each chromosome is made up of 2 chromatids joined by a centromere.
3- The centrosomes duplicate and transform into asters.
4- A spindle of fibers forms ( achromatic spindle).

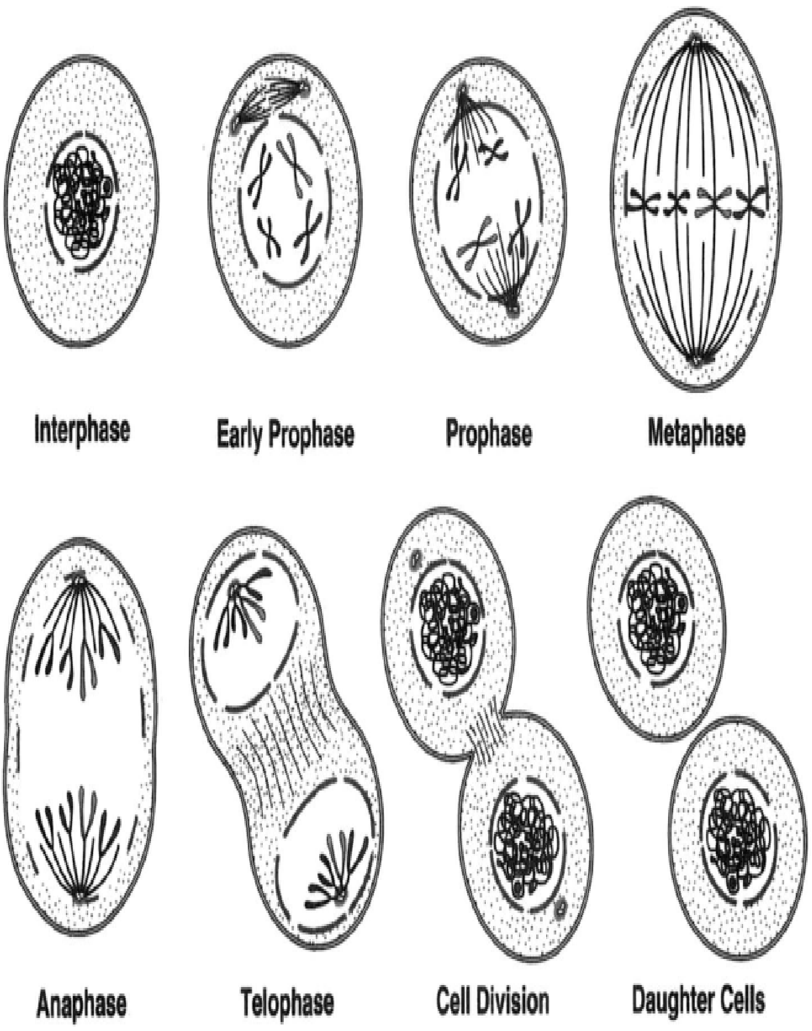
***Metaphase** 1-Chromosomes line up at the center of the cell.

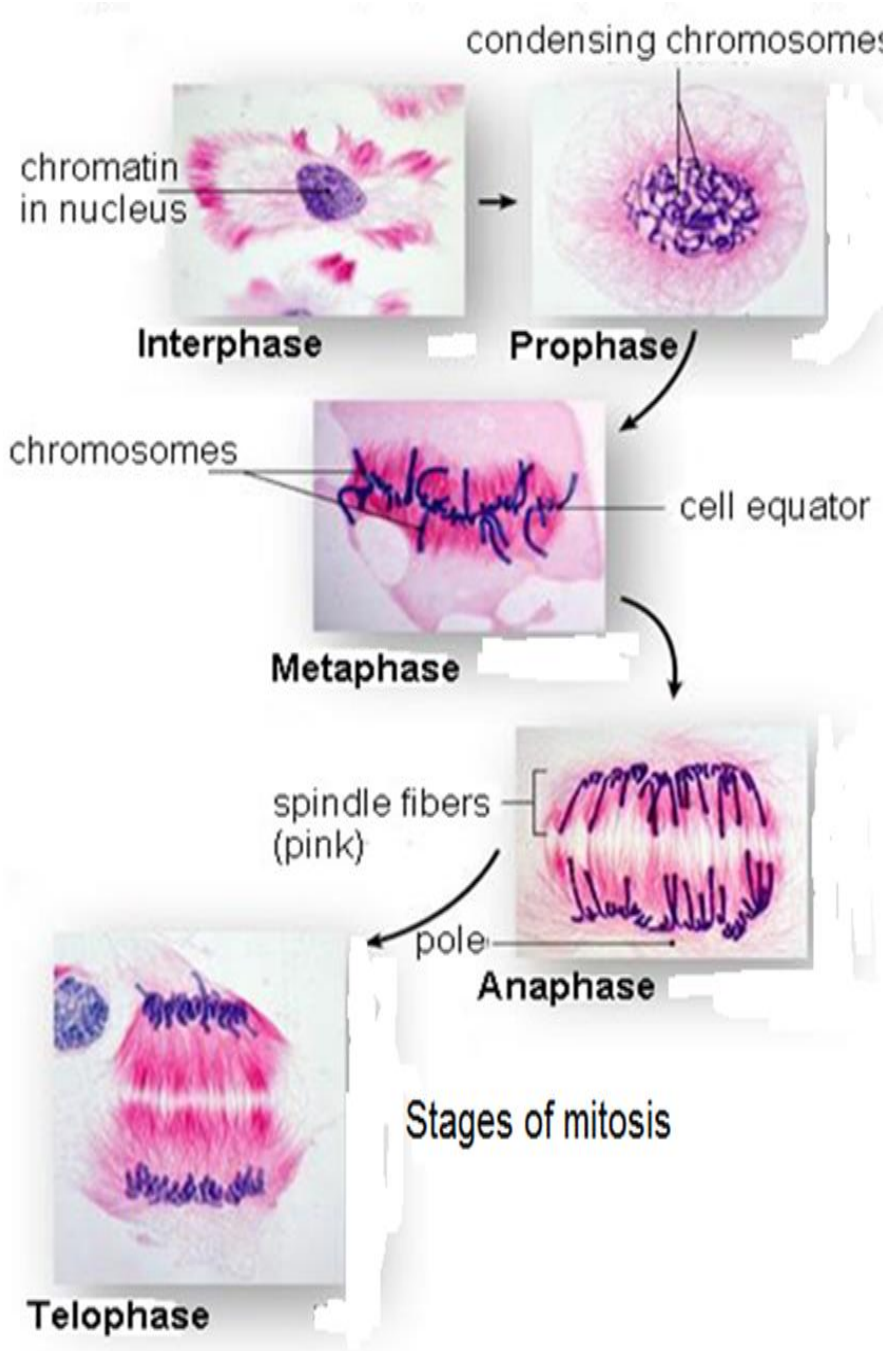
2-Spindle fibers attach from daughter cells to chromosomes at the centromere.

***Anaphase** 1- The centromeres split and the sister chromatids separate. Each chromosome is now formed of one chromatid with a centromere.
2- Sister chromatids move towards opposite poles of the cell.

***Telophase** 1- The chromosomes **de-condense** and go back to the **chromatin** state (tall and thin).
2-A new nuclear membrane forms.
3- All the spindle fibers break down and disappear.
4- The cytoplasm of the mother cell will be cleaved in half, and the cell membrane will finish its constriction at the middle of the cell to divide the mother cell into two separate daughter cells that enter into a new interphase.

## MITOSIS




condensing chromosomes
chromatin
in nucleus
Interphase
Prophase
chromosomes
cell equator
Metaphase
spindle fibers
(pink)
pole
Anaphase
Telophase
Stages of mitosis

# Lab 3:

# Cell Division (Meiosis)

## 2-b-Meiosis:

Meiosis is the process in eukaryotic, sexually-reproducing animals that reduces the number of chromosomes in a cell before reproduction. Many organisms package these cells into gametes, such as egg and sperm. The gametes can then meet, during reproduction, and fuse to create a new zygote. Because the number of alleles was reduced during meiosis, the combination of two gametes will yield a zygote with the same number of alleles as the parents. In diploid organisms, this is two copies of each gene.

## Phases of Meiosis:

Two successive nuclear divisions occur, Meiosis I (Reduction) and Meiosis II (Division). Meiosis produces 4 haploid cells. Mitosis produces 2 diploid cells. The old name for meiosis was reduction/ division. Meiosis I reduces the ploidy level from 2n to n (reduction) while Meiosis II divides the remaining set of chromosomes in a mitosis-like process (division). Most of the differences between the processes occur during Meiosis I.

- Meiosis is divided into :

1. **Meiosis I**
   a-Prophase I
   b-Metaphase I
   c-Anaphase I
   d-Telophase I
2. **Meiosis II**
   a-Prophase II
   b-Metaphase II
   c-Anaphase II
   d-Telophase II

## 1-Meiosis I:

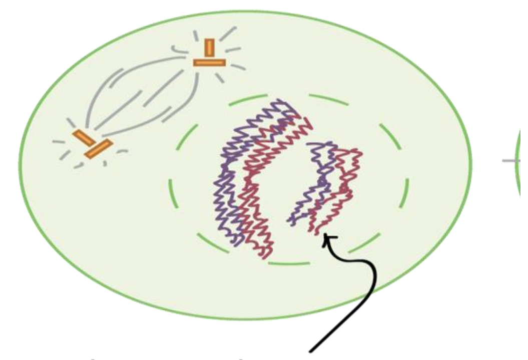
Meiosis I segregates homologous chromosomes, producing two haploid cells (n chromosomes, 23 in humans). Because the ploidy is reduced from diploid to haploid, meiosis I is referred to as a **reductional division**.

**A-Prophase I**

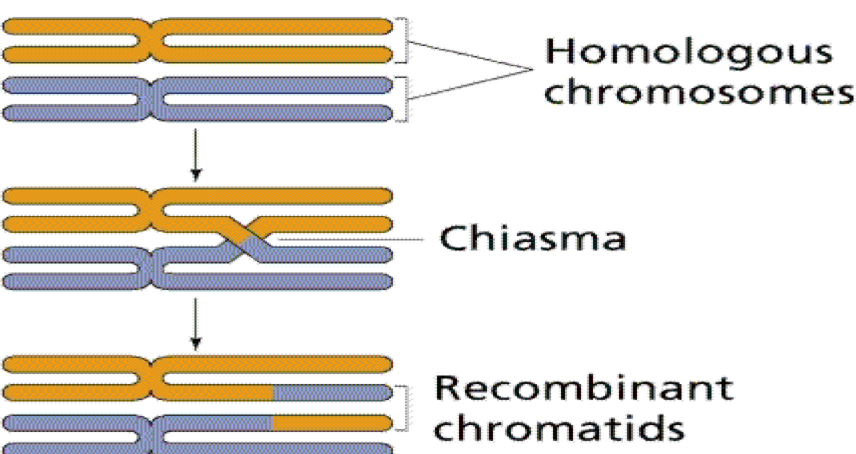
- Each chromosome duplicates and remains closely associated. These are called sister chromatids.
- Cross- Over can occur during the latter part of this stage.
  *(**chiasma**) is the point of contact, the physical link, between two (non-sister) chromatids belonging to homologous chromosomes.

Prophase I

starting cell is diploid ($2n = 4$)

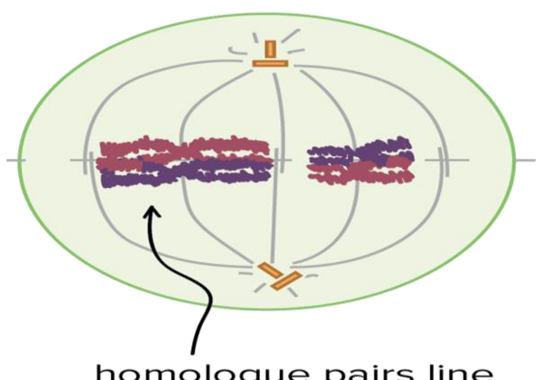


homologous chromosomes pair up and exchange fragments (crossing over)



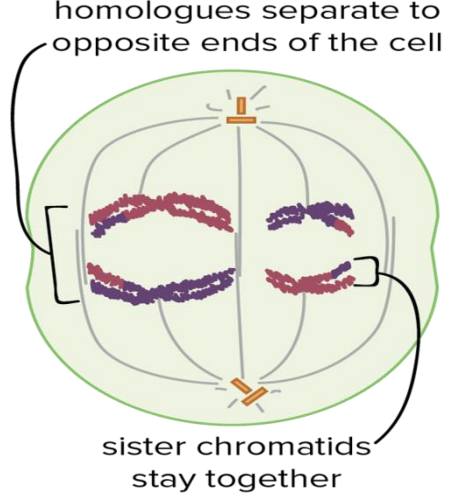

**B-Metaphase I**

- Homologous chromosome move together along the metaphase plate.
- Chromosomes line up at the center of the cell.



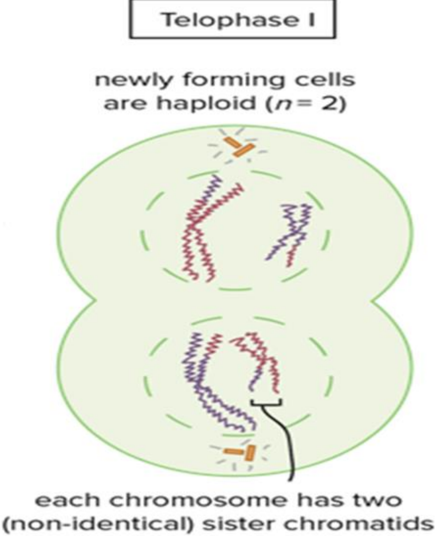

## C-Anaphase I

- The homologues chromosome are pulled apart and move apart to opposite ends of the cell.
- The sister chromatids of each chromosome, however, remain attached to one another and don't come apart.




## D-Telophase I

- Two daughter cells are formed with each daughter containing only one chromosome of the chromosome pair.

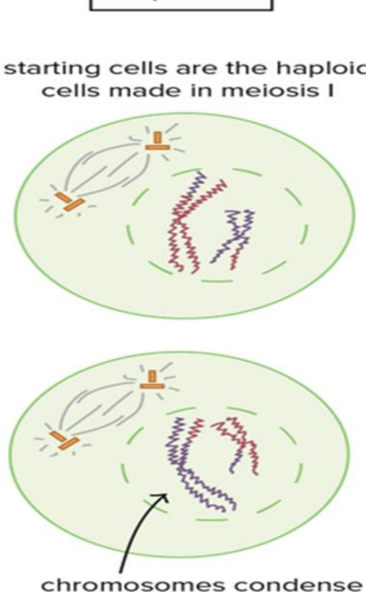



## 2-Meiosis II

Second division of meiosis .Meiosis II is an equational division analogous to mitosis, in which the sister chromatids are segregated, creating four haploid daughter cells.

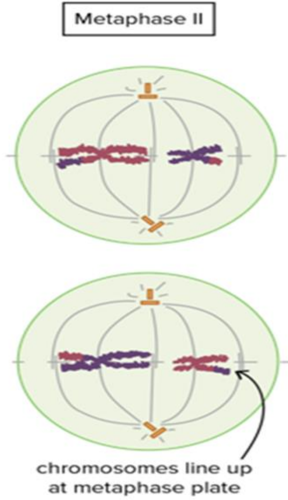
### A-Prophase II

- Chromosomes condense.
- Nuclear membrane dissolves.
- Centrosomes move to opposite poles.
- DNA does not replicate.



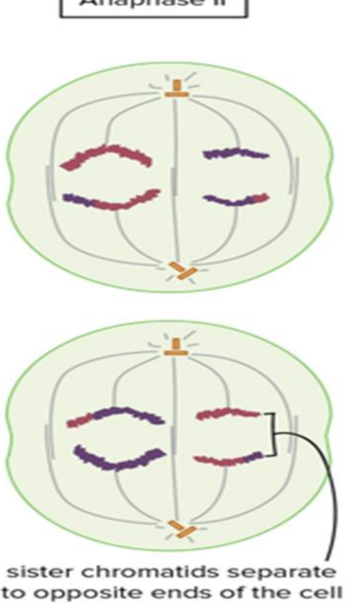
**B-Metaphase II**

- chromosome align at the equatorial plate
- Spindle fibres from opposing centrosomes attach to chromosomes (at centromere).



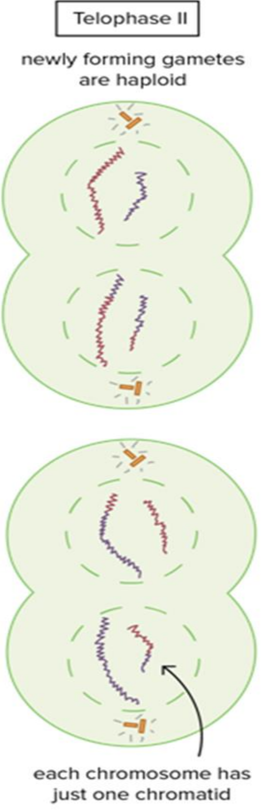

**C-Anaphase II**

- Spindle fibres contract and separate the sister chromatids.
- chromatids (now called chromosomes) move to opposite poles.



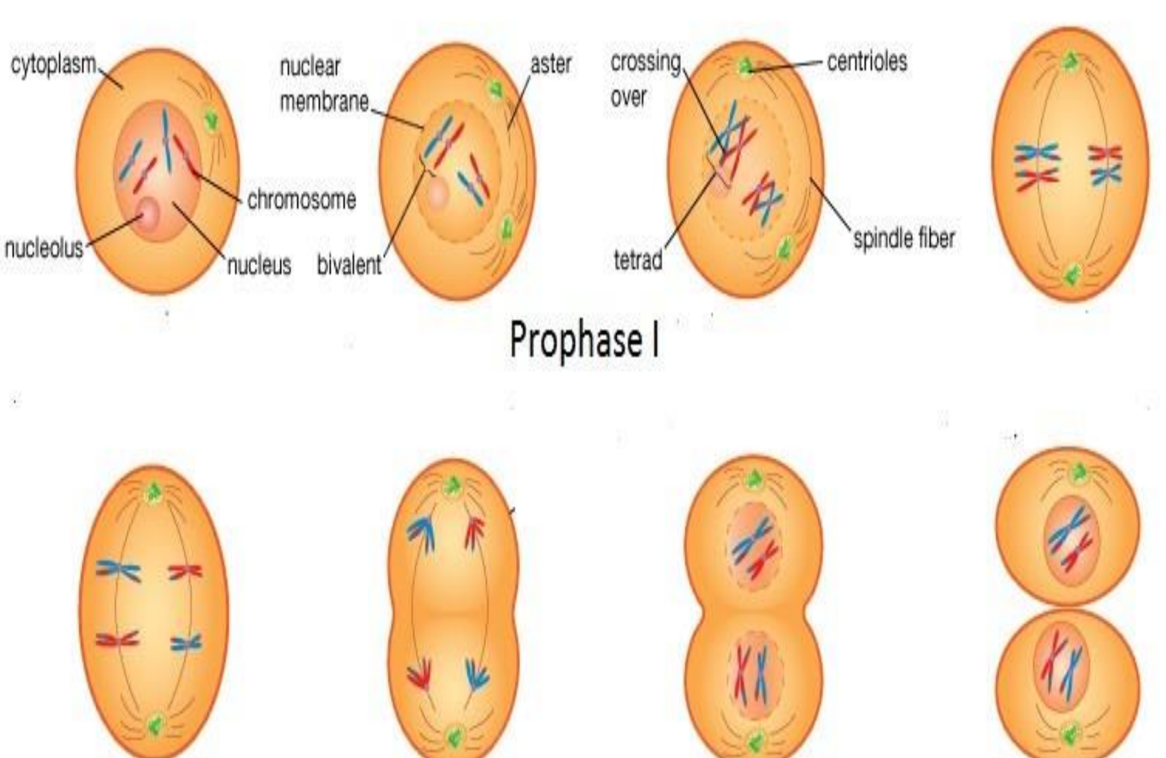
## D-Telophase II

- Nuclear envelopes reform.
- Cells divide (cytokinesis) to form four haploid daughter cells .

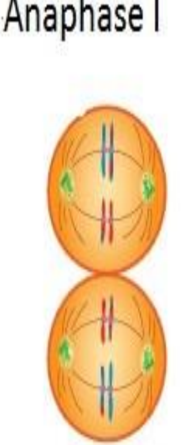
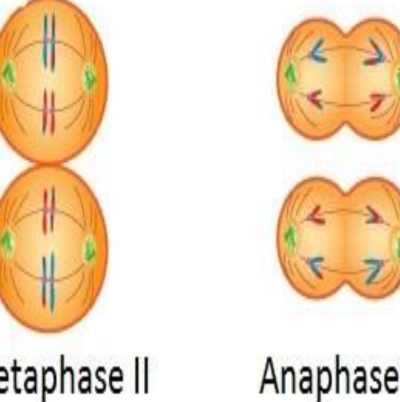
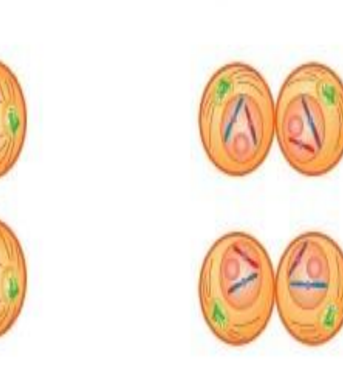



## 3- Cytokinesis:

- Starts in **mid-Anaphase** and ends by the end of **Telophase** dividing the cell into 2 daughter cells.
- Occurs by **invagination of the cell membrane** in animal cells & by **cell plate method** in plant cells.




Metaphase I Anaphase I Telophase I Cytokinesis

Prophase II Metaphase II Anaphase II Telophase II

# Comparison between Mitosis and Meiosis

| MITOSIS | MEIOSIS |
|---|---|
| • Occurs in somatic (body) cells | • Occurs in germ (sex) cells |
| • Produces identical cells | • Produces gametes |
| • Diploid cell → diploid cells | • Diploid cell → haploid cells |
| • 1 cell becomes 2 cells | • 1 cell becomes 4 cells |
| • Number of divisions: 1 | • Number of divisions: 2 |

# Lab:4

## Cytogenetics

It is a branch of genetics that is concerned with the study of the structure and function of the cell, especially the chromosomes.

In the routine laboratory environment, human cytogenetic is always concerned with light microscope studies of chromosomes.

**SOURCES OF SPECIMENS FOR CHROMOSOME ANALYSIS**

Chromosome preparations for cytogenetic analysis are made from **dividing cells**, either directly from **tissue samples** (e.g. bone marrow, testis, chorionic villi, neoplastic tissue) or after **cell culture** (biopsy of skin or almost any other living tissue including amniotic fluid cells).

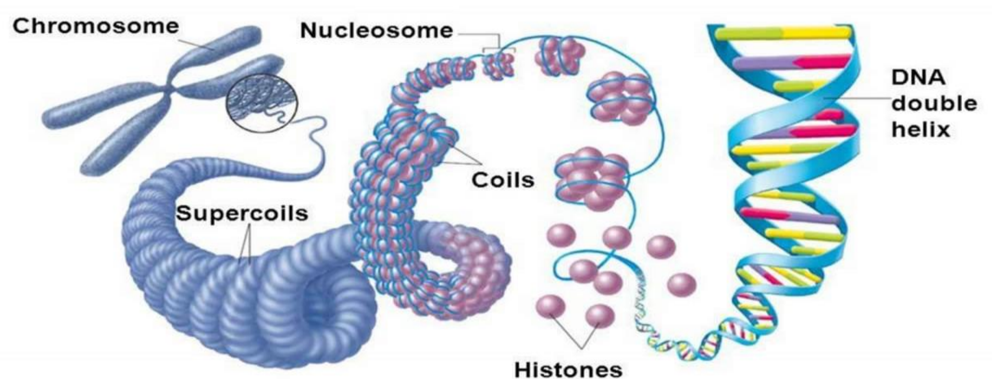
**The human chromosome:**

The human chromosome is the basic building block of life and is one of the most important components of the cell to be transmitted from generation to generation. It is essentially an organized structure of DNA that exists within the nucleus of all human cells and comprises a single chain of DNA that is coiled and super coiled to form dense thread like pieces.

-It is complexed with many structural proteins called **histones** as well as associated transcription factors and several other macromolecules.

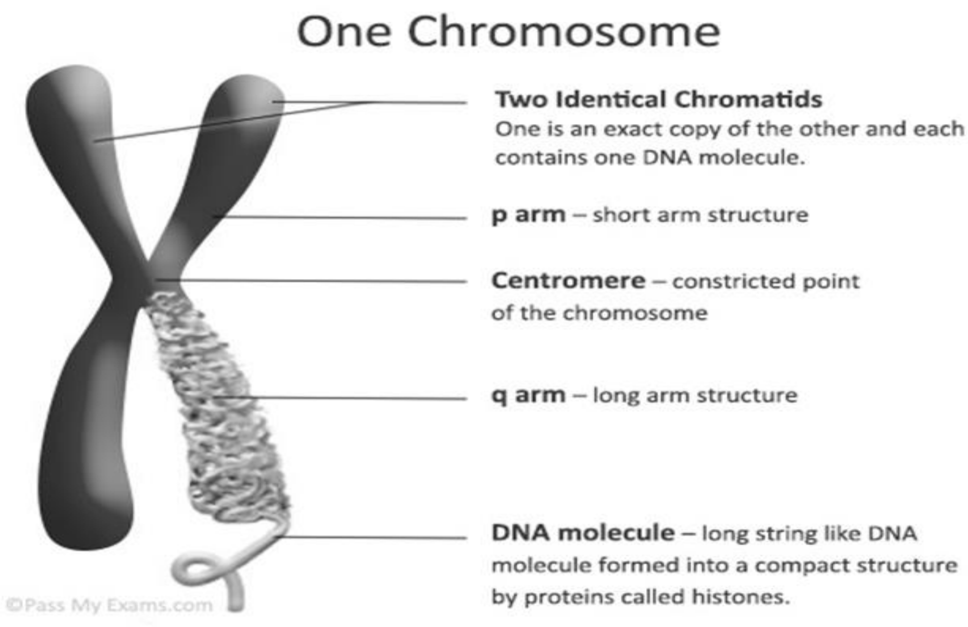
-Chromosomes are normally visible under a light microscope only when the cell is undergoing mitosis.

-In eukaryotes, nuclear chromosomes are packaged by proteins into a condensed structure called **chromatin**. This allows the very long DNA molecules to fit into the cell nucleus.




*The **centromere** is the part of a chromosome that links sister chromatids. During mitosis, spindle fibers attach to the centromere via the **kinetochore**.

Each chromosome has **two arms**, labeled **p** (the shorter of the two) and **q** (the longer).




**The Classification of chromosome:**

All chromosomes are classified according to:

**1-** Number of centromeres (centromere: primary constriction of the chromosome).

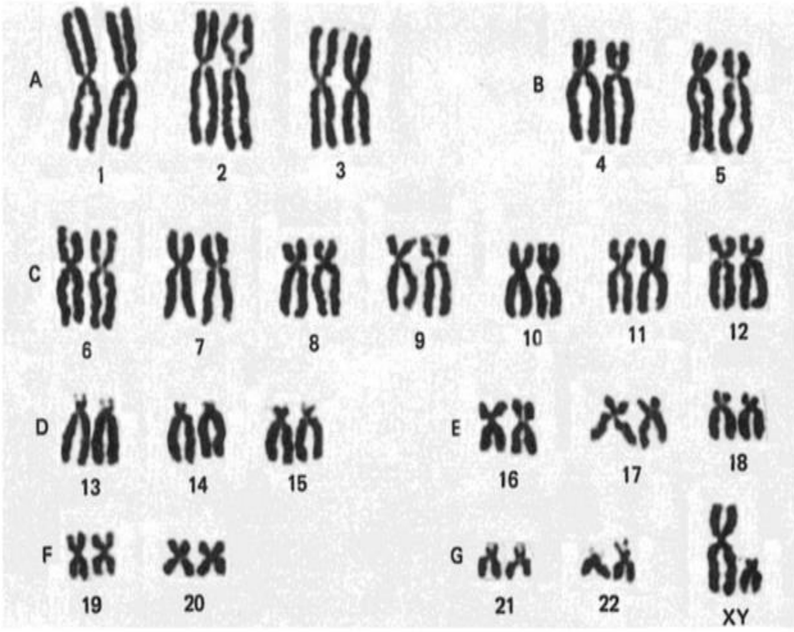
- **Monocentric chromosome:** only one centromere.
- **Diecentric chromosome:** two centromeres.
- **Polycentric chromosome**: more than two centromeres.

**2-**Based on location of centromeres:

| Centromere location | Designation | Metaphase shape |
|---|---|---|
| Middle | Metacentric | Sister chromatids, Centromere |
| Between middle and end | Submetacentric | p arm, q arm |
| Close to end | Acrocentric | |
| At end | Telocentric | |

**3-** Chromosomes are categorized into seven groups on the of size and centromere location:

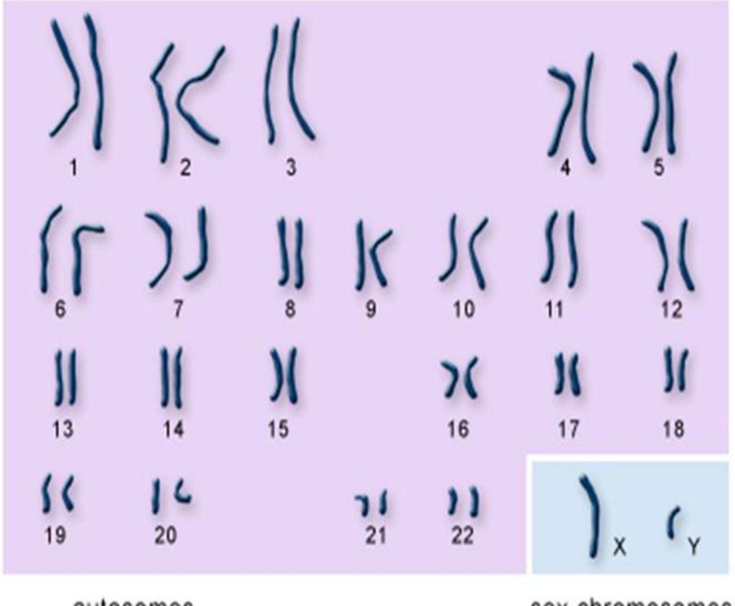
- Group A : chromosomes 1-3 are largest with metacentric
- Group B : chromosomes 4-5 are large with submetacentric .
- Group C: chromosomes 6-12 are medium sized with submetacentric .
- Group D: chromosomes 13-15 are medium sized with acrocentric .
- Group E: chromosomes 16-18 are short with submetacentric.
- Group F: chromosomes 19-20 are short with metacentric .
- Group G: chromosomes 21-22 are very short with acrocentric .




**4- Sexual and non-sexual classification:**

**- Gonosomes SEX CHROMOSOME:** X and Y chromosome which differ in male and female and responsible for sex determination (female XX, male XY).

**- AUTOSOMAL CHROMOSOME:** There are 22 pairs of autosomes in humans. These code for most of the genetic traits in the body.



# Lab:5

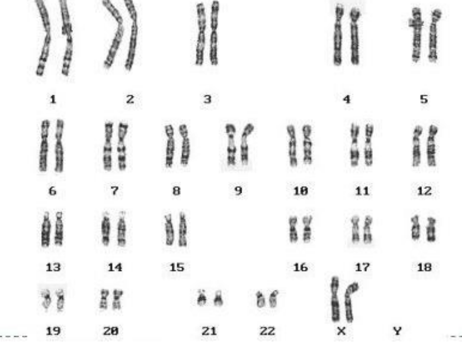
# Karyotyping and Chromosome Banding

## Karyotyping:

**Karyotype** is a profile of organisms chromosomes which are organized by their size, number and shape.

KARYOTYPES

- Test to examine chromosomes
- Can help identify genetic problems by:
  - Counting the number of chromosomes
  - Looking for structural changes in chromosomes
- The test can be performed on almost any tissue:
  - Amniotic fluid
  - Blood
  - Bone marrow
  - Tissue from the placenta

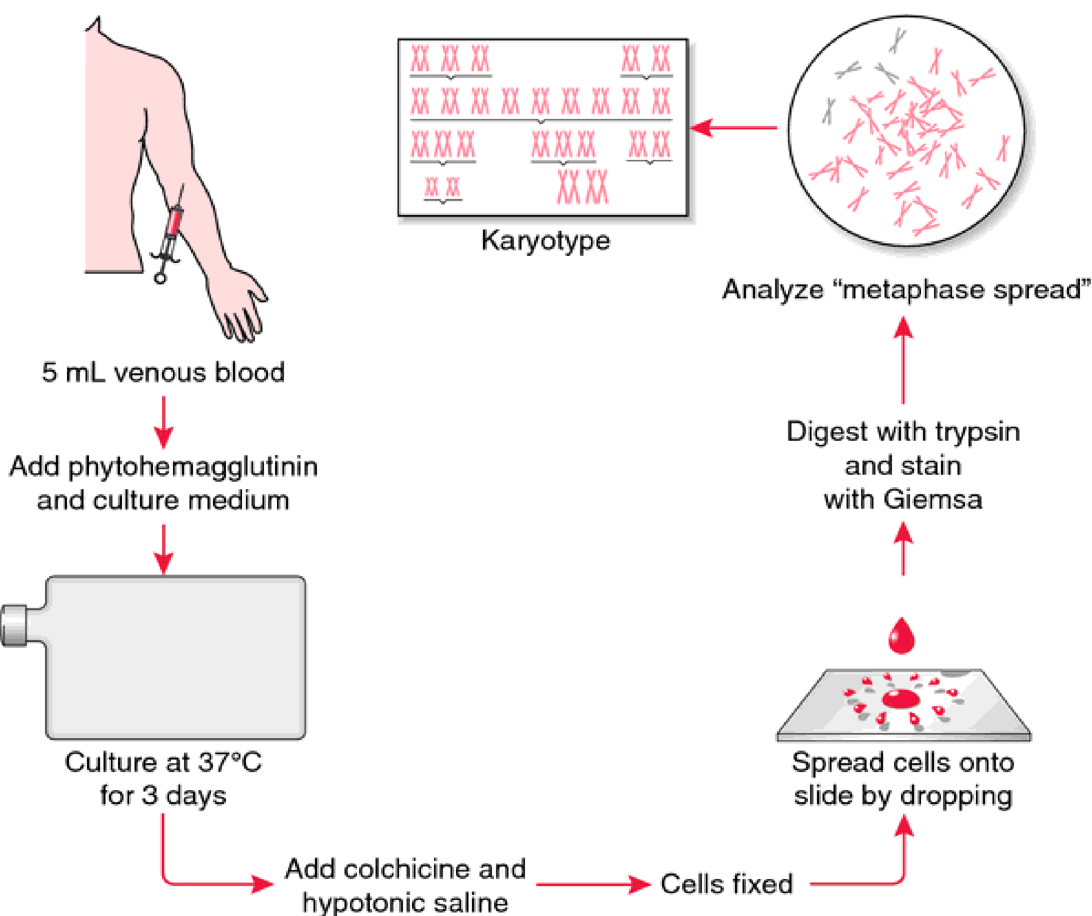



Humans have 22 pairs of autosomal chromosomes (numbered 1 to 22) and 1 pair of sex chromosomes (X, Y). The common **samples** are blood cells, bone marrow cells or amniotic fluid (prenatal diagnosis).This form is typical for one phase of mitosis **metaphase**. We can stop the growth by using some special chemicals (like colchicine or its synthetic derivates). Also the typical striped pattern is caused by chemicals e.g. by **Giemsa dye** which stains adenine and thymine bases The whole test of karyotype takes 1-3 weeks (depending on the material used) and it is done in a cytogenetics laboratory.

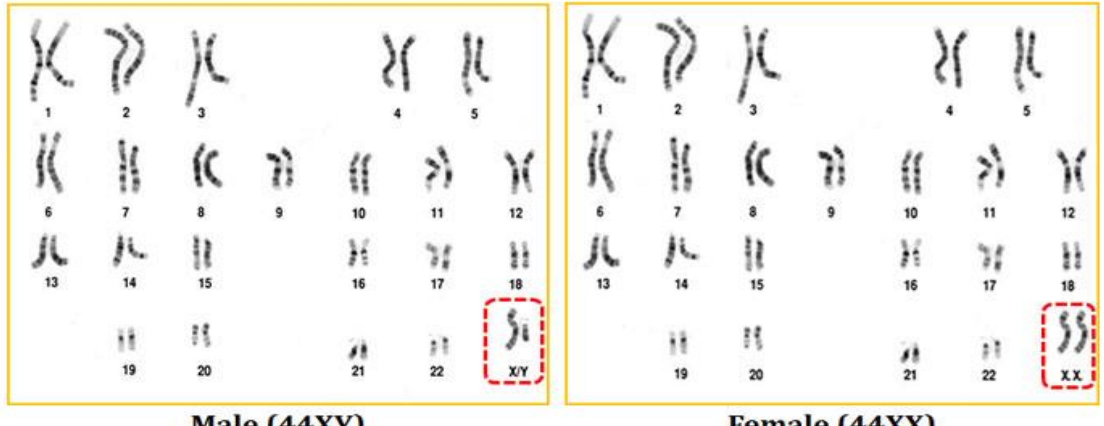
### How Do We Test Karyotype?

1. **Collecting of the samples** – the most common are blood cells (especially the white ones - they are actively dividing).

2. **Growing of the cells** – it is necessary to cultivate the cells on the right medium or cell culture.
3. **Stoping of the growth** – we have to stop division in metaphase.
4. **Dying of chromosomes** – chromosomes are normally colorless so we use various staining and banding methods for visualization.
5. **Sorting of chromosome** – from the largest (chromosome 1) to the smallest (chromosome 22).
6. **Analysing** – structure, pattern, count.




Karyotyping process



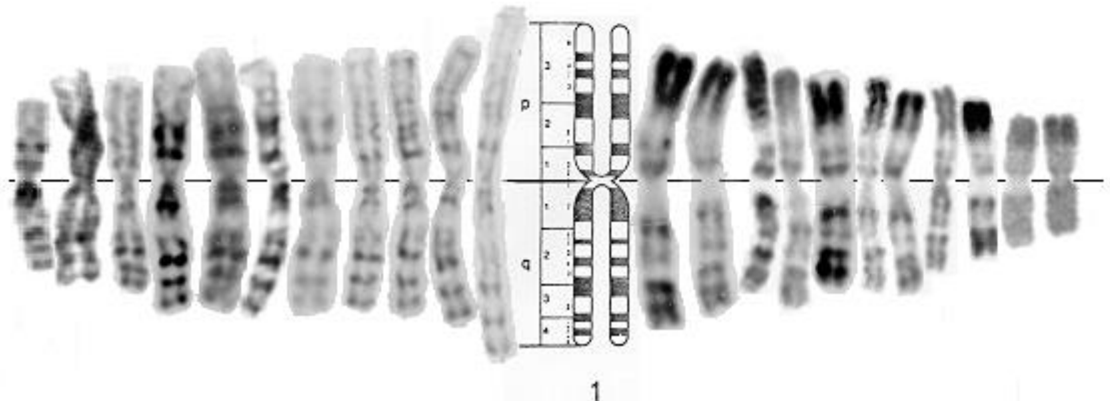

**Chromosome Banding:**

A **band** is defined as that part of a chromosome which is clearly distinguish able from its adjacent segments by appearing darker or brighter with one or more banding techniques. The chromosomes are visualized as consisting of a continuous series of bright and dark bands.

we treat chromosomes with chemicals to stain them and learn about a chromosome by how it stains. There are several different types of stains we can use.

There are several types of **chromosome banding**. Here, we will list a few of the most common types.

- **G-banding** : uses a stain called Giemsa stain. G-banding gives you a series of light regions (tend to GC rich) and dark regions (tend to AT rich) stripes along the length of the chromosome..
- **Q-banding** : uses a stain called quinacrine. Q-banding yields a fluorescent pattern. It is similar in pattern to G-banding, but glows yellow.
- **C-banding** : Giemsa binds to constitutive heterochromatin, so it stains centromeres. The name is derived from centromeric.
- **R-banding (Giemsa stain)** is the reverse of G-banding (the R stands for "reverse"). The dark regions are euchromatic (guanine-cytosine rich regions) and the bright regions are heterochromatic (thymine-adenine rich regions).



# Lab:6

# Chromosomal Abnormalities

Chromosomal abnormalities, which is the addition or subtraction of a chromosome from a pair of homologs or Structural defects in chromosomes are another type of abnormality that can be detected in karyotypes

## How do chromosome abnormalities happen?

Chromosome abnormalities usually occur when there is an error in cell division. There are two kinds of cell division, mitosis and meiosis.

Other factors that can increase the risk of chromosome abnormalities are:

- **Maternal Age:** Women are born with all the eggs they will ever have. Some researchers believe that errors can crop up in the eggs' genetic material as they age. Older women are at higher risk of giving birth to babies with chromosome abnormalities than younger women. Because men produce new sperm throughout their lives, paternal age does not increase risk of chromosome abnormalities.
- **Environment:** Although there is no conclusive evidence that specific environmental factors cause chromosome abnormalities, it is still possible that the environment may play a role in the occurrence of genetic errors.

**There are two type of chromosomal abnormalities:**

- 1-Numerical abnormalities
- 2- Structural abnormalities

**1-Numerical abnormalities**

This is called **aneuploidy** (an abnormal number of chromosomes), and occurs when an individual either is missing a chromosome from a pair (**monosomy**) or has more than two chromosomes of a pair in a **trisomy**, there are three, homologs of a particular chromosome.

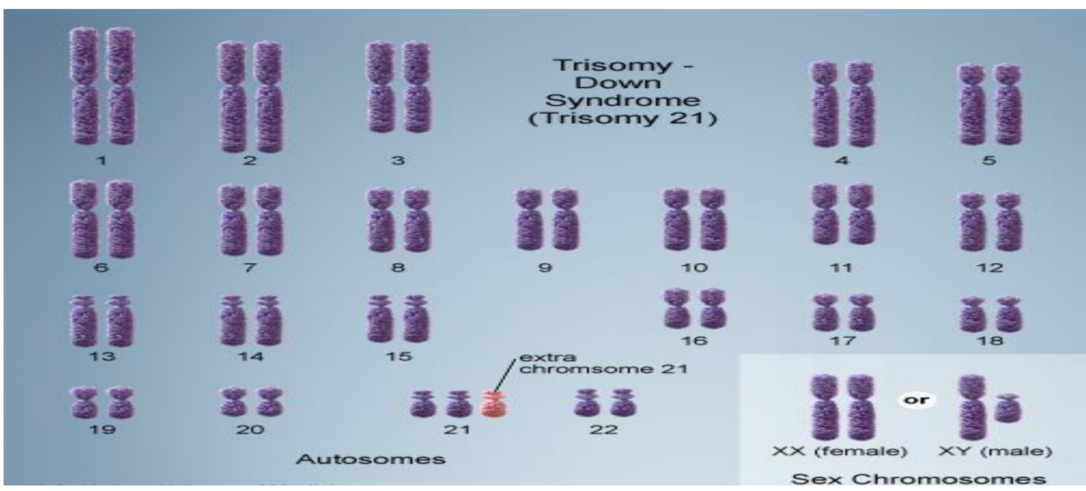
Aneuploidy can arise due to a **non-disjunction** event, which is the failure of at least one pair of chromosomes or chromatids to segregate during mitosis or meiosis. Non-disjunction will generate gametes with extra and missing chromosomes. (**Non-disjunction** occurs when either homologues fail to separate during anaphase I of meiosis, or sister chromatids fail to separate during anaphase II. The result is that one gamete has 2 copies of one chromosome and the other has no copy of that chromosome)

- **a-Autosomes chromosomes**
- **b-Sex chromosomes**

**a-Autosomes chromosomes**

***(Down syndrome):-**

This is an example of trisomy 21 (47, XY or XX, +21) also known as Down syndrome is a genetic disorder caused by the presence of all or part of a third copy of chromosome 21. It is typically associated with physical growth delays, mild to moderate intellectual disability, and characteristic facial features.




***(Edwards Syndrome)**:-

This karyotype demonstrates trisomy 18 (47, XY or XX, +18). It is uncommon for fetuses with this condition to survive, so the incidence is only 1 in 8000 live births**.** Babies with Edwards' syndrome also typically have :heart and kidney problems ,feeding problems – leading to poor growth and breathing problems

***(Patau Syndrome)**:-

The karyotype here demonstrates trisomy 13 (47, XX or XY, +13). Trisomy 13 occurs in about 1 out of every 10,000 newborns. Most cases are not passed down through families (inherited). Instead, the events that lead to trisomy 13 occur in either the sperm or the egg that forms the fetus.

**Symptoms include:**

- Cleft lip or palate.
- Clenched hands (with outer fingers on top of the inner fingers)
- Close-set eyes - eyes may actually fuse together into one.

# Lab:7

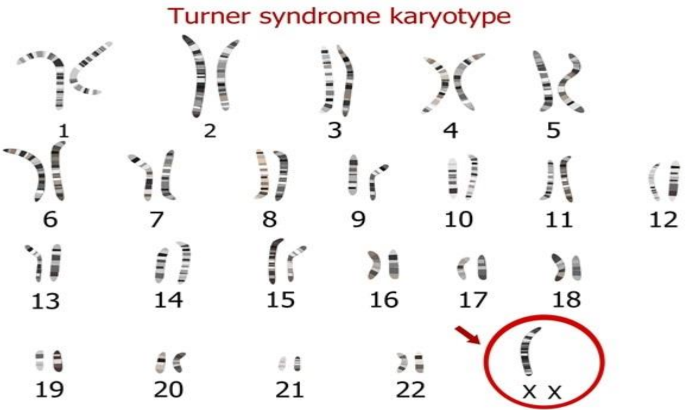
## Chromosomal Abnormalities

### B- Sex Chromosomes Abnormalities:

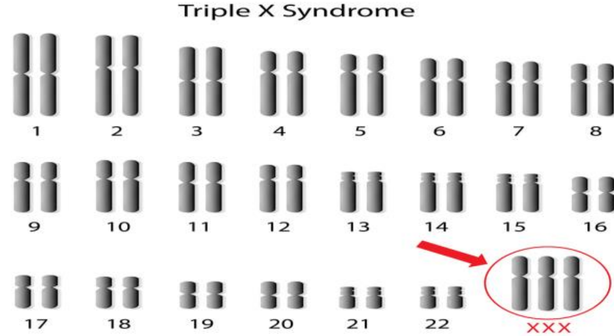
Sex chromosome abnormalities are gender specific. Normal males inherit an X and a Y chromosome while females have two X's. A single Y chromosome is sufficient to produce maleness. Female abnormalities are due to variations in the number of X chromosomes. Male abnormalities are the result of irregular numbers of either the X or the Y chromosome or both.

**(Female Sex Chromosome Abnormalities):**

***Turner syndrome** occurs when females inherit only one X chromosome-their karyotype (45, X0) monosomy X. If they survive to birth, these girls have abnormal growth patterns.They are short in stature, and often have distinctive webbed necks (i.e., extra folds of skin),They generally lack prominent female secondary sexual characteristics.Their ovaries do not develop normally and they do not ovulate. its frequency range from 1 in 2,000 to 1 in 5,000 female infants

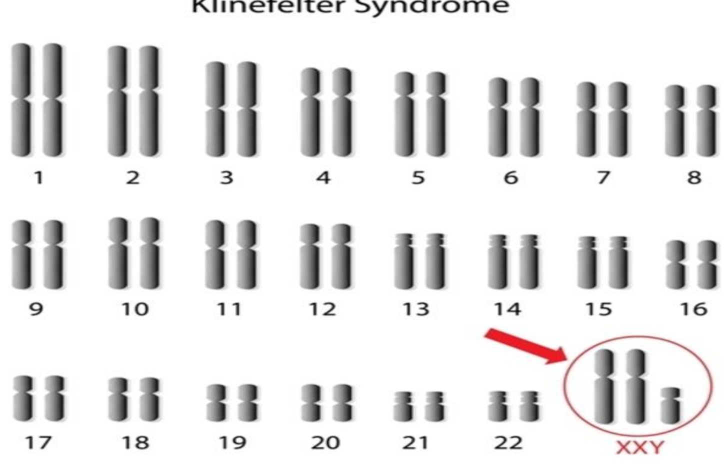



***Trisomy-X syndrome** occurs in women who inherit three X chromosomes their karyotype is (47,XXX) these "super-females", generally are an inch or so taller than average with unusually long legs and slender torsos but otherwise appear normal. They usually have normal development of sexual characteristics and are fertile but tend to have some ovary abnormalities that can lead to premature ovarian failure.The frequency is approximately 1 in 1,000 female infants.

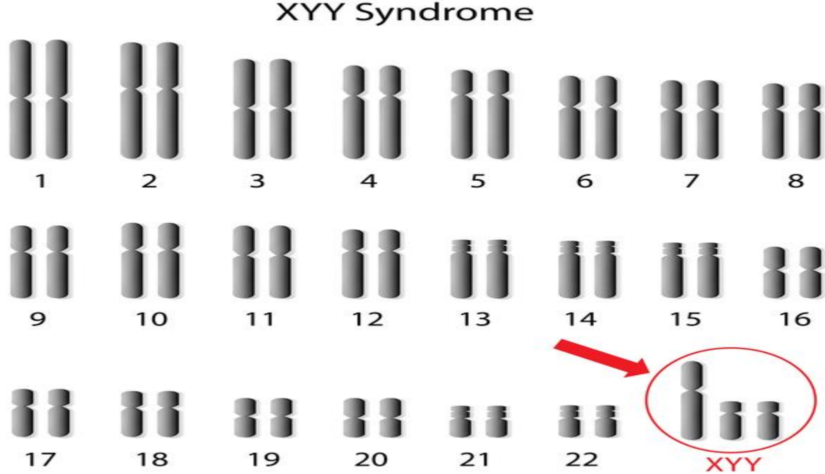



**(Male Sex Chromosome Abnormalities):**

**Klinefelter syndrome:-**This syndrome with a (47, XXY) karyotype**.** males inherit one or more extra X chromosomesIn severe cases, they have relatively high-pitched voices, asexual to feminine body contours as well as breast enlargement, and their testes and prostate gland are small. As a result, they produce relatively small amounts of testosterone.The frequency of Klinefelter syndrome has been reported to be between 1 in 500 and 1 in 1000 male births.This makes it one of the most common chromosomal abnormalities.



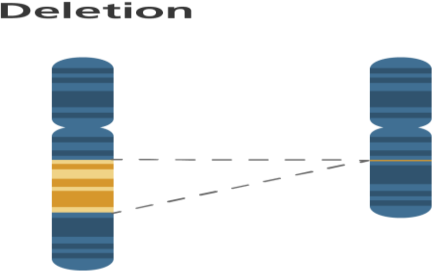

***XYY syndrome** males inherit an extra Y chromosome-their karyotype is (47,XYY). As adults, these "super-males" are usually tall and generally appear and act normal. However, they produce high levels of testosterone. They are usually fertile and lead ordinary lives as adults.It may be as common as 1 in 900 male births to as rare as 1 in 1500 or even 1 in 2,000. XYY syndrome is also referred to as **Jacobs syndrome**.



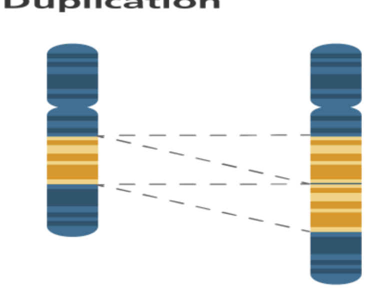

**2-Structural abnormalities:**

This is when large sections of DNA are missing from or are added to a chromosome. Structural abnormalities can take several forms.

**-Deletion:** a mutation causing part of the chromosome to be missing.



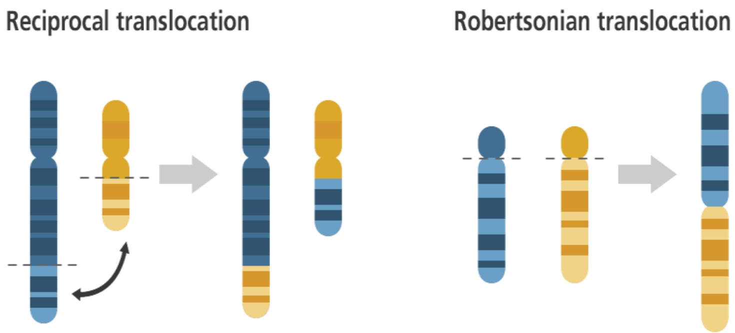

-**Duplication:** a mutation causing part of the chromosome to be repeated, resulting in extra genetic material.



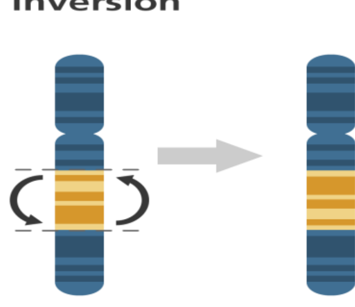

**-Translocation:** a mutation causing one portion of a chromosome to be moved to a different part of the chromosome (intrachromosomal) or to a different chromosome altogether (interchromosomal). There are two key types:

1- Reciprocal: segments from two different chromosomes are exchanged

2-Robertsonian: an entire chromosome attaches to another.




**-Inversion:** a mutation resulting in a portion of a chromosome being in the opposite orientation (inverted).




**-Ring:** when a portion of a chromosome has broken off and formed a circle or ring.

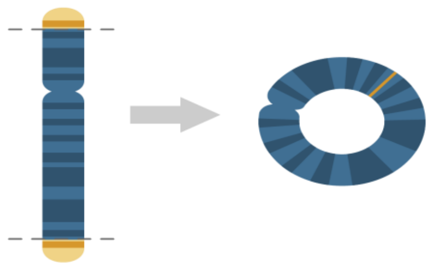


**Table :** Unbalanced structural abnormalities

| Syndrome | Abnormality | Incidence |
|---|---|---|
| **Wolf-Hirschhorn** | Deletion from tip of short arm of chromosome 4 | 1 in 50,000 |
| **Cri-du-chat** | Deletion from tip of short arm of chromosome 5 | 1 in 50,000 |
| **WAGR syndrome** | Microdeletion from short arm of chromosome 11 | 1 in 500,000 to 1 million |
| **Prader-Willi/Angelman** | Microdeletion from short arm of chromosome 15 | 1 in 15,000 |
| **DiGeorge** | Microdeletion from long arm of chromosome 22 | 1 in 4,000 |

# Lab:8

# Pedigree

Pedigrees are formalized ways using standard sets of symbols to depict family trees and lineages.

### Why do Pedigrees Analysis?

- Pedigrees are used to determine the mode of inheritance of genetic diseases.
- To study the inheritance of genes in humans.
- Pedigree analysis is also useful when studying species with a long genration time.

**Basic Symbols**

male (unaffected)
affected male
female (unaffected)
affected female
unknown sex
Dead

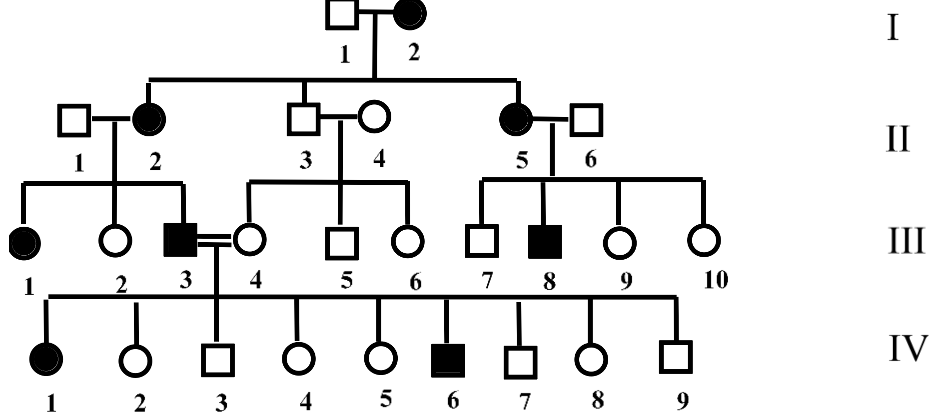
Mating
Consanguineous mating
Monozygotic Twins
Dizygotic Twins

No Offspring

### Categories of inhertance

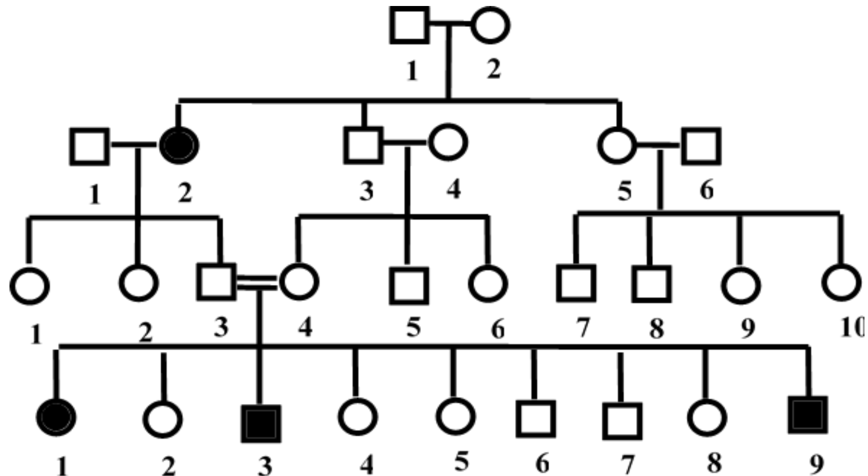
**a- Autosomal:**

**1- Autosomal dominant:**




- both sexes affected (males and females).
- trait does NOT skip generations.
- e.g.Noonan syndrom.

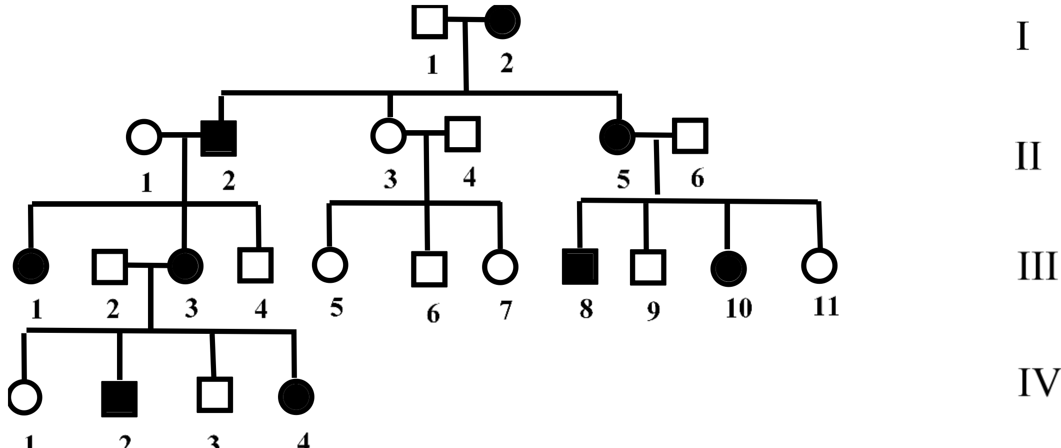
## 2- Autosomal Recessiv e




- Both sexes affected (males and females)
- trait can skip generations
- e.g. cystic fibrosis, albinism

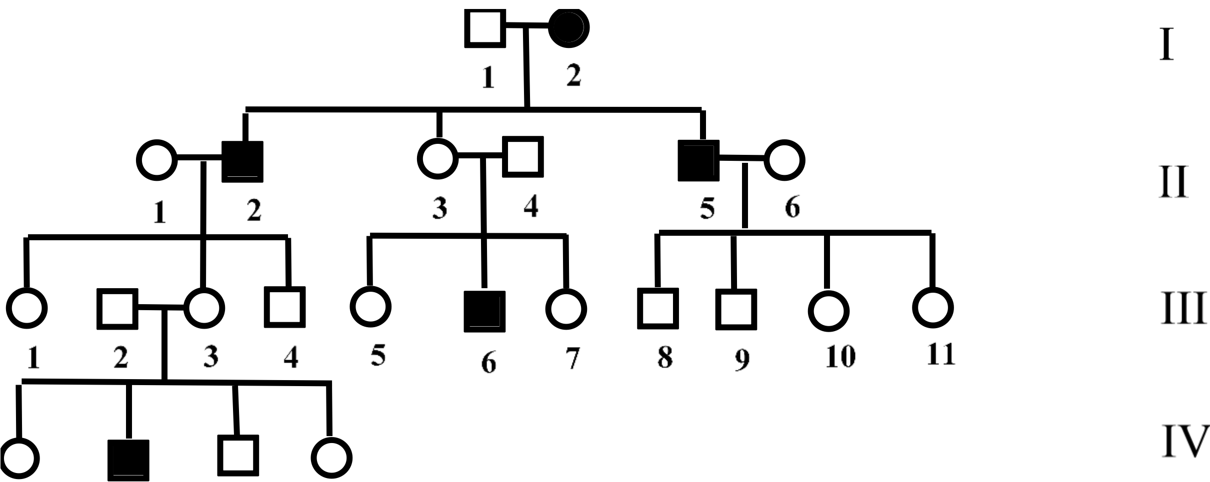
## b- X Chromosome – linked.

### 1- X chromosome linked dominant



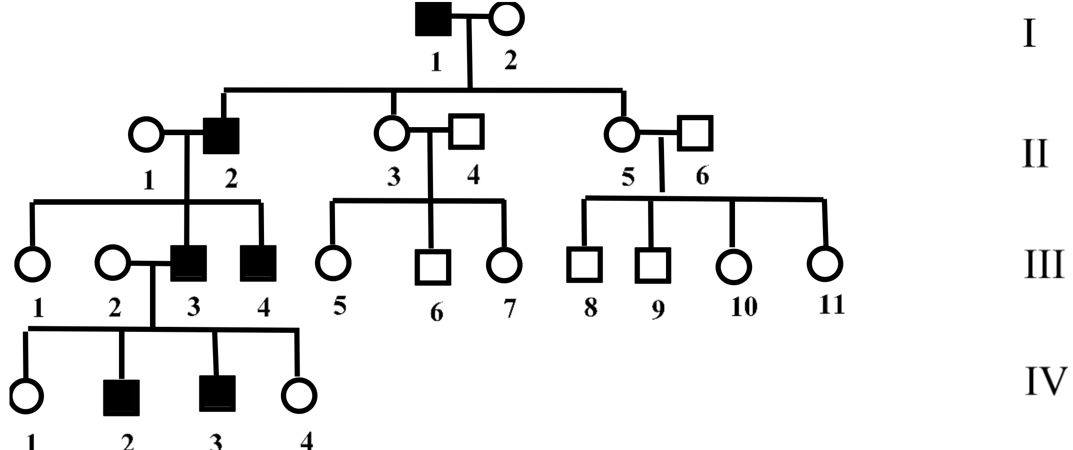

- both sexes affected (males and females)
- females transmit to daughters and sons
- males ALWAYS transmit to daughters, but NOT at ALL to sons
- trait does NOT skip generationse.g. Rett Syndrome (mental retardation, neural degeneration).

## 2-X chromosome linked recessive




- more affected males than females.
- males never transmit to sons
- trait can skip generations, e.g. when females are heterozygotes.
- daughters of affected males always inherit (recessive) mutation, thus are “carriers”.
- trait can skip generations, e.g. when females are heterozygotes (“carriers”) e.g. hemophilia.

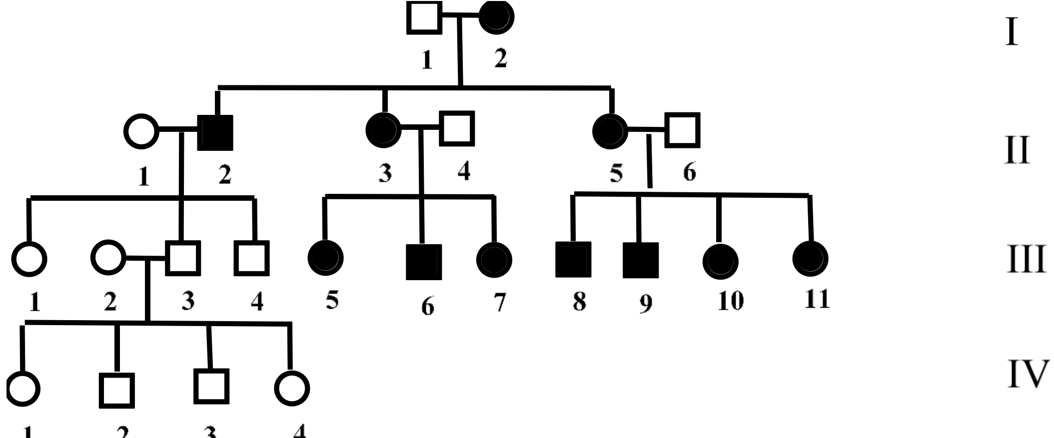
## c- Y chromosome linked




- only males are affected
- males ALWAYS transmit to sons.
- E.g Auricular hypertrichosis (Hairy pinna).

---

## d- mitochondrial inheritance




- both sexes are affected
- females transmit to ALL of their progeny
- males do NOT transmit to any of their progeny.
- E.g MELAS syndrome.

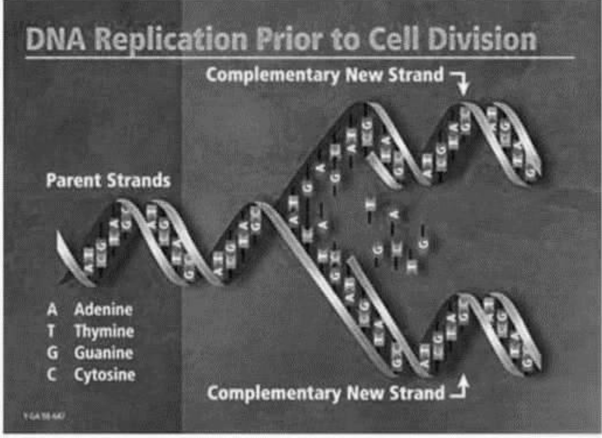
## problems in constructing a pedigree:

- poor medical records
- scattering of family members
- inaccurate and anecdotal information
- miscarriages and still births
- concealed adoptions

# Lab:9

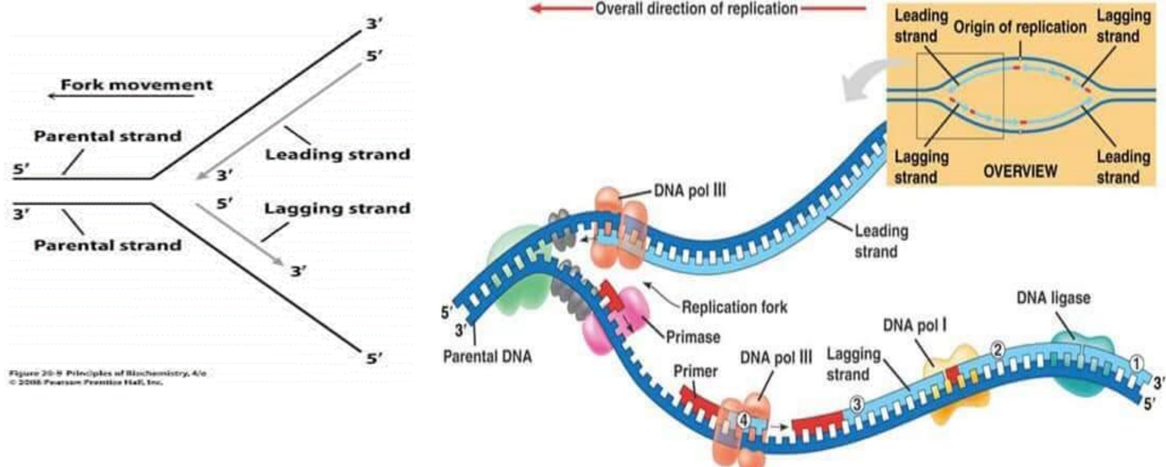
## DNA Replication

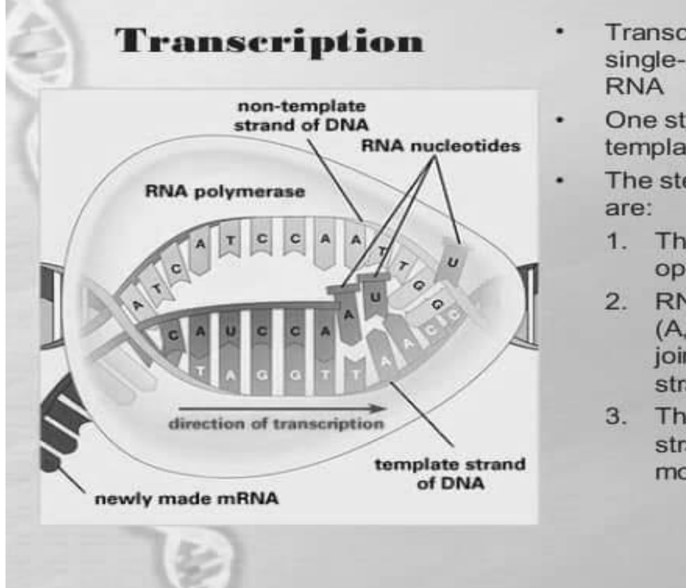
- DNA Replication is the process in which DNA makes an exact copy of itself.
- A single strand of DNA serves as a template (pattern) for a new strand.
- Replication occurs following the base-pair rules (A = T and C = G).
- DNA is replicated during Interphase (S – Synthesis) of the cell cycle.
- Each somatic cell (body cell) gets a complete set of identical DNA.




## The Steps of DNA Replication

| | |
|---|---|
| 1 | **DNA Gyrase** : unwinds the double helix |
| 2 | **DNA Helicase**: Unzips the double helix |
| 3 | **Single Stranded Binding Proteins**: Stabilize the phosphodiester bonds between the sugar & phosphate preventing its breaking |
| 4 | **Primase**: Lays down RNA primer from which nucleotide polymerization can begin |
| 5 | **DNA Polymerase III**: Lays down complementary nucleotides in the 5' to the 3' direction of the growing strand |
| 6 | **DNA Polymerase I**: Removes the RNA primers and lays down DNA nucleotides instead, however it leaves phosphodiester gaps towards the 3' end |
| 7 | **In Case of the Lagging Strand**: DNA Ligase seals the gaps in between the sugar-phosphate backbone (between the Okazaki fragments) |



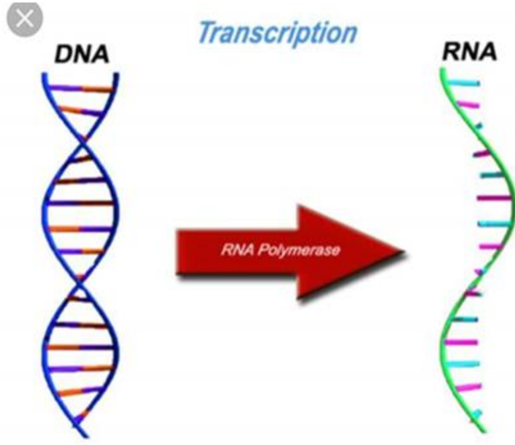

- Transcription produces a single-stranded molecule of RNA
- One strand of DNA is the template or pattern
- The steps of transcription are:
  1. The DNA molecule opens up along a gene
  2. RNA nucleotides (A,U,C,G) match up and join the open DNA strand
  3. The complete RNA strand is released and moves to the cytoplasm

---

## Steps of DNA Transcription

### Making mRNA from DNA

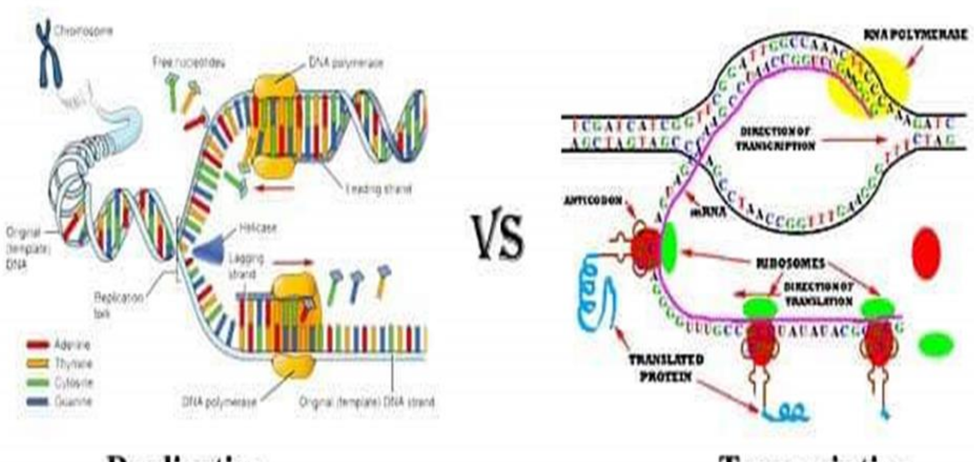
1. Helicase does NOT unzip DNA at the gene of interest
2. RNA polymerase unwinds and matches RNA nucleotide bases to DNA, using one side as a template.
3. The mRNA strand is created. It now compliments the original DNA strand (G-C and A-U).
4. Ligase helps the strand of DNA to close and again.
5. mRNA strand moves out of nucleus to ribosomes, and the DNA zips up.




| DNA Replication | Transcription |
|---|---|
| Copies both strands making 2 new DNA molecules | Copies one strand of DNA making 1 mRNA |
| Uses DNA Polymerase | Uses RNA polymerase |
| Happens when cell splits | Happens all the time |

## Difference Between

# Replication and Transcription




Replication Transcription

## Replication vs. Transcription

| Replication | Transcription |
|---|---|
| • DNA-DNA | • DNA-RNA |
| • Starts at replication origins | • Starts at promoter regions |
| • Unwinds with Helicase | • Does not need Helicase to unwind |
| • DNA polymerase | • RNA polymerase |
| • Proofreads | • No proofreading |
| • Start with 1 DNA<br>End with 2 DNA:<br>½ new, ½ old | • Start with 1 DNA<br>End with same DNA and 1 RNA |

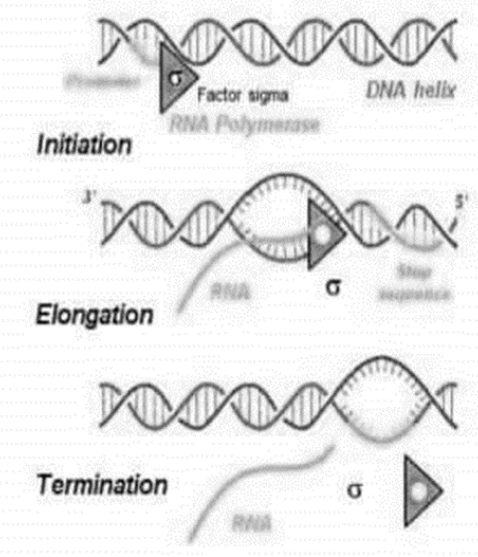
## Similarities between Replication and Transcription

The processes of DNA and RNA synthesis are similar in that they involve-

(1) the general steps of initiation, elongation, and termination with 5' to 3' polarity;

(2) large, multicomponent initiation complexes; and

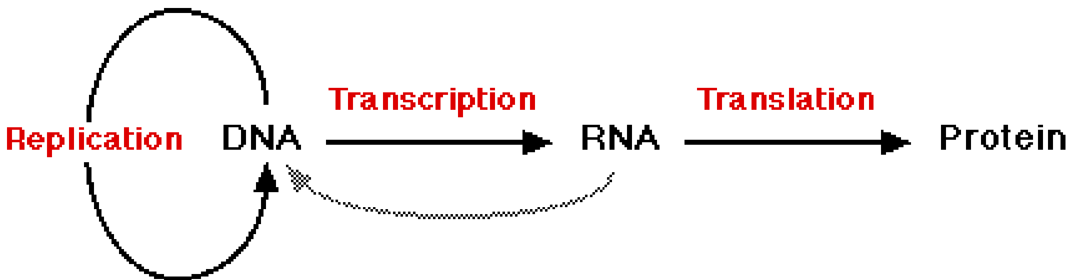
(3) adherence to Watson-Crick base-pairing rules.



# Lab:10

## Translation (Protein Synthesis)

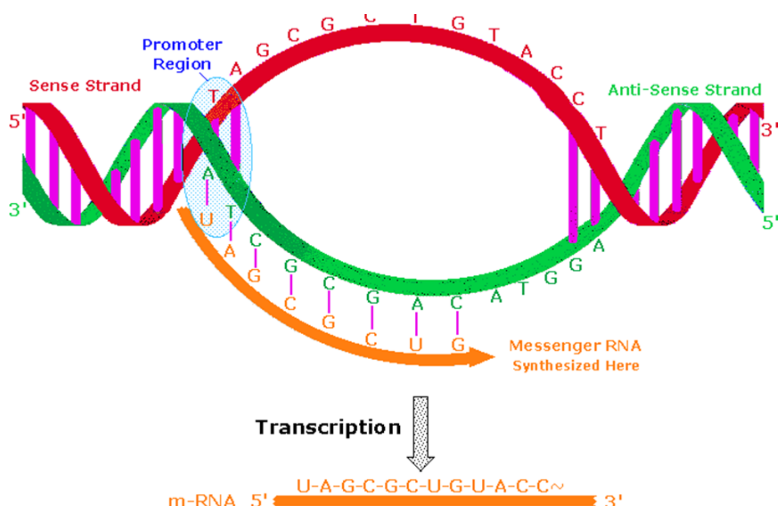
**The Central Dogma of Molecular Biology**




**Transcription** is carried out by **RNA polymerase**
**Translation** is performed on **ribosomes**
**Replication** is carried out by **DNA polymerase**
Reverse transcriptase copies RNA into DNA

*Making a protein Many RNAs needed mRNA, tRNA, rRNA

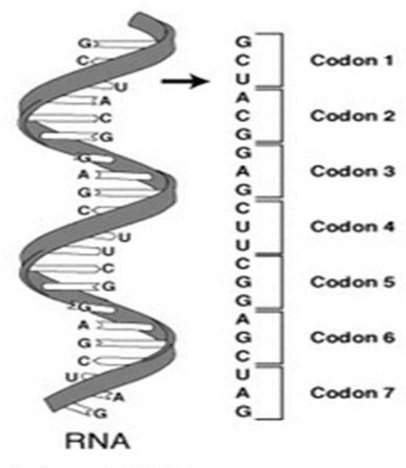
1-Messenger RNA (mRNA):
Carries coded instructions for protein synthesis (translation)
From the DNA in the nucleus to the ribosome.




**The genetic code** instructions for making a protein, a series of three nucleotides on the mRNA each codon signifies start, stop, or an amino acid.

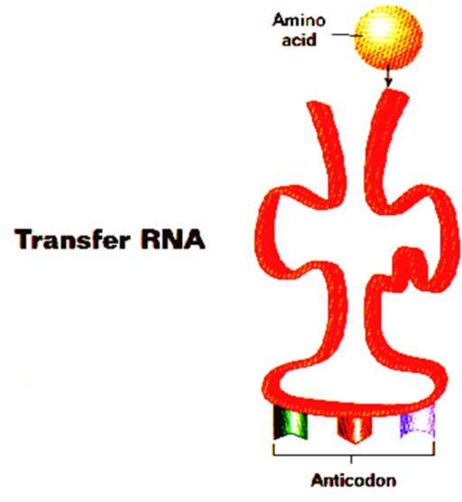
Second Position

| First Position (5' end) | U | C | A | G | Third Position (3' end) |
|---|---|---|---|---|---|
| U | UUU<br>UUC ] Phe<br>UUA<br>UUG ] Leu | UCU<br>UCC<br>UCA<br>UCG ] Ser | UAU<br>UAC ] Tyr<br>UAA Stop<br>UAG Stop | UGU<br>UGC ] Cys<br>UGA Stop<br>UGG Trp | U<br>C<br>A<br>G |
| C | CUU<br>CUC<br>CUA<br>CUG ] Leu | CCU<br>CCC<br>CCA<br>CCG ] Pro | CAU<br>CAC ] His<br>CAA<br>CAG ] Gln | CGU<br>CGC<br>CGA<br>CGG ] Arg | U<br>C<br>A<br>G |
| A | AUU<br>AUC<br>AUA ] Ile<br>AUG Met | ACU<br>ACC<br>ACA<br>ACG ] Thr | AAU<br>AAC ] Asn<br>AAA<br>AAG ] Lys | AGU<br>AGC ] Ser<br>AGA<br>AGG ] Arg | U<br>C<br>A<br>G |
| G | GUU<br>GUC<br>GUA<br>GUG ] Val | GCU<br>GCC<br>GCA<br>GCG ] Ala | GAU<br>GAC ] Asp<br>GAA<br>GAG ] Glu | GGU<br>GGC<br>GGA<br>GGG ] Gly | U<br>C<br>A<br>G |

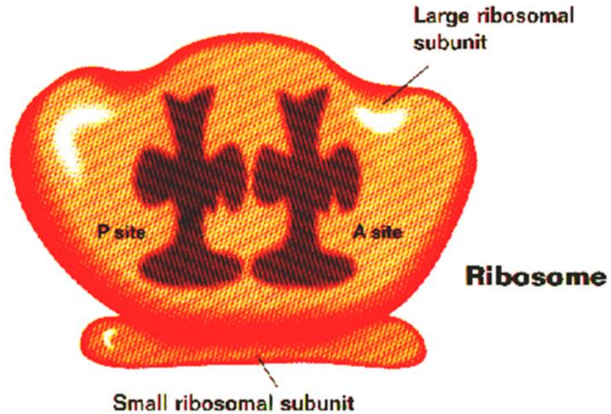



## 2-**Transfer RNA (tRNA)**

Brings amino acids to the ribosome so it can build proteins It has Anticodons 3 nucleotide sequence complementary to the mRNA codon.




**3-Ribosomal RNA (rRNA):** Makes up ribosomes

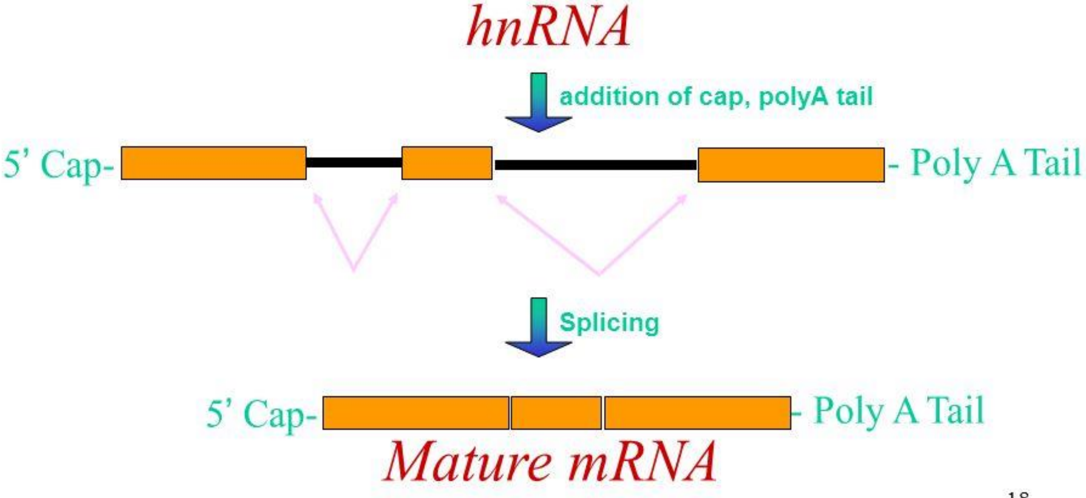



# Making a protein

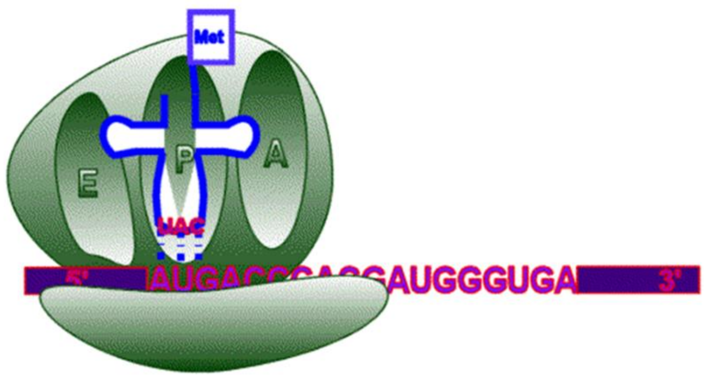
## RNA Splicing

Primary transcripts (in eukaryotes) are sometimes "spliced" to remove non-coding regions "introns" from coding regions "exons"

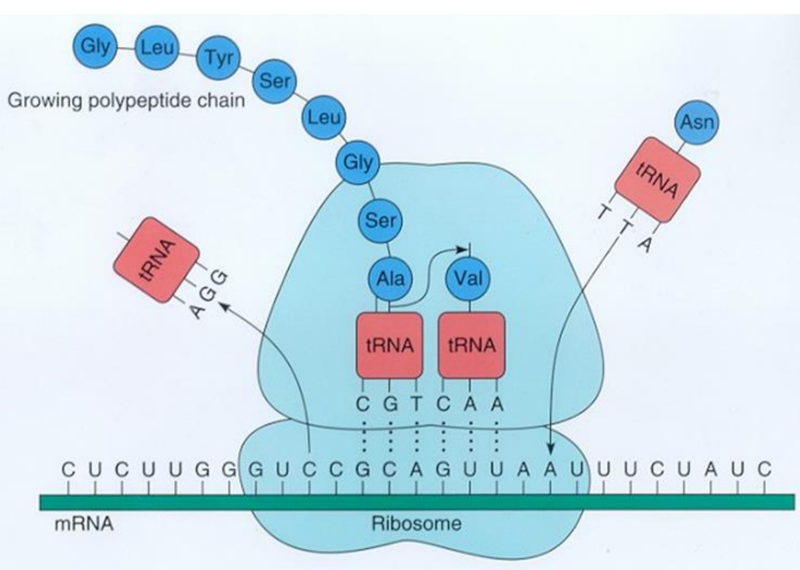
The exon regions are spliced together to form the mature mRNA




b-mRNA from nucleus Through cytoplasm to the ribosome mRNA start codon AUG signals beginning of protein.




C-tRNA with the complementary anticodon carries amino acid (a.a.) to bind to the codon.

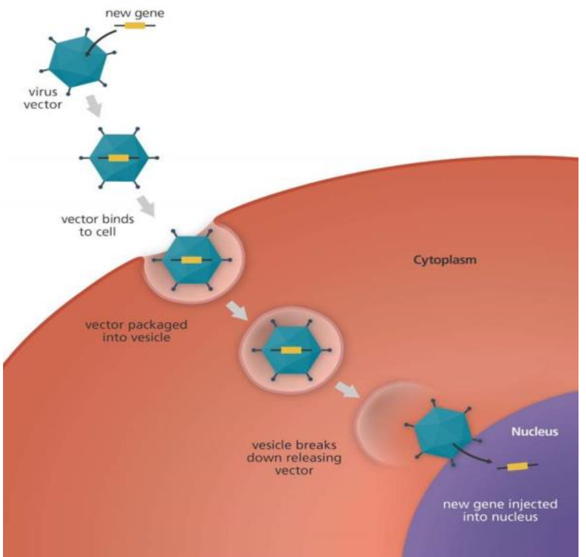


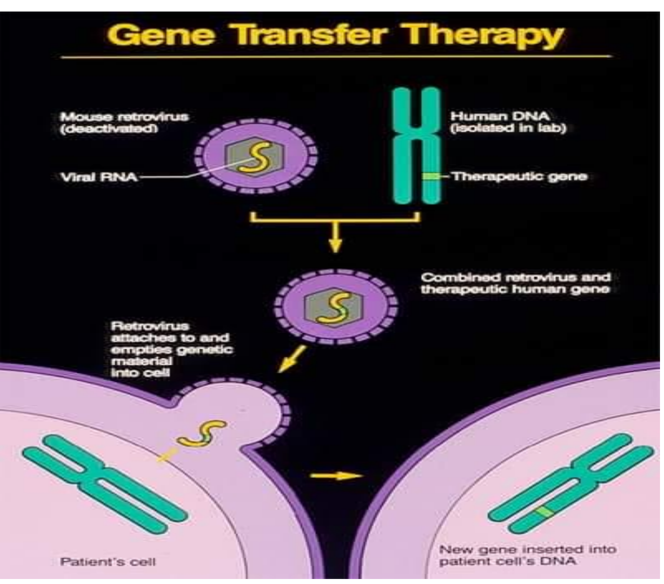

# What is Gene Therapy

- It is a technique for correcting defective genes that are responsible for disease development.

OR

- The treatment of disease by either replacing damaged or abnormal genes with normal ones, or by providing new genetic instructions to help fight disease.




## Diseases for applying gene therapy

| Disease | | Target cell |
|---|---|---|
| Severe combined immunodeficiency | | Bone marrow cells or T-lymphocytes |
| Hemophilia | | Liver, muscle |
| Cystic fibrosis | | Lung Cells |
| Cancer | | Many cell types |
| Neurological diseases | Parkinson's/Alzheimers | Nerve Cells |
| Infectious diseases | AIDS, hepatitis B | White Blood Cells |

## problem of gene therapy

Some of these risks may include:

1. The immune system may respond to the working gene copy that has been inserted by causing inflammation.
2. The working gene might be slotted into the wrong spot.
3. The working gene might produce too much of the missing enzyme or protein, causing other health problems.

# Lab:11

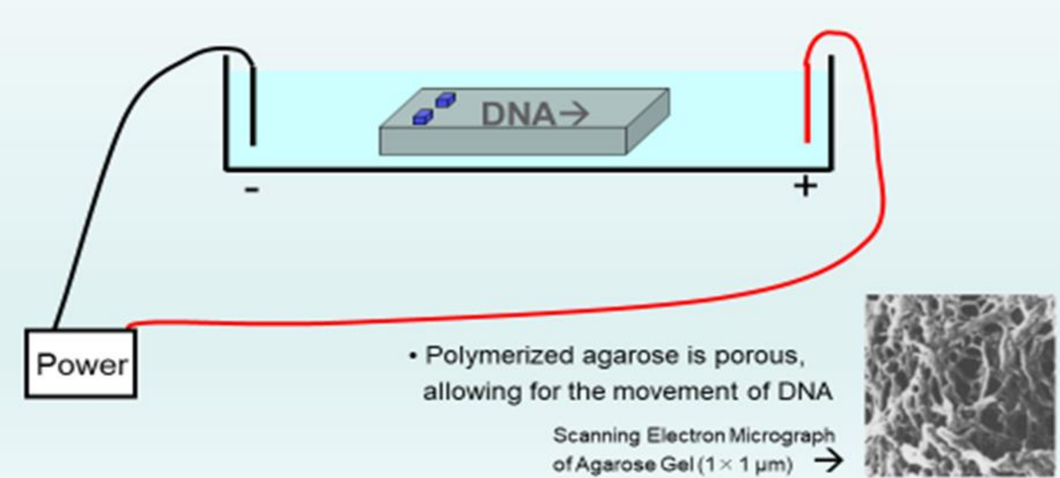
## Agarose Gel Electrophoresis

Gel electrophoresis is a widely used technique for the analysis of **nucleic acids** and **proteins**. Agarose gel electrophoresis is routinely used for the preparation and analysis of DNA.

Gel electrophoresis is a procedure that separates molecules on the basis of their rate of movement through a gel under the influence of an electrical field.

We will be using agarose gel electrophoresis to determine the presence and size of PCR products. PCR products indicate the presence of Wolbachia.

- DNA is negatively charged.
- When placed in an electrical field, DNA will migrate toward the positive pole (anode).
- An agarose gel is used to slow the movement of DNA and separate by size



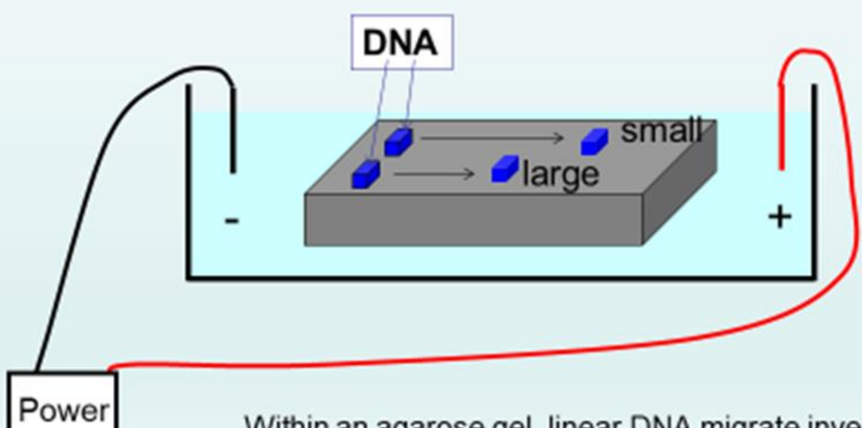
## How fast will the DNA migrate?

strength of the electrical field, buffer, density of agarose gel…

Size of the DNA!

*Small DNA move faster than large DNA

…gel electrophoresis separates DNA according to size




Within an agarose gel, linear DNA migrate inversely proportional to the log10 of their molecular weight.

**Agarose**

D-galactose 3,6-anhydro L-galactose

•Sweetened agarose gels have been eaten in the Far East since the 17th century.

•Agarose was first used in biology when Robert Koch* used it as a culture medium for Tuberculosis bacteria in 1882

***Lina Hesse**, technician and illustrator for a colleague of Koch was the first to suggest agar for use in culturing bacteria

Agarose is a linear polymer extracted from seaweed.

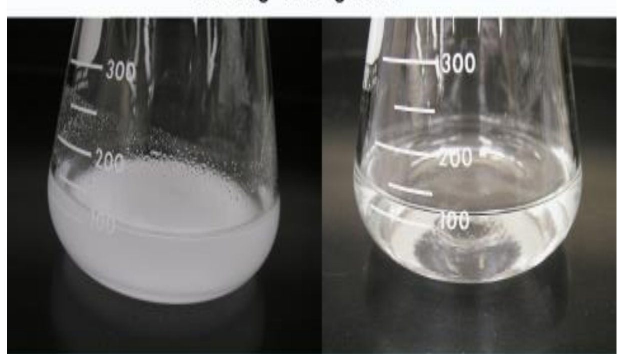
**Making an Agarose Gel:**

An agarose gel is prepared by combining agarose powder and a buffer solution.

Agarose Buffer Solution

Combine the agarose powder and buffer solution. Use a flask that is several times larger than the volume of buffer.

## Melting the Agarose

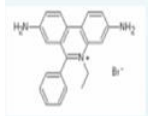


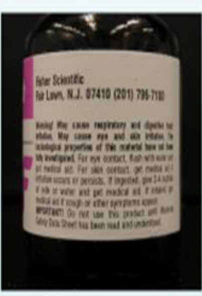

Agarose is insoluble at room temperature (left).
The agarose solution is boiled until clear (right).

Gently swirl the solution periodically when heating to allow all the grains of agarose to dissolve.
***Be careful when boiling - the agarose solution may become superheated and may boil violently if it has been heated too long in a microwave oven.

## Staining the Gel

- Ethidium bromide binds to DNA and fluoresces under UV light, allowing the visualization of DNA on a Gel.
- Ethidium bromide can be added to the **gel** and/or **running buffer** before the gel is run or the gel can be stained after it has run.






***CAUTION! Ethidium bromide is a powerful mutagen and is moderately toxic. **Gloves should be worn at all times**.

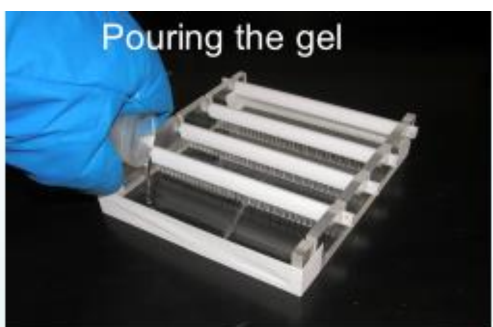
## Safer alternatives to Ethidium Bromide

- Methylene Blue
- BioRAD - Bio-Safe DNA Stain
- Ward's - QUIKView DNA Stain
- Carolina BLU Stain

…others

advantages
Inexpensive
Less toxic
No UV light required
No hazardous waste disposal

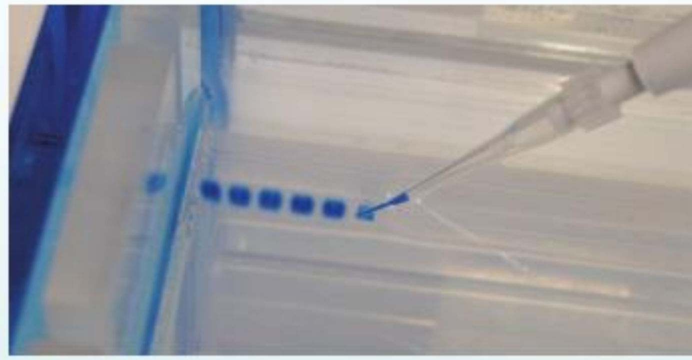
disadvantages
Less sensitive
More DNA needed on gel
Longer staining/destaining time



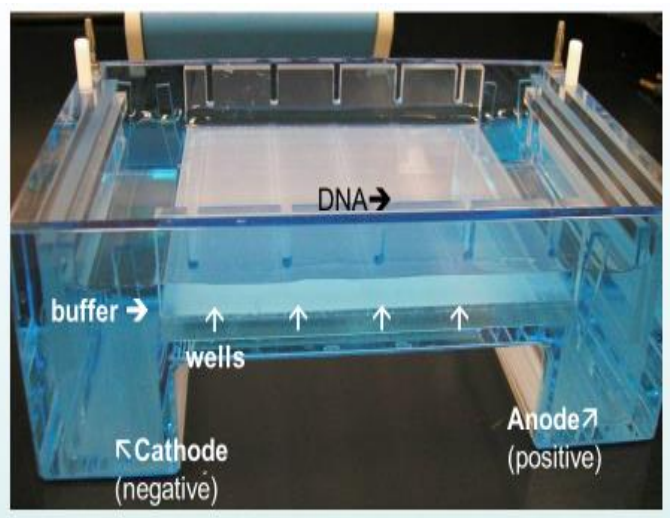

Allow the agarose solution to cool and then carefully pour the melted agarose solution into the casting tray. Avoid air bubbles.

It should appear lighter in color when completely cooled (30-45 minutes). Carefully remove the combs and tape.

## Loading the Gel

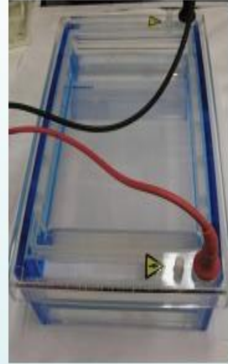


Carefully place the pipette tip over a well and gently expel the sample. The sample should sink into the well. Be careful not to puncture the gel with the pipette tip.

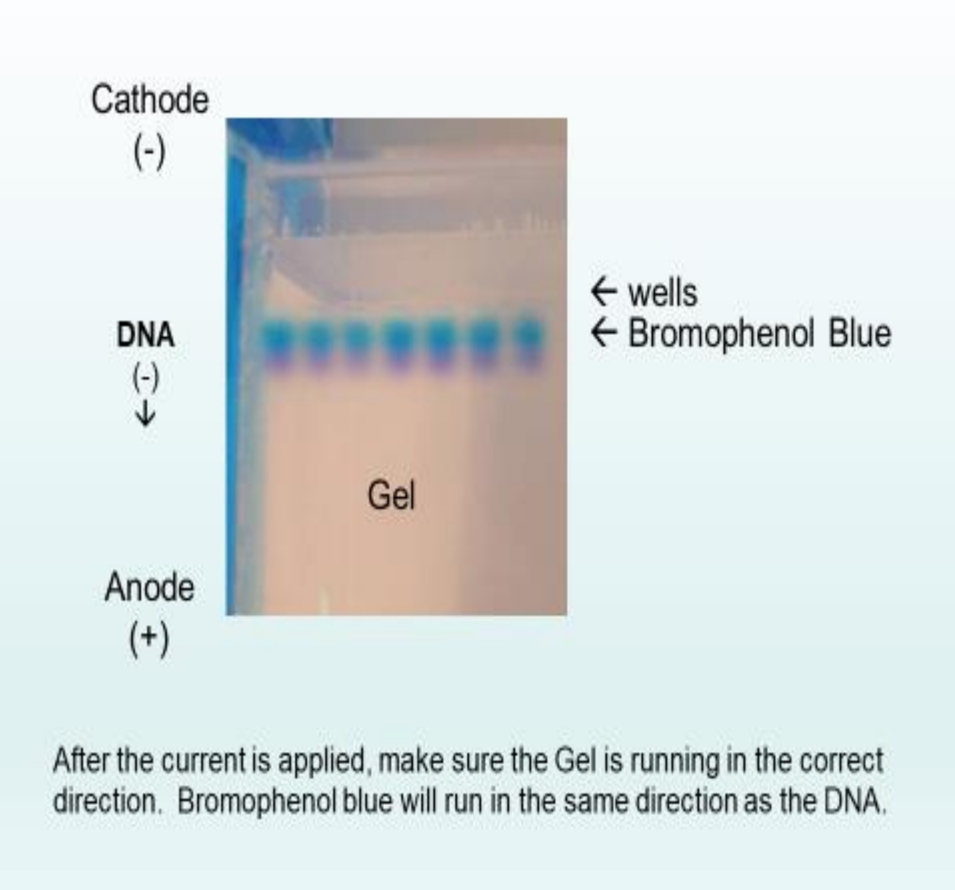


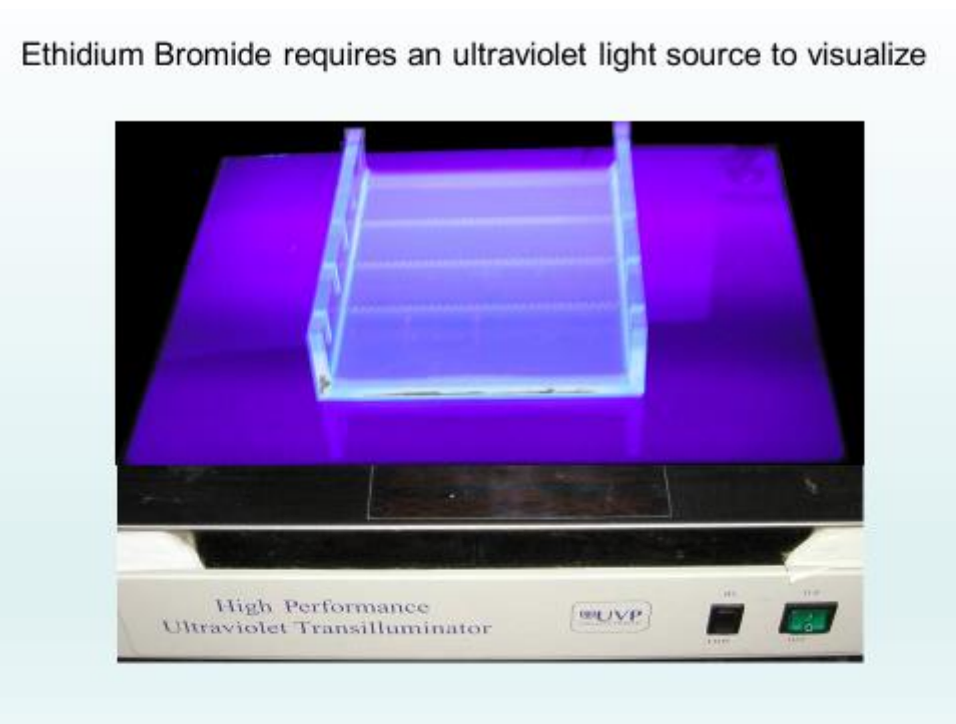

Place the gel in the electrophoresis chamber and Add enough electrophoresis buffer to cover the gel to a depth of at least 1 mm.Make sure each well is filled with buffer.

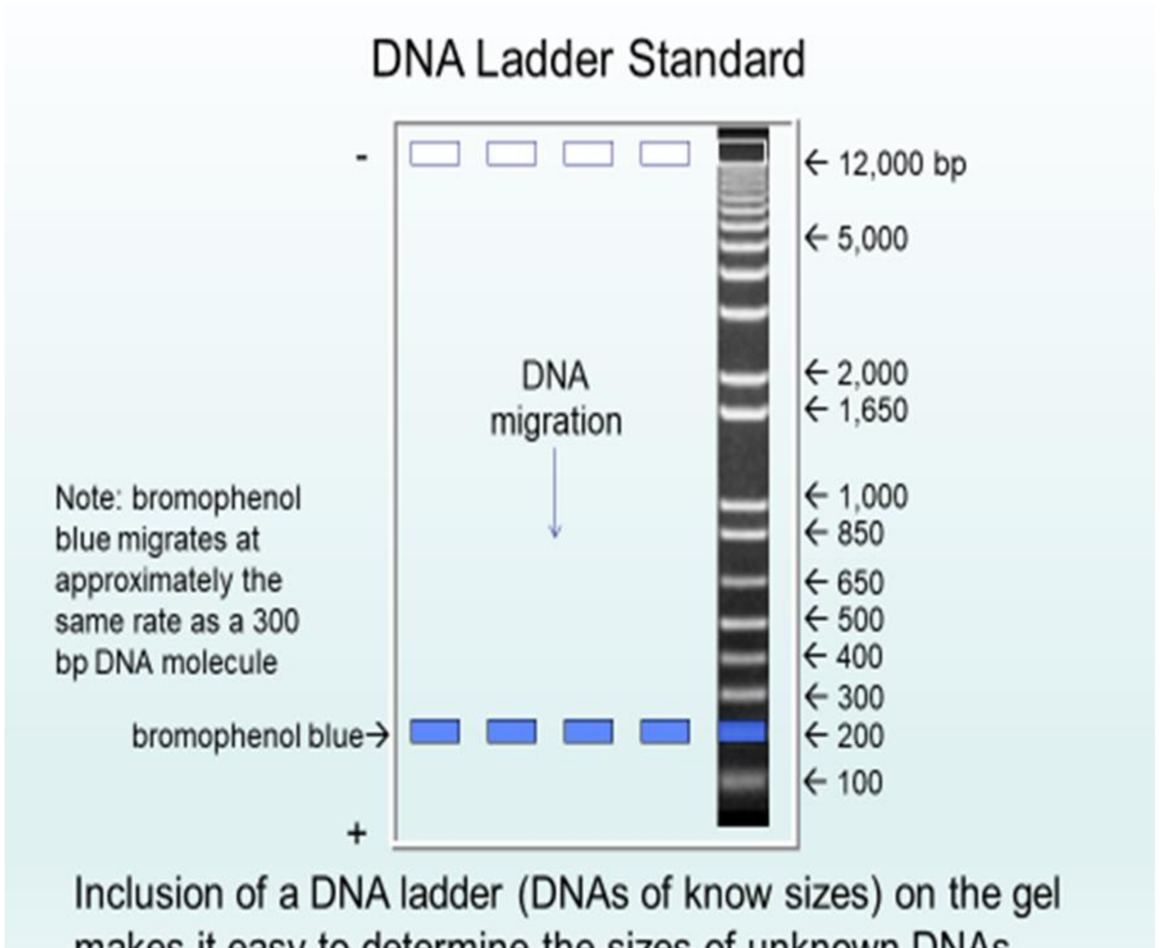
## Running the Gel



Place the cover on the electrophoresis chamber, connecting the electrical leads. Connect the electrical leads to the power supply. Be sure the leads are attached correctly - DNA migrates toward the anode (red). When the power is turned on, bubbles should form on the electrodes in the electrophoresis chamber.


Cathode
(-)
DNA
(-)
← wells
← Bromophenol Blue
Gel
Anode
(+)
After the current is applied, make sure the Gel is running in the correct direction. Bromophenol blue will run in the same direction as the DNA.


Ethidium Bromide requires an ultraviolet light source to visualize
High Performance
Ultraviolet Transilluminator
UVP


DNA Ladder Standard
-
+
← 12,000 bp
← 5,000
← 2,000
← 1,650
← 1,000
← 850
← 650
← 500
← 400
← 300
← 200
← 100
DNA migration
Note: bromophenol blue migrates at approximately the same rate as a 300 bp DNA molecule
bromophenol blue→
Inclusion of a DNA ladder (DNAs of know sizes) on the gel makes it easy to determine the sizes of unknown DNAs.

# Lab:12

## Polymerase Chain Reaction (PCR)

The polymerase chain reaction. PCR, the quick, easy method for generating unlimited copies of any fragment of DNA Amplify specific nucleic acids in vitro.

PCR will allow a short stretch of DNA (usually fewer than 3000 base pairs) to be amplified to about a million fold This amplified sample then allows for size determination and nucleotide sequencing ,Introduced in 1985 by Kary Mullis Millions of copies of a segment of DNA can be made within a few hours.

**Reaction Components:**

1- DNA template :DNA containing region to be sequenced
2- Primers: 2 sets of primers Generally 20-30 nucleotides and 40-60% GC content preferred for better annealing, complimentary to the 3' ends of target DNA
3- Enzyme: Usually Taq Polymerase or anyone of the natural or Recombinant thermostable polymerases.
4- All four nucleotide triphosphates dNTPs (A, T, G, C)
5- $Mg2+$
6- buffers

**The PCR Cycle Comprised of 3 steps:**

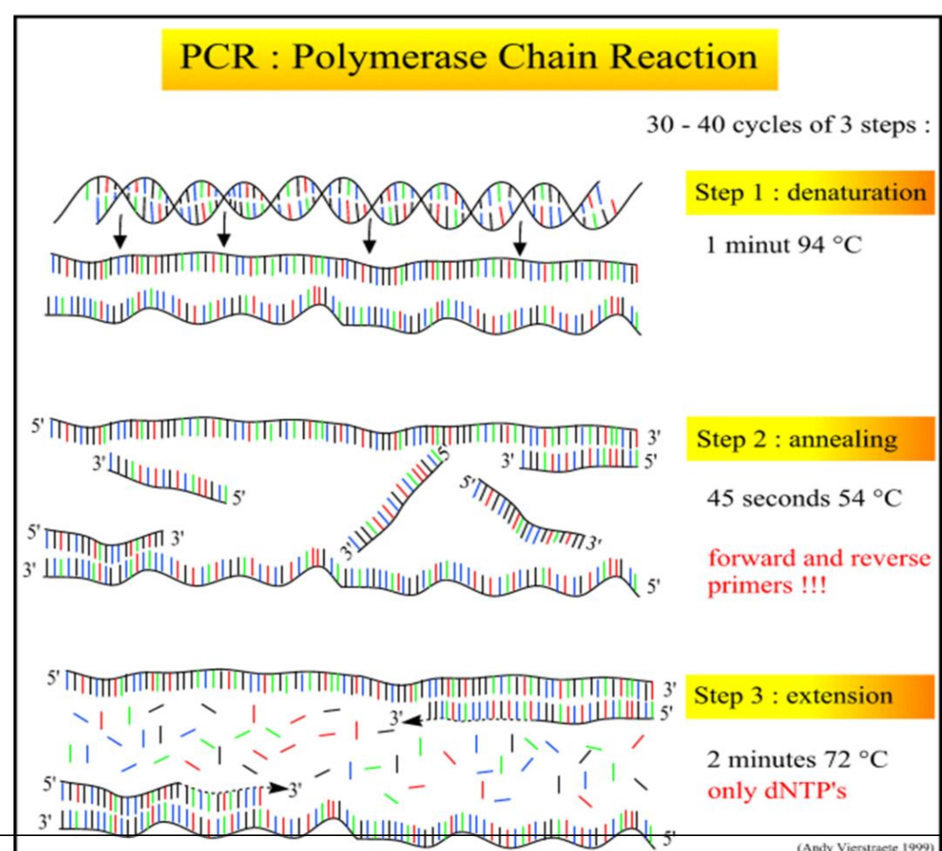
1- **Denaturation** this occurs at 94 ºC mimicking the function of helicase in the cell.
2- **Annealing** or Primers Binding at $(40\text{-}50)^0$C .Primers bind to the complimentary sequence on the target DNA. Primers are chosen such that one is complimentary to the one strand at one end of the target sequence and that the other is complimentary to the other strand at the other end of the target sequence.
3- DNA synthesis (Primer **extension**) at $72^0$C. DNA polymerase catalyzes the extension of the strand in the 5-3 direction, starting at the primers, attaching the appropriate nucleotide (A-T, C-G).

**Detection of amplification products:**

(Gel electrophoresis, Sequencing of amplified fragment , Southern blot ,etc...)

## Applications

1- Genome mapping and gene function determination
2- Biodiversity studies ( e.g. evolution studies)
3- Diagnostics (prenatal testing of genetic diseases, early detection of cancer, viral infections...)
4- Detection of drug resistance genes
5- Forensic (DNA finger printing)



# Lab:13

## Nucleic acid hybridization:

Nucleic acid hybridization A technique in which single-stranded nucleic acids (DNA or RNA) are allowed to interact so that complexes called hybrids are formed by molecules with similar, complementary sequences.

A hybridization probe is a fragment of DNA or RNA of variable length (usually 100–1000 bases long) which can be radioactively labeled. It can then be used in DNA or RNA samples to detect the presence of nucleotide sequences (the DNA target) that are complementary to the sequence in the probe. The probe thereby hybridizes to single-stranded nucleic acid (DNA or RNA) whose base sequence allows probe–target base pairing due to complementarity between the probe and target.

Hybrids are detected by various means: visualization in the electron microscope; by radioactively labelling one component and removing non-complexed DNA; or by washing or digestion with an enzyme that attacks single-stranded nucleic acids and finally estimating the radioactivity bound.

## Applications:

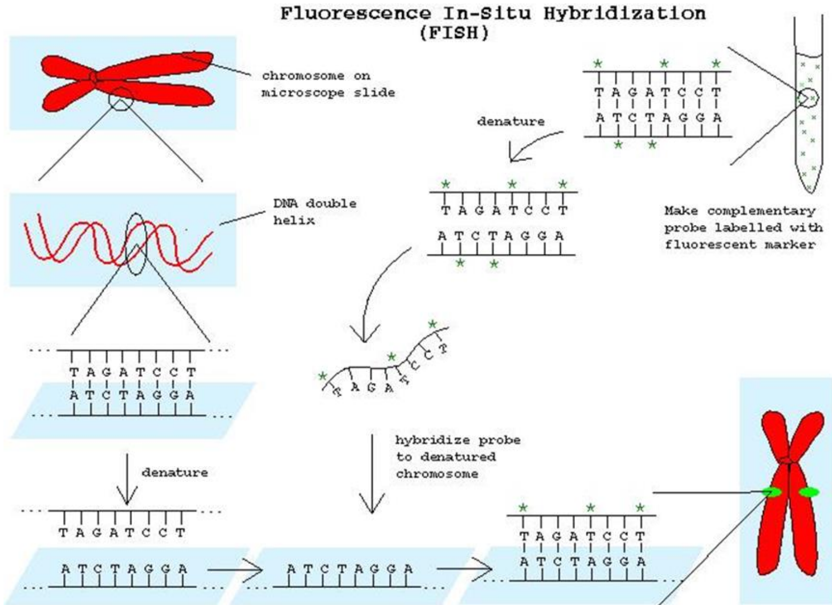
1- **A Southern blot** is a method used in molecular biology for detection of a specific DNA sequence in DNA samples. Southern blotting combines transfer of electrophoresis-separated DNA fragments to a filter membrane and subsequent fragment detection by probe hybridization.

2- **The northern blot**, or RNA blot, is a technique used in molecular biology research to study gene expression by detection of RNA (or isolated mRNA) in a sample.

*In 1&2 The hybridization can be carried out in solution or with one component immobilized on a gel or, most commonly, on nitrocellulose paper.

3- **Fluorescence in situ hybridization (FISH)**
Fluorescence in situ hybridization (FISH) is a technique used to detect specific chromosomes or chromosomal regions through hybridization (attachment) of fluorescently labeled DNA probes to denatured chromosomal DNA. (FISH) can be used to test for the

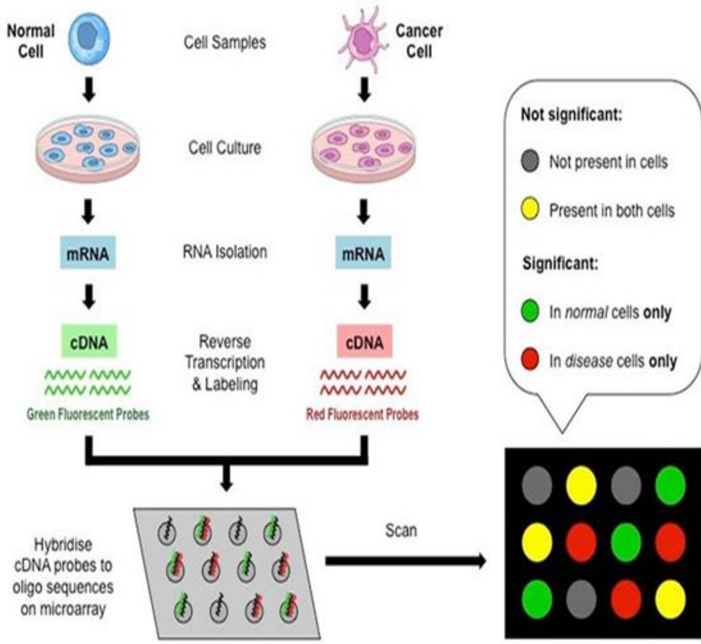
presence or absence of specific chromosome regions and is often used to detect small chromosome deletions such as Williams syndrome.




## 4-Microarray

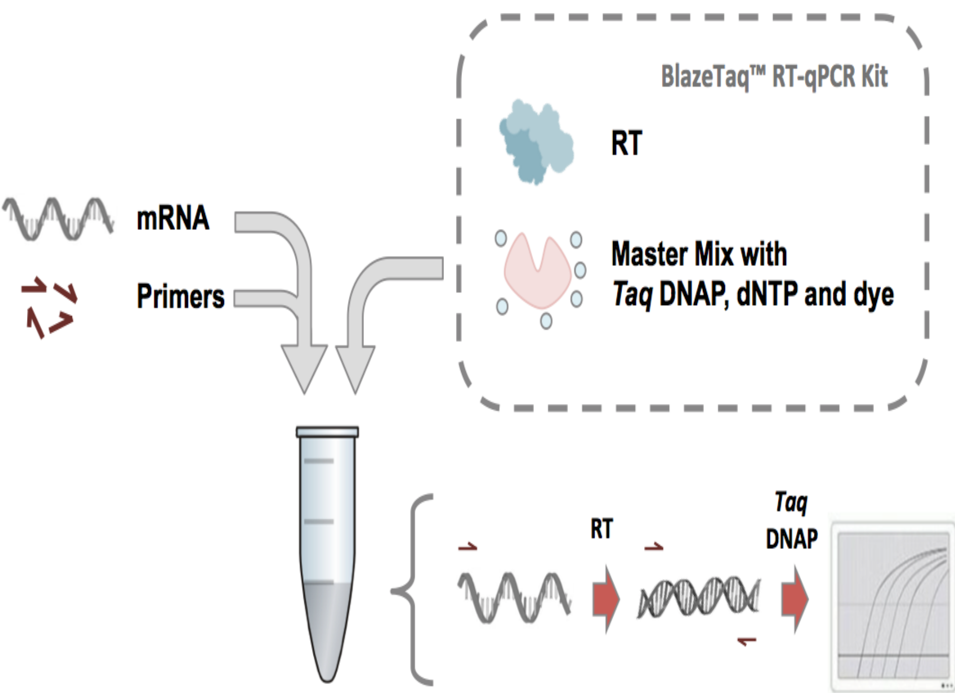
A laboratory tool used to analyze large numbers of genes or proteins at one time (Scientists use DNA microarrays to measure the expression levels of large numbers of genes simultaneously). In a microarray, biologic molecules such as DNA, RNA, or protein are placed in a pattern onto a surface such as a glass slide. Other substances are added to these slides to detect specific patterns of molecules. Microarrays are being used to help diagnose diseases, such as cancer, and to develop treatments for them.

## Microarray Technique




## 4- RT-PCR (Real Time PCR)

Real time PCR assays are now easy to perform, have high sensitivity, more specificity, and provide scope for automation. Real time PCR is also referred to as real time RT PCR which has the additional cycle of reverse transcription that leads to formation of a DNA molecule from a RNA molecule. This is done because RNA is less stable as compared to DNA ,This occurs due to the accumulation of the PCR product with each cycle of amplification. These fluorescent reporter molecules include dyes that bind to the double-stranded DNA (i.e. SYBR Green ) or sequence specific probes.




Real Time PCR Applications Include

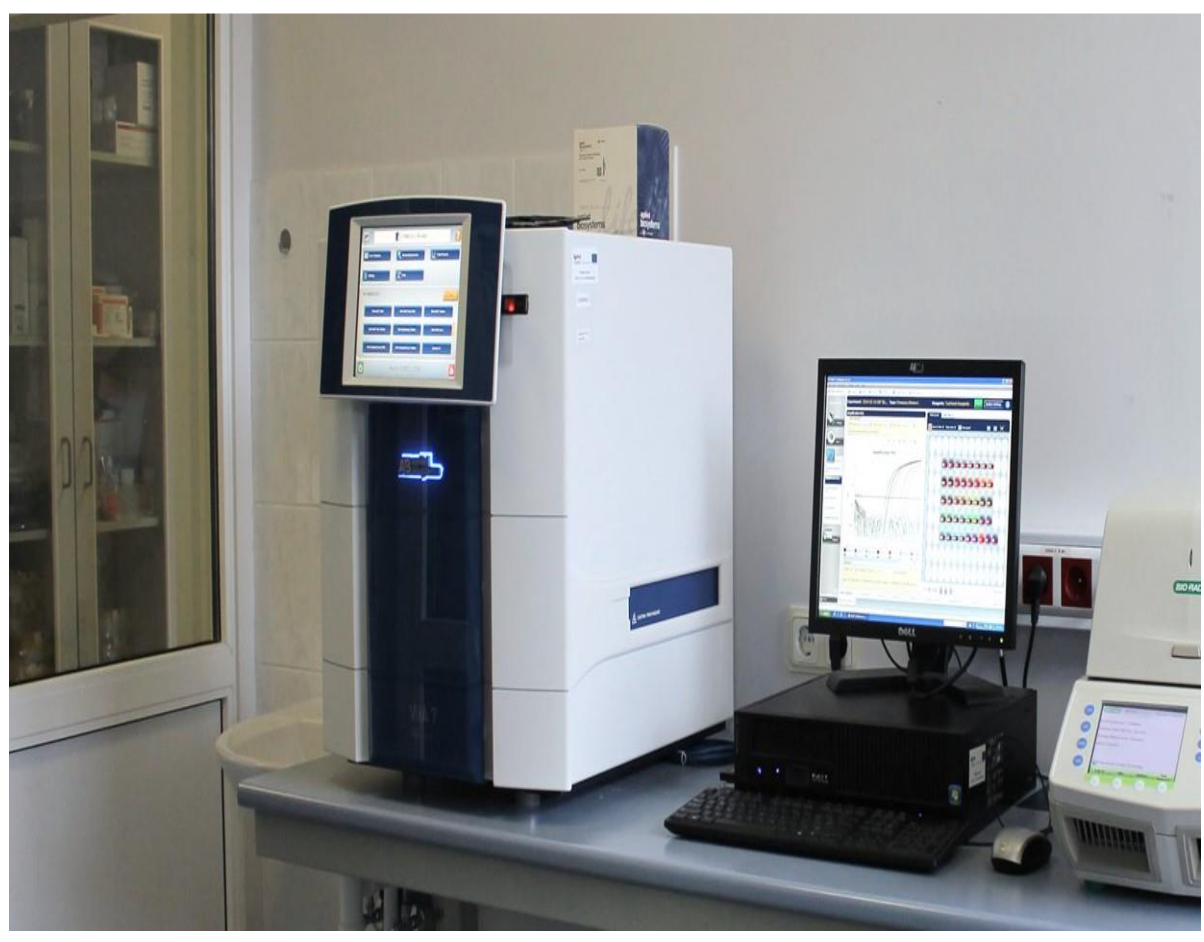
1- Quantitative mRNA expression studies

2-DNA copy number measurements in genomic or viral DNAs .

3-Allelic discrimination assays or SNP genotyping .

4-Verification of microarray results .

5-DNA damage measurement .



Arrayit
Arrayit

# Lab:14

## Forensics DNA Fingerprinting

DNA fingerprinting is a method used to identify an individual from a sample of DNA by looking at unique patterns in their DNA. On average, about 99.9 percent of the DNA between two humans is the same. The remaining percentage is what makes us unique (unless you are an identical twin).

**A Short Tandem Repeat (STR) analysis:** Is a tool in forensic analysis that evaluates specific STR regions found on nuclear DNA. it is incredibly sensitive so it only needs a tiny amount of someone's DNA to produce an accurate result.

**How is a DNA profile (STR) produced?**

1- DNA is extracted from a biological sample. As a result the DNA can be extracted from a wider range of biological samples, including blood, skin, bones, saliva, hair, Semen, Urine, Teeth and Tissue.

2- In STR analysis the primers used in the PCR are designed to attach to either end of the STR sequence of interest .The primers for each STR is labelled with a specific coloured fluorescent tag.This makes it easier to identify and record the STR sequences after PCR.

3- Once enough copies of the sequence have been produced by PCR, electrophoresis is used to separate the fragments according to size.

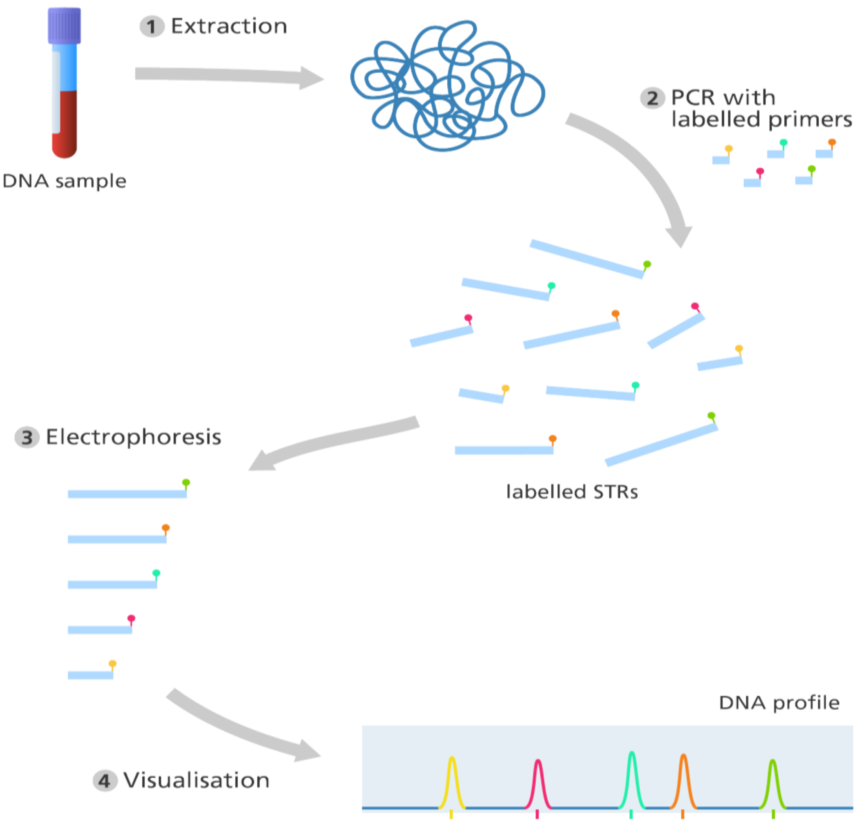
4- Each fragment passes by a laser which causes the fragments with fluorescent tags to glow with a specific colour. The output is displayed as a series of coloured peaks (as shown in the image below) highlighting the colour and length of each STR sequence.

**There are two main applications of DNA analysis in forensic medicine:**

**1- Criminal investigation:** DNA profiles are very useful in forensics because only a tiny sample of human material left behind after a crime may be sufficient to identify someone. A match made between a crime scene profile and an individual profile identifies a possible suspect. The

police may use this DNA evidence to support other evidence to help prosecute someone for a crime

**2-Paternity testing**: DNA profiling can also be used to identify victims of crime or major disasters and help bring separated families back together.




**DNA profile (STR)**

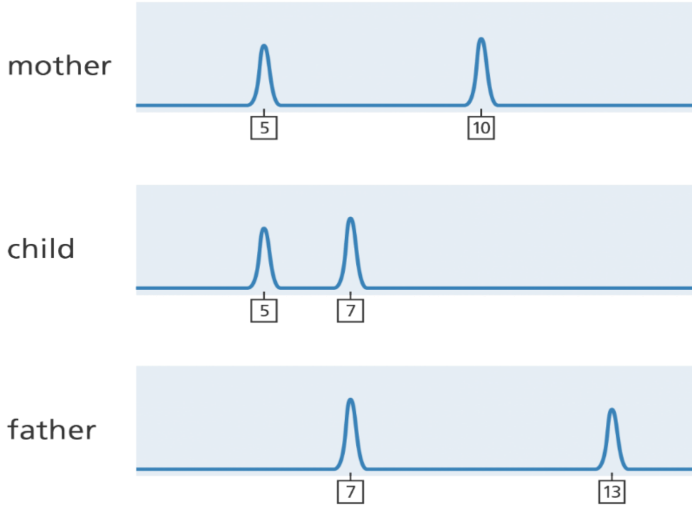



Illustration comparing the DNA profiles of two parents and their child. You can see which STRs in the child have been inherited from which parent.