



# **MEDICAL BIOLOGY and GENETICS LABORATORY BOOK**

**FACULTY OF MEDICINE  
DEPARTMENT OF MEDICAL BIOLOGY and GENETICS**

**Izmir, 2020**

# **MEDICAL BIOLOGY and GENETICS LABORATORY BOOK**

**FACULTY OF MEDICINE  
DEPARTMENT OF MEDICAL BIOLOGY and GENETICS**

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# **INFORMATION PAGE OF THE STUDENT**

Of the Student:

Name-Surname:

Student Number:

Department:

Other information:

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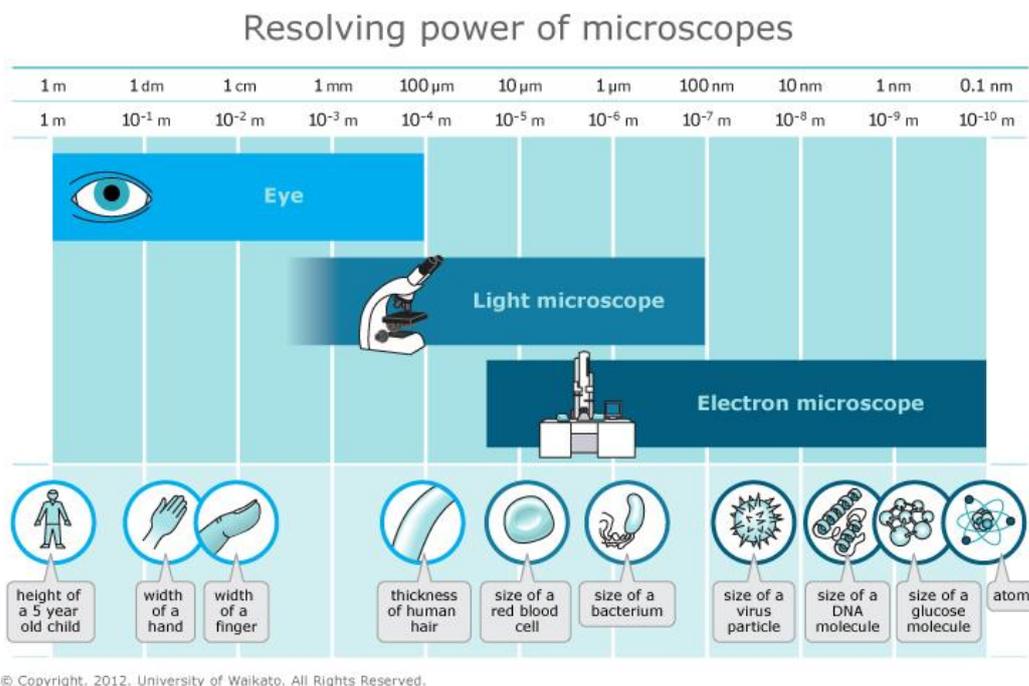
# LAB.1- INTRODUCTION OF MICROSCOPE TYPES and THE USE OF LIGHT MICROSCOPE

**MICROSCOPE:** An optical device magnifies the objects, which are too small to be seen with the naked eyes, with a lens system called ocular and objectives, and reflects the image of the material properly to the eye. Our eye cannot distinguish objects smaller than 0.1 mm.

There are two types of microscopes according to the classification based on the illumination source:

1. Light Microscopes
  - a) Bright field microscope
  - b) Phase contrast microscope
  - c) Ultraviolet microscope
  - d) Fluorescence microscope
  - e) Dark field microscope
  - f) Stereo-microscope (dissection microscope)
2. Electron Microscopes
  - a) TEM (Transmission Electron Microscope )
  - b) SEM (Scanning Electron Microscope)

There are three types of microscopes according to the number of oculars: monocular, binocular, and trinocular. The microscope consists of an optical part that magnifies the object under the microscope and the mechanical parts that carry this optical part.



**Figure 1.1** Alignment of microscopes according to their resolving power (<http://www.boruhealthmachine.org/the-resolving-power-of-a-microscope-is-a-function-of.html>)

**\*\*In this laboratory, light microscopy will be used in preparation examinations.**

## LIGHT MICROSCOPE

### Parts of the microscope:

It is an optical device consisting of lens systems. It consists of two main parts: A) Mechanic part B) Optical part

**A) Mechanic part:** It supports optical part. Mainly, this part consists of:

Microscope base: It is in the shape of a horseshoe and allows the microscope to stand firmly on the stage.

Microscope arm: Microscope is carried by holding this arm. It carries microscope tube at the top and microscope stage at the bottom.

Microscope tube: It carries ocular at the top and objectives at the bottom. It is linked to microscope arm and is moved vertically with the help of a gear. It is stable in some of the microscopes. In this situation, vertical movement is achieved by microscope stage. The stage should be brought closer and removed.

Revolver (Nose piece): It is a rotating circle-shaped adjustment knob, which is attached to the lower end of the microscope tube, carries various lenses on it and allows the desired lens to reach the axis of vision easily.

Coarse adjustment knob: It moves the microscope tube fast and provides a rough adjustment.

Fine adjustment knob: It moves the microscope tube very slowly. Therefore, it is used to sharpen the adjustment.

Microscope stage: It is a plate with a hole in the middle on which the preparation to be examined is placed. It can be fixed or mobile. The preparation is fixed on the stage with the help of two clips.

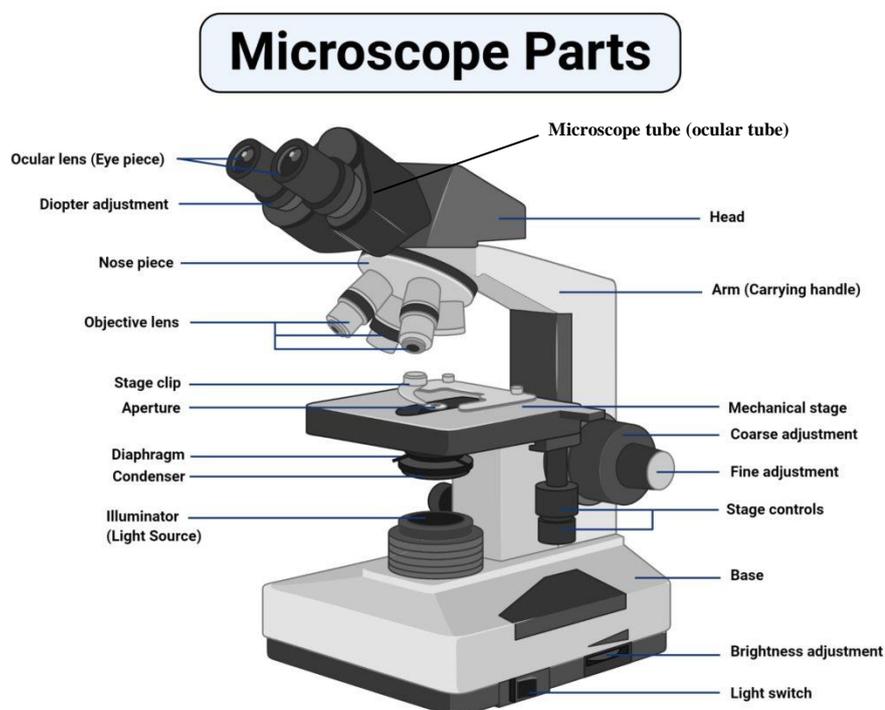


Figure: Parts of a microscope, Image Copyright © Sagar Aryal, www.microbenotes.com

Figure 1.2 General parts of a light microscope (<https://microbenotes.com/parts-of-a-microscope/>)

## B) Optical part:

**Illuminator/Light source:** Some microscopes have an illumination lamp, while others have a mirror. While the illumination lamp is used as a direct light source, the mirror serves to direct the light coming from the light source onto the object.

**Condenser:** The zoom, located under the microscope table, is a system made of two strong lenses. It is used to collect the light coming from the light source on the object.

**Diaphragm:** It consists of a curtain located under the condenser that can narrow and expand the space in the middle at any rate. Adjusts the diameter of the light beam coming from the light source to the condenser.

The most valuable parts of the microscope and the parts that show the object in a magnified way are objective and ocular.

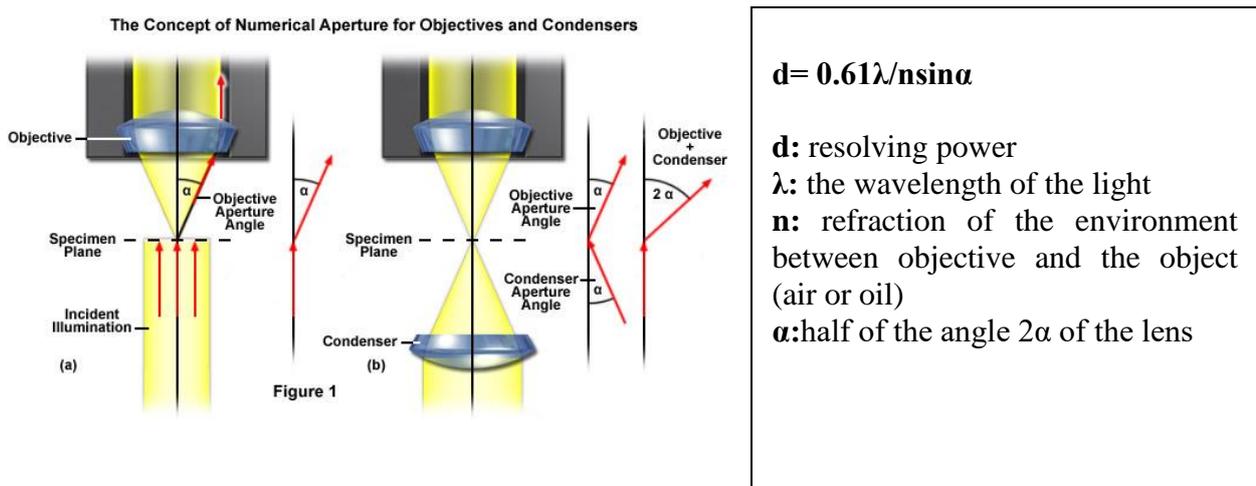
**Objective:** Lenses are a system made of one or more lenses screwed into the holes of the revolver located at the lower end of the microscope tube. It is installed in the order of increase in strength. While adjusting the image, one passes from weak lenses to stronger lenses by rotating the revolver one by one. The magnifying ability of the lens is written on itself. Weaker lenses are shorter, strong lenses are longer. They give a real, inverted and magnified image from the object.

**Ocular:** It is a system made of two lenses placed at the end of the microscope tube close to the eye. Its task is to transmit the image by magnifying the image given by the lens. Magnification capabilities are written on the ocular.

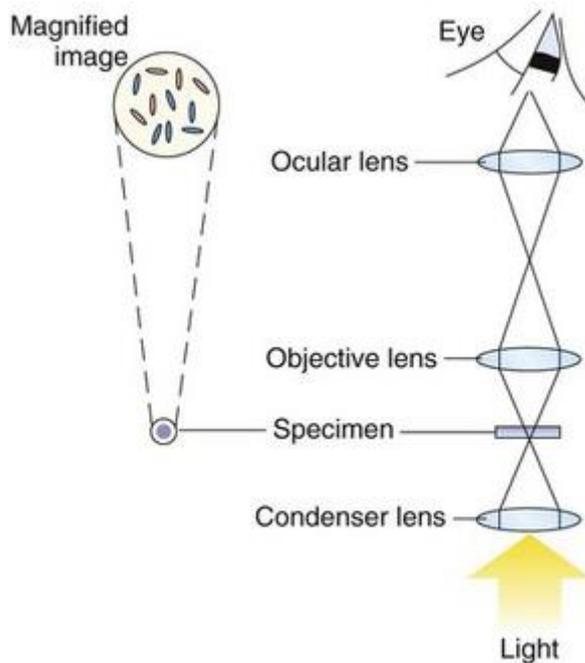
It is important to know how much you magnified the object you observe with a microscope. To find the magnification, simply multiply the numbers on the lens you use with the eyepiece.

In example; if the ocular power is 10X (ten times magnification) and objective power is 40X (40 times magnification), total magnification is  $10 \times 40 = 400$ .

The most important feature of a microscope is its resolving power. It is the ability to clearly select two side by side point shaped objects. When the apex angle of the cone formed by the rays is shown as  $2\alpha$ , the resolving power ( $d$ ) is calculated by the following formula.



**Figure 1.3** Refraction of the light by lenses. a) without condenser, b) with condenser (<http://zeiss-campus.magnet.fsu.edu/articles/basics/resolution.html>)



**Figure 1.4** Formation of the image (<https://clinicalgate.com/role-of-microscopy/>)

Zooming the lens to the object causes the  $\alpha$  angle to increase, thus reducing the separation power, in other words, the image looks larger and clearer. Refractive index ( $n$ ) is accepted as  $n = 1.0$  for air. In immersion oil, this value is  $n = 1.5$  and it strengthens the resolving power 1.5 times more. In addition, the shorter the wavelength of the light used, the stronger the separating power. Currently, the best microscope has  $\alpha$  angle is  $70^\circ$ . The shortest visible rays are blue and the wavelength is  $\lambda = 450\text{nm}$ , since the refractive index of the air is  $n = 1.0$ ,  $d$  is equal to  $292\text{nm}$  (approximately  $300\text{nm}$ ). If the immersion oil is used,  $d$  will be equal to  $194\text{ nm}$  (approximately  $200\text{ nm}$ ).

No matter what type of lens is used, no matter how magnified the image is, objects closer to each other than  $0.2\ \mu\text{m}$  or objects smaller than  $0.2\ \mu\text{m}$  in length cannot be selected well with normal light microscopes.

The microscope is a sensitive instrument that requires careful maintenance. When using the microscope, you should always observe the following general rules:

- Always carry the microscope with both hands, holding it firmly under the arm with one hand and the arm with the other hand.
- Do not put your microscope too close to the edge of the table and remove unnecessary things on the table beforehand.
- If you are using a preparation made with water, bring the base of the microscope to a perfectly horizontal position.
- Wipe the lens of the microscope with a soft cheesecloth.
- The adjustment knobs of the microscope should not be forced.
- While making a coarse adjustment, care should be taken not to hit the lens.
- When you are finished with your work, put the small lens into use before inserting the cover of the microscope.

### **Preparation of the microscope for the work**

- • Put the small lens into use. The correct fit of the lens is evident from a click.
- Adjust the mirror to brighten the space in the center of the table.
  - Most microscopes have a diaphragm that reduces and increases light.
  - Some objects are better studied in bright light, others in low light.
- Check the cleanliness of the lenses and wipe if necessary.



**PART 1 QUESTIONS**  
**(The questions will be given at the end of the lesson)**

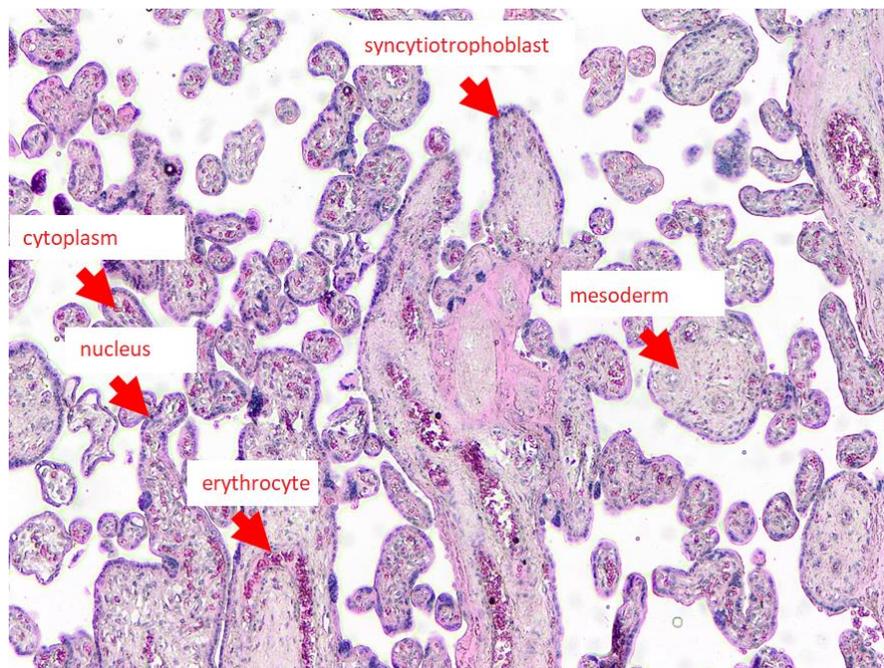
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## LAB 2. THE PREPARATION and THE INVESTIGATION OF HUMAN CELL PREPARATES

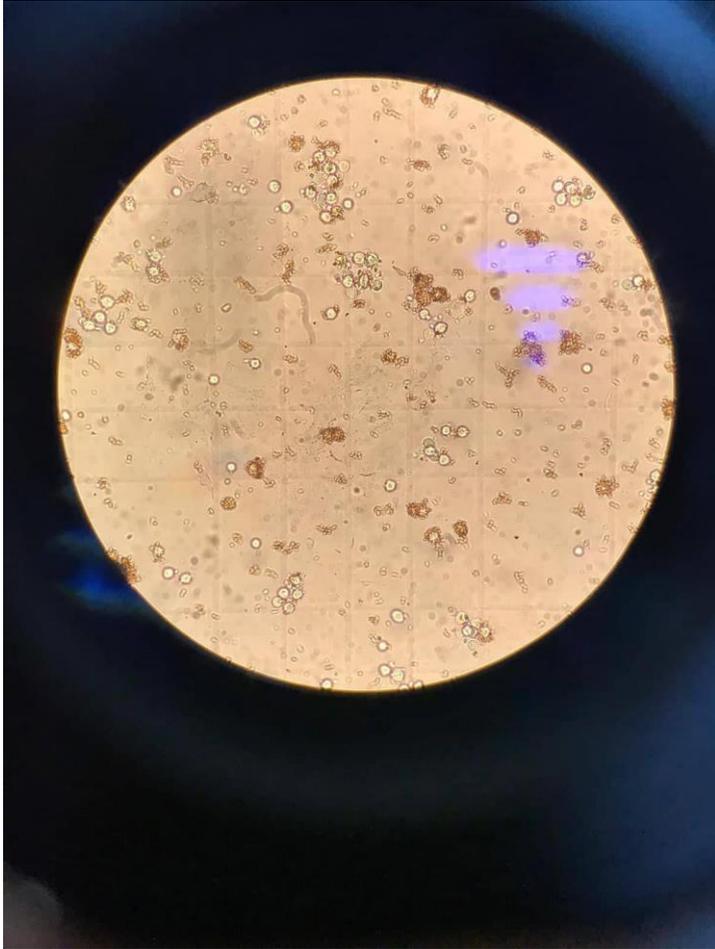
In this laboratory lesson, different cell types in preparates obtained from human spleen tissue and placenta will be investigated. In addition, nucleus-cytoplasm separation and cell size measurement will be performed.



**Figure 2.1** Hemotoxylene-eosine dying from plasenta tissue section



**Figure 2.2** Hemotoxylene-eosine dying from spleen tissue section



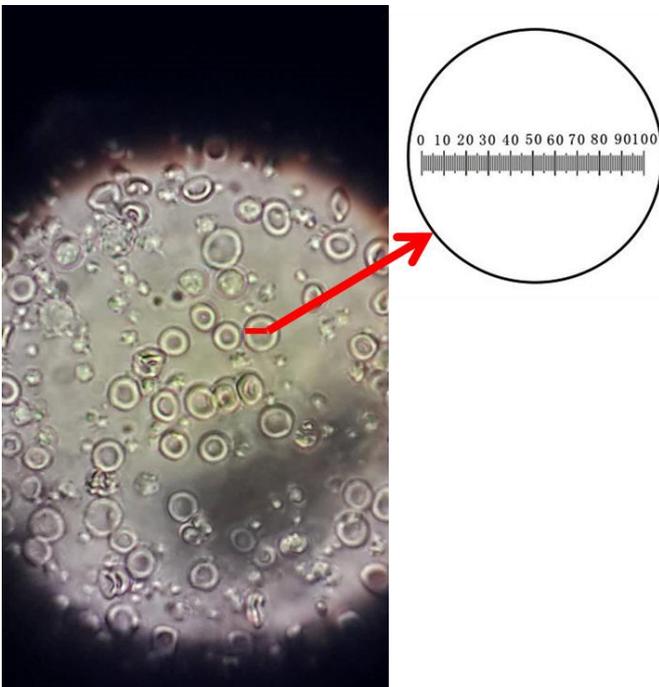
**Figure 2.3** The view of the B cells isolated from spleen under the light microscope

## The measurement of the Cell Size

- Ocular and objective micrometers are used to measure the cell size.
- Ocular micrometer is a round, made of thin glass, and the line on it is divided into 100 equal parts. It is placed in the ocular tube.
- Objective micrometer carries 1/100 mm split line. The space between two line =  $1/100 \text{ mm} = 0.01 \text{ mm} = 10 \mu\text{m}$ . It is placed on the microscope stage.
- The objective micrometer is placed in the center of the field of view using the lens with the lowest magnification capacity.
- Superimpose the baseline of the ocular micrometer with the baseline of the objective micrometer.
- Overlapping lines are found as far as possible from the starting points by following the regions of both micrometers.
- The regions between the baseline and coincident lines are counted in both ocular and objective micrometers.
- Based on the lens used, the distance between the two lines of the ocular micrometer is calculated.

**Example:** Let the 90th Line of the ocular micrometer coincide with the 11th Line of the objective micrometer. Since each chamber of the objective micrometer is  $10 \mu\text{m}$ ,  $11 \text{ aperture} = 110 \mu\text{m}$ . In this case, each chamber in the ocular micrometer is  $110/90 = 1.22 \mu\text{m}$  in length.

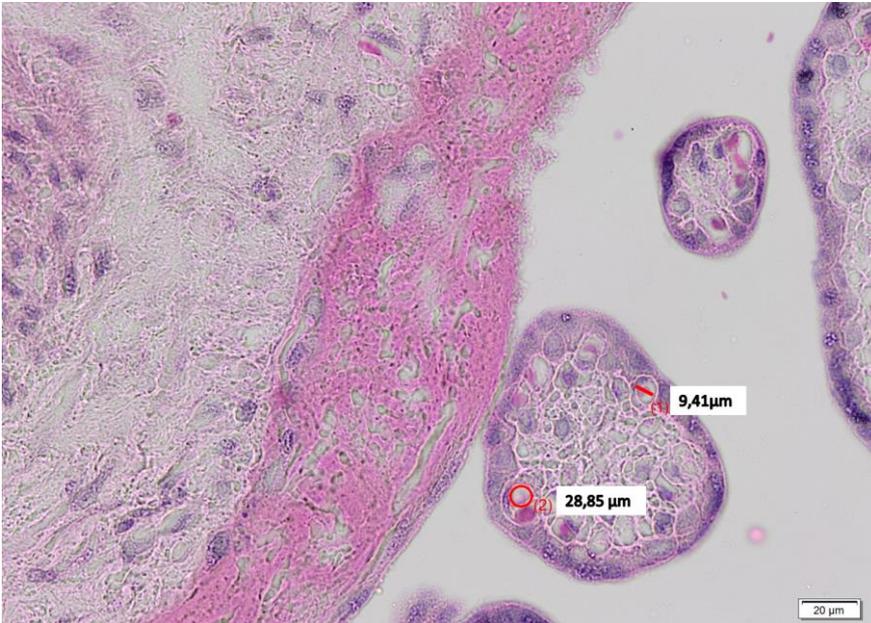
- This calibration process is done separately for each lens, after the adjustment is made, the lens micrometer is removed and the preparation is replaced.
- If the cell to be measured occupies the number of regions of the ocular micrometer, this value is multiplied by 1.22 and the microorganism size is found as  $\mu\text{m}$ .



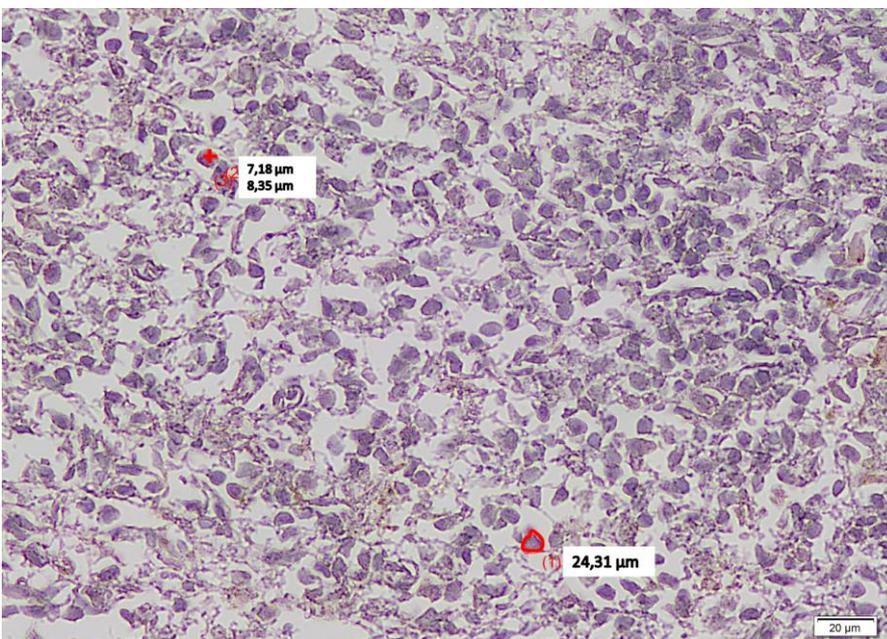
**Figure 2.4** Imaging and measuring the size of erythrocyte cells under light microscope

The diameter of the erythrocytes in a human is on average  $6\text{-}8 \mu\text{m}$ .

Today, computer programs are also used in cell size measurement. The prepared preparation is placed on the microscope stage. The image projected on the screen with the help of the camera is opened with the program. Scale up of the microscope is introduced to the program. The cells to be measured are marked and specified in the program. The measured image is saved.



**Figure 2.5** The cell size measurement in plasenta tissue section



**Figure 2.6** The cell size measurement in spleen tissue section



**PART 2 QUESTIONS**  
**(The questions will be given at the end of the lesson)**

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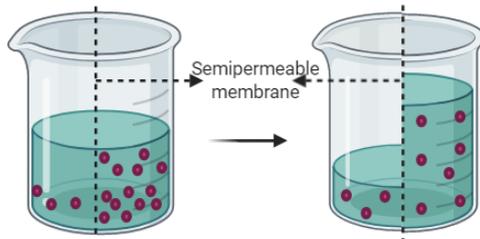
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## LAB.3- THE INVESTIGATION OF OSMOSIS

In this laboratory lesson, osmosis will be investigated with intestine membrane experiment.

If two solutions of different concentrations are separated by a semi-permeable membrane (impermeable to large soluble molecules and permeable to small solvent molecules), water (or liquid substance) moves from the lower substance concentration region to the higher substance concentration region. This process is called osmosis.



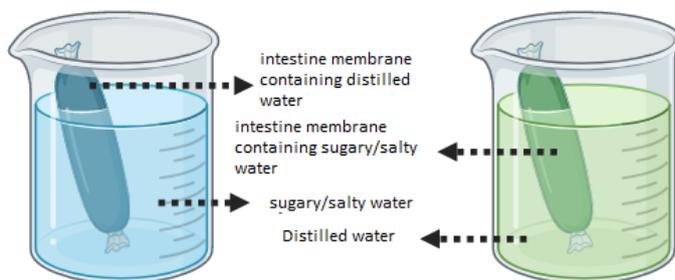
**Figure 3.1** Osmosis (prepared by BioRender.com)

Two ends of the intestinal membrane are tied up. Distilled water is put into one membrane and sugary/salty water is put into the other. The other ends of the membranes are tied, and the membranes with the solutions are weighed in a precision scale.

Two beakers are taken; distilled water is put into one beaker, sugary/salty water is put into the other.

The intestine membrane containing distilled water is submerged in the beaker containing sugary/salty water, whereas the membrane containing sugary/salty water is submerged in the beaker with distilled water. The membranes are incubated for 15-20 minutes.

The membranes are weighed in a precision scale again. The differences between the pre- and post-incubation values are evaluated.



**Figure 3.2** The laboratory experiment of osmosis (prepared by BioRender.com)



## **PART 3 QUESTIONS**

**(The questions will be given at the end of the lesson)**

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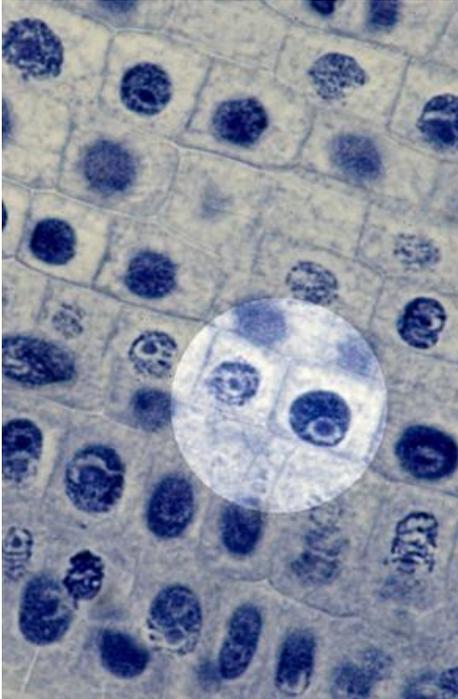
**Reviewed by:**



## LAB.4- THE INVESTIGATION OF MITOSIS

In this lesson, the ready-to-use preparates of the mitosis phases will be investigated.

### Interphase

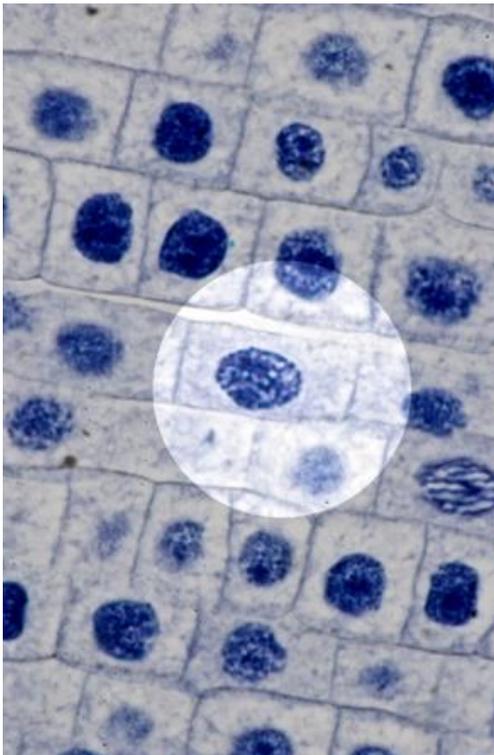


Cell size enlarges, DNA is replicated, and nucleus is apparent.

**Figure 4.1** The microscope view of interphase in an onion stem cell  
(<http://www.microscopy-uk.org.uk/mag/indexmag.html?http://www.microscopy-uk.org.uk/mag/artnov04macro/ironionroot.html>)

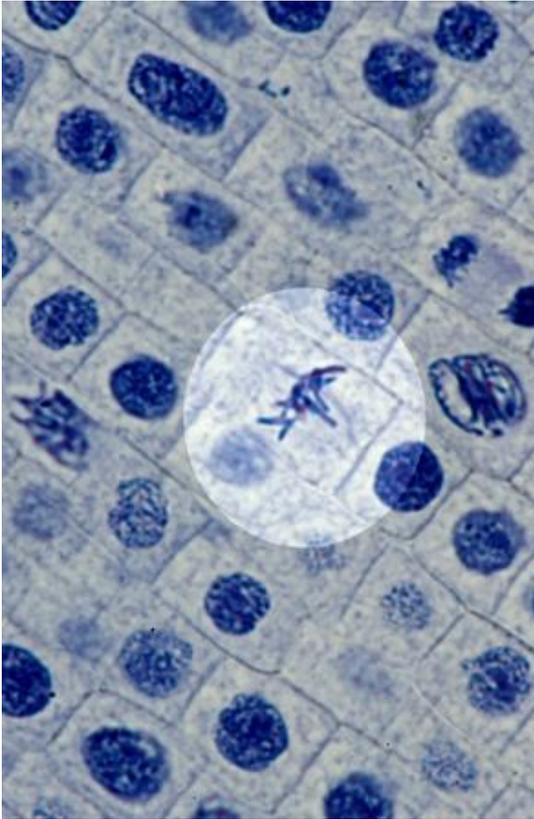
### Prophase

The paired chromatins become chromosomes by shortening and thickening. Nucleus membrane, nucleolus, and the organelles completely disappear. Chromosomes begin to move to the equatorial region of the cell.



**Figure 4.2** The microscope view of prophase in an onion stem cell  
(<http://www.microscopy-uk.org.uk/mag/indexmag.html?http://www.microscopy-uk.org.uk/mag/artnov04macro/ironionroot.html>)

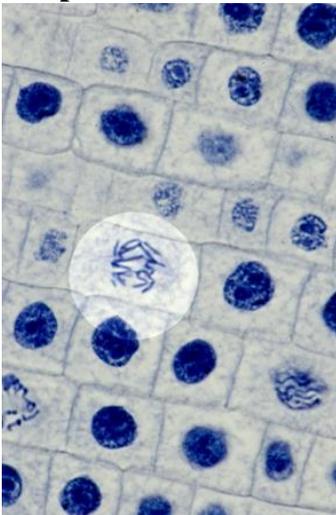
### Metaphase



The sister chromatids are arranged sometimes like a chamber sometimes mixed in the equatorial plane. Centrosomes begin to send their spindle fibers to the chromosomes. An articulation occurs in the middle of the cell. Spindle fibers attach to the sister chromatids. The chromosomes are seen most clear in this phase.

**Figure 4.3** The microscope view of metaphase in an onion stem cell (<http://www.microscopy-uk.org.uk/mag/indexmag.html?http://www.microscopy-uk.org.uk/mag/artnov04macro/jronionroot.html>)

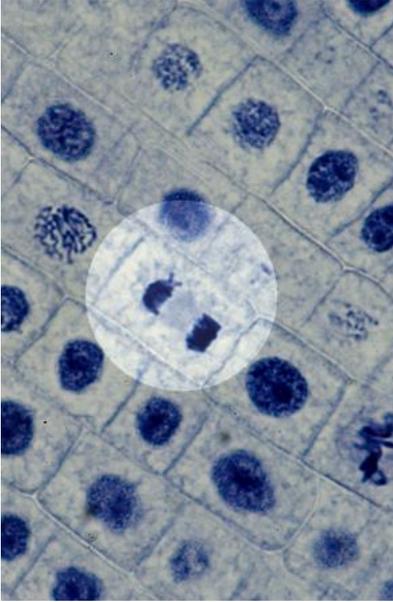
## Anaphase



The sister chromatids are completely separated by the division of centromeres into two at the same time. The sister chromatids move to the opposite poles of the cells by attaching spindle fibers. This phase is completed when the sister chromatids arrive the opposite poles.

**Figure 4.4** The microscope view of anaphase in an onion stem cell (<http://www.microscopy-uk.org.uk/mag/indexmag.html?http://www.microscopy-uk.org.uk/mag/artnov04macro/jronionroot.html>)

## Telophase

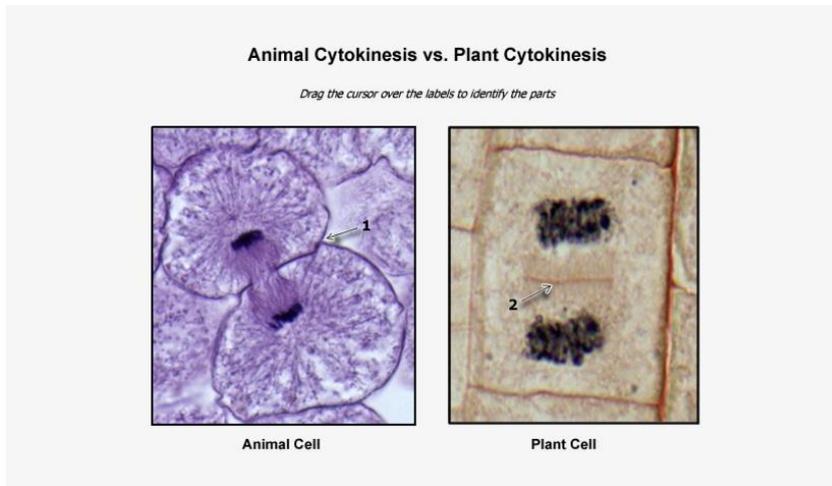


The nucleus membrane, nucleolus, and the organelles re-appear again. The chromatids at the opposite polars are surrounded with nucleus membrane. In brief, the phenomena that occur in this phase are the opposite of the phenomena in prophase. Cytokinesis occurs after this phase.

**Figure 4.5** The microscope view of telophase in an onion stem cell (<http://www.microscopy-uk.org.uk/mag/indexmag.html?http://www.microscopy-uk.org.uk/mag/artnov04macro/jonionroot.html>)

## Cytokinesis (Cytoplasmic division)

After the karyokinesis (nucleus division), two new cells are produced by articulation in animal cells and intermediate lamella in plant and other eukaryotes with cell wall.



**Figure 4.6** Hayvan ve bitki hücrelerinde mitoz bölünmenin aşamaları ([https://www.nicepng.com/ourpic/u2e6a9w7w7u2o0a9\\_plant-cytokinesis-images-cytokinesis-in-plant-cells-microscope/](https://www.nicepng.com/ourpic/u2e6a9w7w7u2o0a9_plant-cytokinesis-images-cytokinesis-in-plant-cells-microscope/))



**PART 4 QUESTIONS**  
**(The questions will be given at the end of the lesson)**

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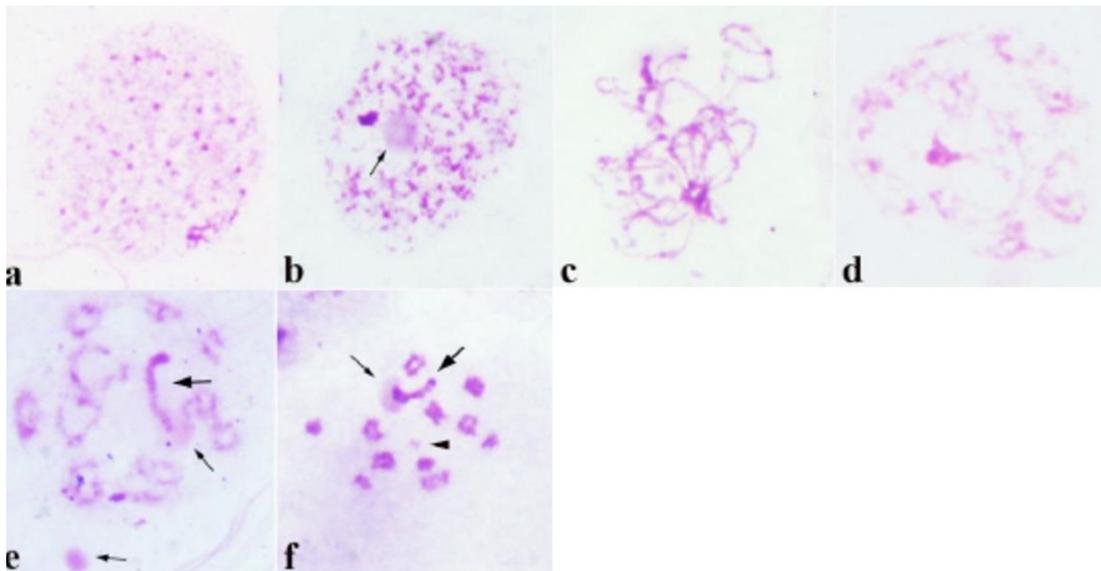
## LAB 5- THE INVESTIGATION OF MEIOSIS

There are four phases in meiosis like mitosis such as prophase, metaphase, anaphase, and telophase. These phases occur two times one after the other without interphase between them. In the end, four daughter cells are produced with two different types of genetic features. The most important difference between mitosis and meiosis is observed in prophase. Meiosis occurs in two steps: meiosis I and II.

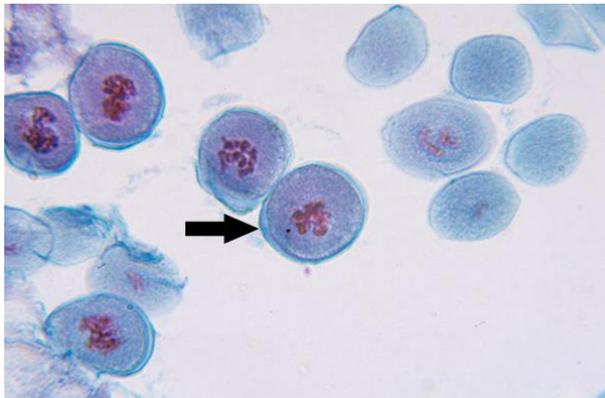
### MEIOSIS I

#### Prophase I

- It begins with the shortening and thickening of DNA strands. This phase is investigated in five sub-phases:
- ✓ **Leptotene:** It starts when the chromosomes are visible with a microscope. Two identical chromatids are coiled together.
- ✓ **Zygotene:** Similar homologous chromosomes, one from the mother and the other from the father, start to pair together. This pairing continues from one end to the other. At this stage, when two chromosomes, each bearing two chromatids, stand side by side, it is seen as if the cell carries  $4n$  chromosomes. This structure is called tetrate.
- ✓ **Pachytene:** The matching of homologous chromosomes is complete, but the chromosomes continue to shorten. In addition, unlike mitosis, there is an exchange of genetic material between tetrates at this phase. This is called **crossing-over**. This event takes place in the overlapping part (chiasma "chiasma") of the homologous chromosomes.
- ✓ **Diplotene:** Centromeres of chromosomes are not separated. There are two centromeres for four chromatids. Homologous chromosomes in the tetrate begin to separate from each other. However, there is no separation in the chiasma regions and the chiasma starts to slide towards the tip.
- ✓ **Diakinesis:** The chromosomes take their final form. The nucleolus disappears. The nucleus membrane breaks up.



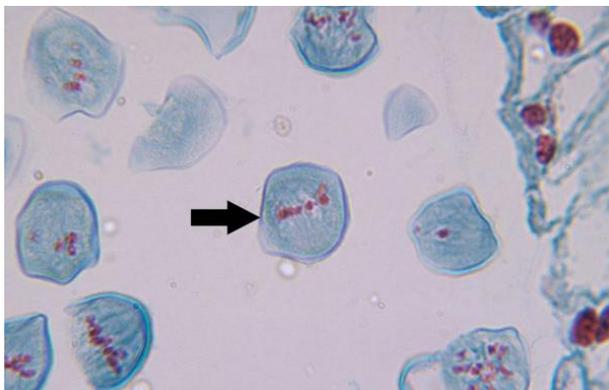
**Figure 5.1** Microscope views of prophase I sub-phases in meiosis: (a) chromatin fiber; (b) leptotene-zygotene; (c) pachytene; (d) dispersion step; (e) diplotene; (f) diakinesis (<https://biology.stackexchange.com/questions/51192/how-do-i-identify-the-different-stages-of-meiosis-under-microscope>)



**Figure 5.2** General microscope view of Prophase I in oleander reproductive cell (<http://sciences.usca.edu/biology/zelmer/122/celldivision/meiosis/>)

## Metaphase I

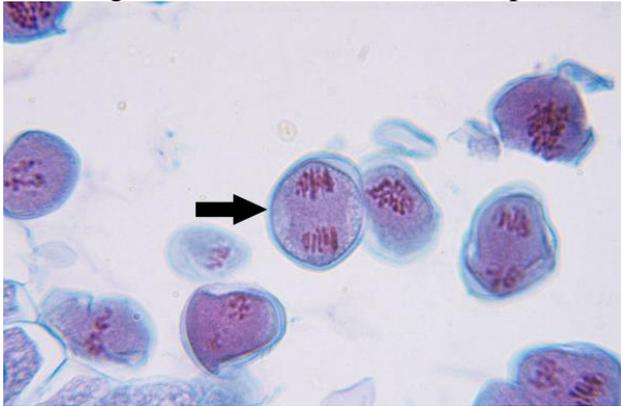
- Homologous chromosomes are arranged opposite each other on the equatorial plate. The chromosomes attach to the spindle fibers with their centromeres.



**Figure 5.3** Microscope view of Metaphase I in oleander reproductive cell (<http://sciences.usca.edu/biology/zelmer/122/celldivision/meiosis/>)

## Anaphase I

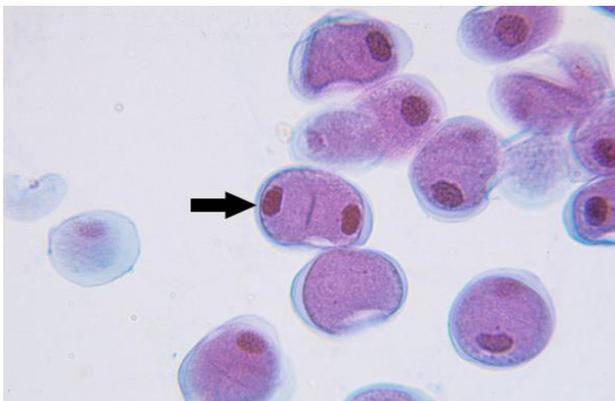
- Homologous chromosomes are separated and move to the opposite poles.



**Figure 5.4** Microscope view of Anaphase I in oleander reproductive cell (<http://sciences.usca.edu/biology/zelmer/122/celldivision/meiosis/>)

## Telophase I

- Chromosomes in two poles of the cell begin to elongate and become thin. Nucleus membrane is formed around them. With the articulation of the cytoplasm, two daughter cells with haploid number of chromosomes are formed.
- In animal cells: Cytoplasm divides into two by articulation after the nucleus division is completed, and two different cells are produced.
- In plant cells: An intermediate lamella occurs in the middle of the cell with two nuclei and arrives to the cell wall. Thus, two adjacent cells are produced. The chromosomes at the opposite poles begin to elongate and become thin. Nucleus membrane occurs around them. Two daughter cells with haploid chromosome are produced by articulation of the cytoplasm.

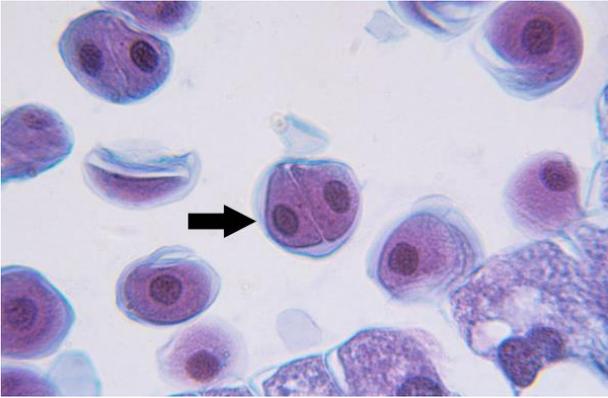


**Figure 5.5** Microscope view of Telophase I in oleander reproductive cell (<http://sciences.usca.edu/biology/zelmer/122/celldivision/meiosis/>)

## Cytokinesis I

Two daughter cells are produced by cytokinesis.

The events that have passed here are called meiosis-I. After that, unlike in mitosis, meiosis-II begins with the onset of prophase-II, with no interphase in between. Meiosis-II is almost identical to mitosis. By preserving the haploid chromosome number in the cells, prophase-II, metaphase-II, anaphase-II and telophase-II occur, and at the end of meiosis, 4 daughter cells with  $n$  chromosome number are formed.

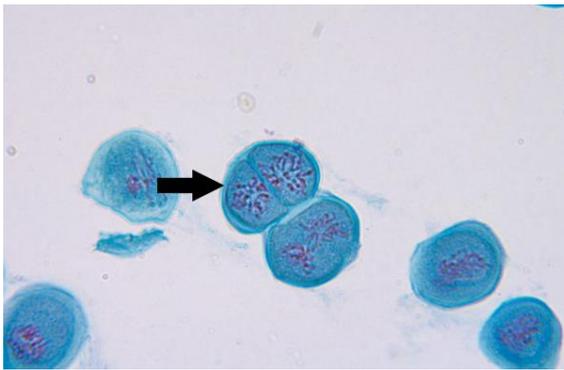


**Figure 5.6** Microscope view of cytokinesis I in oleander reproductive cell (<http://sciences.usca.edu/biology/zelmer/122/celldivision/meiosis/>)

## MEIOSIS 2

### Prophase II

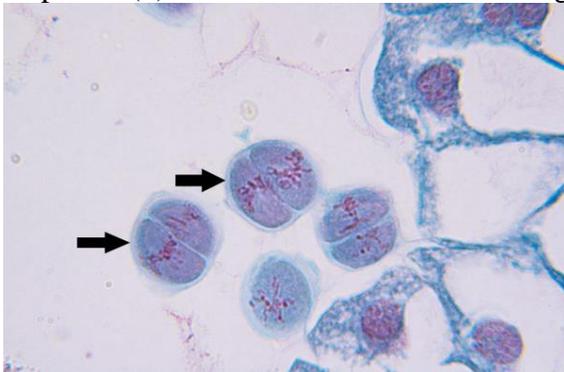
- Nuclear membrane is broken without an interphase between the telophase of first meiosis and the meiosis II. Birinci bölünmenin telofazı ile ikinci bölünme arasında bir dinlenme evresi olmadan çekirdek zarı parçalanır. New spindle strands are formed perpendicular to the direction of the first spindle strands.



**Figure 5.7** Microscope view of prophase II in oleander reproductive cell (<http://sciences.usca.edu/biology/zelmer/122/celldivision/meiosis/>)

### Metaphase II

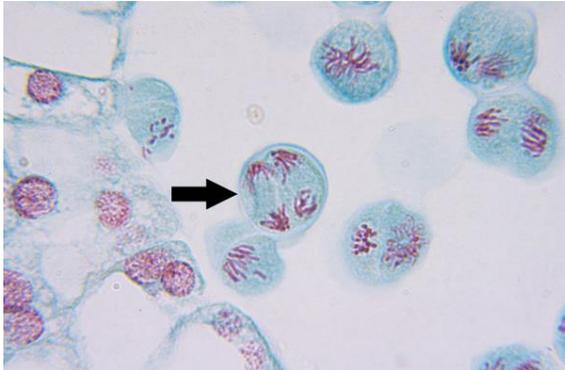
- Haploid ( $n$ ) chromosome of each daughter cell arrange in the equatorial plate.



**Figure 5.8** Microscope view of metaphase II in oleander reproductive cell (<http://sciences.usca.edu/biology/zelmer/122/celldivision/meiosis/>)

## Anaphase II

- It is also called an intermediate stage. This stage is similar to the anaphase stage in mitosis. However, while sister chromatids are found regularly in the anaphase in mitosis, they are found irregularly at this stage. This provides genetic diversity in Meiosis.

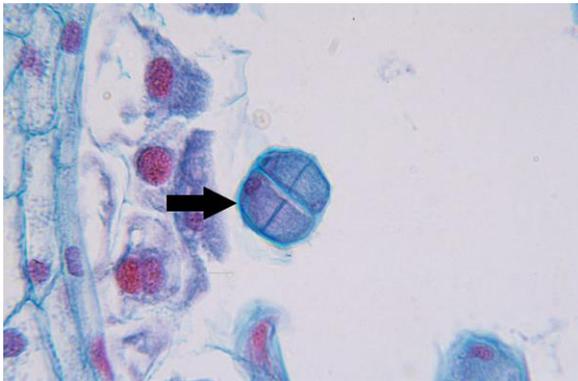


**Figure 5.9** Microscope view of anaphase II in oleander reproductive cell

(<http://sciences.usca.edu/biology/zelmer/122/celldivision/meiosis/>)

## Telophase II

- The coils of the chromosomes are opened, so they become invisible. Nucleus membranes are formed and the cytoplasm divides. Thus, four haploid cells are formed from a single cell.



**Figure 5.10** Microscope view of telophase II in oleander reproductive cell

(<http://sciences.usca.edu/biology/zelmer/122/celldivision/meiosis/>)

## Cytokinesis II

- At the end of meiosis, four cells (gametes) with  $n$  chromosome are produced.



**Figure 5.11** Microscope view of cytokinesis II in oleander reproductive cell

<http://sciences.usca.edu/biology/zelmer/122/celldivision/meiosis/>



**PART 5 QUESTIONS**  
**(The questions will be given at the end of the lesson)**

**Prepared by:**

**Comments:**

**Reviewed by:**



## LAB.6- DNA ISOLATION

Genomic DNA can be obtained from either materials such as blood, saliva, hair or directly from the cells with nucleus.

DNA isolation consists of three main steps:

1. Uncovering the DNA with high molecular weight by breaking down the cell
  2. Separating the DNA-protein complex by denaturation or proteolysis, and making DNA soluble
  3. Removing proteins, RNA, and the other macromolecules from DNA by enzymatic and/or chemical methods
- **In this lesson, DNA will be isolated from blood sample using a commercial DNA isolation kit.**



**Figure 6.1** The DNA isolation Kit that we use in our laboratory (<https://www.thermofisher.com/order/catalog/product/K182001>)

### The contents of the commercial kit

- **RNase A:** It digests RNA molecules in the sample.
- **Proteinase K:** It digests proteins. Thus, it provides the cell lysis.
- **Lysis/binding buffer:** It lyses cells and attaches DNA molecule to the column.
- **Alcohol:** It precipitates DNA.
- **Wash buffer:** It removes the wastes.
- **Elution buffer:** It elutes DNA during the last step.
- **Spin-column tubes:** It is used to remove protein and RNA wastes without losing DNA by filtration method.

## DNA ISOLATION PROTOCOL

1. Adjust the heater to 55 °C.
2. Add 200 µl blood sample into sterile 1.5 ml eppendorf tube.
3. Add 20 µl Proteinaz K on the sample.
4. Add 20 µl RNaz A on the sample and incubate for 2 minutes at room temperature.
5. After incubation, add 200 µl Lysis/Binding buffer on the sample and vortex it properly.
6. Incubate the sample at 55 °C for 10 minutes.
7. After incubation, add 200 µl %96-100 ethanol.
8. Homogenize the sample by vortexing for 5 seconds.
9. Put the lysate into the spin coloumn.
10. Centrifuge the sample at 10.000xg for 1 minute. Discard the collection tube and put the coloumn into a new collection tube.
11. Add 500µl wash buffer 1 on the sample and centrifuge at 10.000xg for 1 minute.
12. Discard the collection tube and put the coloumn into a new collection tube.
13. Add 500µl wash buffer 2 on the sample and centrifuge the sample at maximum rate for 3
14. Discard the collection tube and put the spin-coloumn into a new 1.5 ml Eppendorf tube.
15. Add 25 µl elution buffer in the coloumn and incubate it at room temperature for 1 minute.
16. Centrifuge the sample at maximum rate for 1 minute.
17. At the end, 1.5 ml Eppendorf tube contains pure DNA. It can be stored at +4 °C for a short time (a week, two weeks), whereas it is stored at -20 °C for long periods (six months).

## Determination of DNA concentration and purity

Since the heterocyclic rings of nucleotides have the ability to absorb light at 260 nm wavelength, the degree of absorption at this wavelength is a measure of the amount of nucleic acids. Proteins absorb at 280 nm wavelength. Accordingly, the amount and purity of the DNA can be determined from the values obtained in the spectrophotometer (nanodrop instrument in our laboratory) at 260 and 280 nm wavelengths. DNA concentration is calculated with this formula:

$$C_{\text{DNA}} (\mu\text{g/ml}) = (A_{260} \times \text{Dilution coefficient} \times 50 \mu\text{g/ml})$$

The ratio in 260 and 280 nm wavelengths ( $A_{260} / A_{280}$ ) gives an idea about the purity and quality of DNA. This ratio is about 1.8 - 2.0 in well-purified DNA. If phenol and protein are high in the sample, the ratio will be lower than these values.



Figure 6.2 Nanodrop measuring system (<https://www.thermofisher.com/order/catalog/product/ND-2000>)

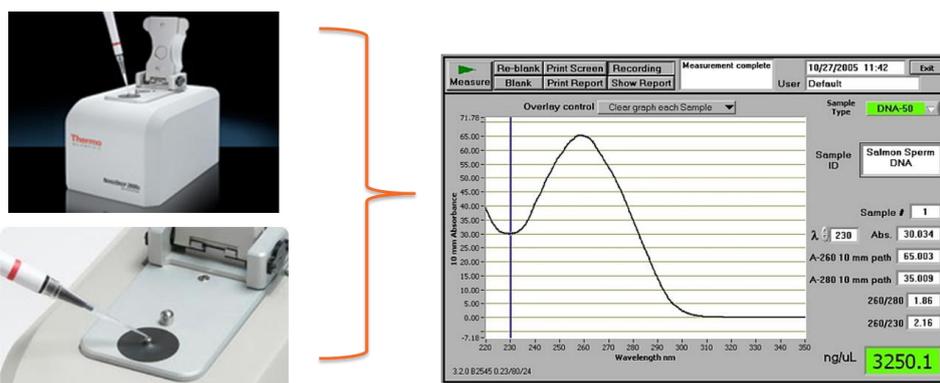


Figure 6.3 Loading the sample on the Nanodrop instrument and analysis of the results (<https://www.biocompare.com/Product-Reviews/41292-NanoDrop-ND-1000-From-Thermo-Scientific/>, <https://www.isogen-lifescience.com/NanoDrop-2000>, [http://uoqasim.edu.iq/e\\_Learning/lec\\_file/CHAPTER%204.pdf](http://uoqasim.edu.iq/e_Learning/lec_file/CHAPTER%204.pdf))



**PART 6 QUESTIONS**  
(The questions will be given at the end of the lesson)

**Prepared by:**

**Comments:**

**Reviewed by:**



# LAB.7- POLYMERASE CHAIN REACTION (PCR) and AGAROSE GEL ELECTROPHORESIS

## LAB.7.1 POLYMERASE CHAIN REACTION (PCR)

PCR is based on the binding of specific oligonucleotide primers to target sequences on a double stranded DNA molecule and amplification of the target regions.

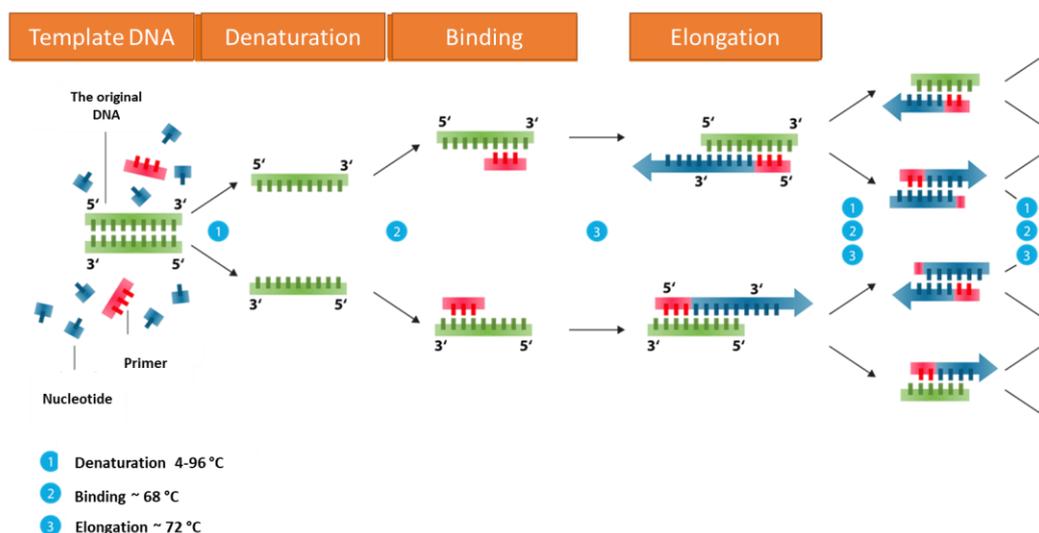
PCR occurs by repeating the three steps:

**1. Denaturation:** Double stranded DNA becomes single stranded at high temperatures for the binding of the primers. In general, the most effective denaturation temperature is 92-95 °C.

**2. Annealing (Binding of the primers):** Primers hybridize with the complementary sequences on single stranded DNA molecules. In addition, primers provide a free 3'OH for DNA polymerase. In this step, T<sub>m</sub>/binding temperature ratio is very important for the PCR reaction.

**3. Extension:** Taq DNA polymerase elongates the primers from their 3'OH ends in the presence of proper buffer and four types of deoksiribonucleoside triphosphate (dNTP). In this step, it is usually accepted that 72°C is the most suitable temperature for the polymerization activity of the Taq DNA polymerase enzyme.

DNA fragments amplify exponentially by consecutively repeating denaturation, annealing, and extension steps as shown in figure 7.1. The reason for the exponential increase is the fact that the product of a cycle acts as a template for the primers in the next cycle.

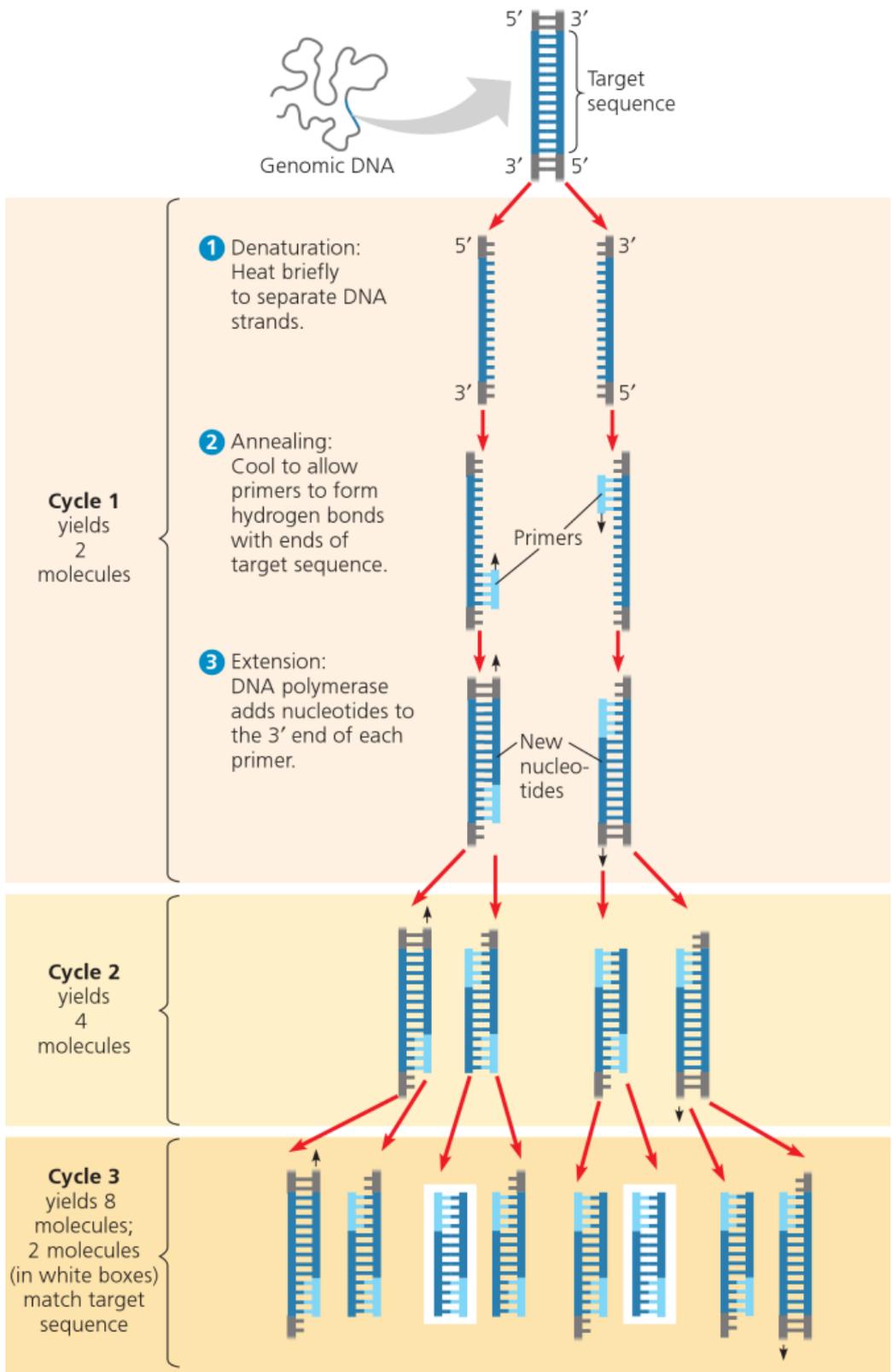


**Figure 7.1** PCR steps (<https://microbeonline.com/polymerase-chain-reaction-pcr-steps-types-applications/>)

The number of amplicon is calculated with this formula:

$$\text{Amplicon number} = 2^n$$

n: the number of repeating cycle

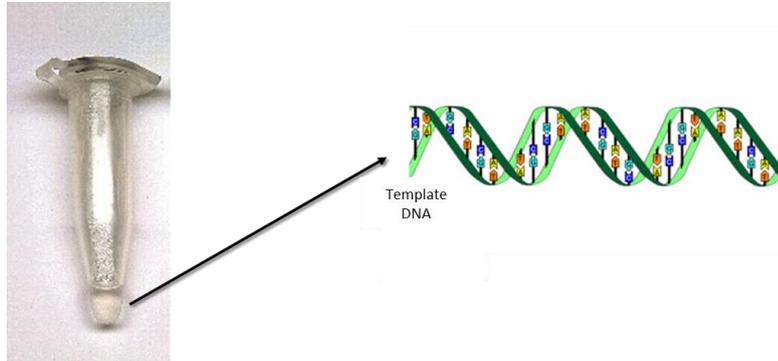


**Figure 7.2** The cycles of PCR reaction (<https://laboratoryinfo.com/polymerase-chain-reaction-pcr/>)

## MAIN COMPONENTS OF PCR REACTION

### 1. Template DNA:

In a PCR reaction, genomic DNA, plasmid and phage DNA, various genes or any DNA fragment can be used as a template. In this lesson, we will use the DNA sample that we isolated in previous lesson.



**Figure 7.3** Template DNA for PCR reaction

<https://seraplaekolaybiyoloji.wordpress.com/2017/12/19/replikasyon-dnanin-kendini-eslemesi/>

### 2. Polymerases:

DNA polymerase enzymes catalyze the synthesis of polypeptide chains longer than 4 types of dNTPs by using the base information in the template chain to form a DNA chain complementary to the template chain. These enzymes need primers for this process. The direction of the synthesis is from 5' to 3' end, and by the nucleophilic effect of the dNTPs in the environment to the free 3'OH end of the primer, the catalysis of the phosphodiester bonds and the polymerization of the new DNA chain are provided.

Thermostable DNA polymerases are preferred in the PCR reaction. The most common polymerase is Taq/Amplitaq DNA polymerase. This enzyme was isolated from a bacterium called *thermus aquaticus*. In this lesson, we will use Taq polymerase for the PCR reaction.



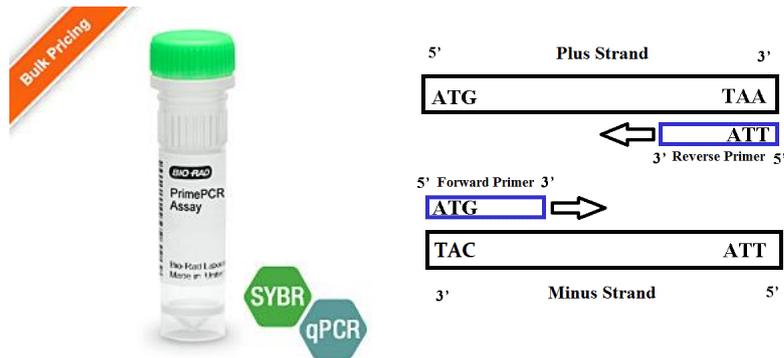
**Figure 7.4** The commercial Taq polymerase

<https://www.thermofisher.com/order/catalog/product/10342020>

### 3. Primers:

The primers in 18-25 nucleotide length are preferred in order to binding to the template with high ratio. You have to be careful for the issues while designing the primers:

1. Whenever possible, four bases should be used in equal numbers.
2. Primers should not contain polypurine, polypyrimidine, or repeated regions.
3. 3' ends of the primers should not be complementary to each other or any region in primer.



**Figure 7.5** A primer example used in PCR reaction

(<https://biology.stackexchange.com/questions/56304/manual-primer-design-for-a-gene-on-the-reverse-strand>, <http://www.bio-rad.com/en-us/prime-pcr-assays/assay/qhsaced0037870-primepcr-sybr-green-assay-mitf-human>)

### 4. dNTP Mix:

Deoxyribonucleoside triphosphates with high purity are provided either one by one or mix of four. The optimum dNTP concentration for the PCR reaction is depends on:

- $MgCl_2$  concentration
- Reaction conditions
- Primer concentration
- The length of the amplicon
- The number of PCR cycle



**Figure 7.6** A commercial dNTP mix

(<https://www.rpicorp.com/products/molecular-biology/zymo-products/dntp-mix-10-mm-500-ul.html>)

## 5. Buffer and MgCl<sub>2</sub> :

In a PCR reaction, DNA polymerase specific buffers are used. Mg<sup>+2</sup> ions make soluble complexes with dNTPs, stimulate polymerase activity, and provide primer/template interaction. Low Mg<sup>+2</sup> concentration leads to decrease in amplicon production, whereas high Mg<sup>+2</sup> concentration leads to non-specific product accumulation.



**Figure 7.7** PCR buffer (<https://www.thermofisher.com/order/catalog/product/4379876>) and MgCl<sub>2</sub> (<http://www.bio-rad.com/en-tr/sku/1708872-mgcl2-solution-for-pcr-50-mm-1-25-ml?ID=1708872>)

### AREA OF PCR USAGE:

- In basic molecular biological researches (cloning, sequence analysis, DNA mapping)
- In clinical medicine for the DNA-based diagnosis of various diseases (sickle cell anemia, cystic fibrosis, fragile x syndrome, leukemia, etc.).
- For the determination of tissue type for tissue transplantation by showing allelic sequence variations.
- In forensic medicine for genetic typing of the samples (maternity-paternity test)
- In the other fields of medicine

**!!! In this lesson, the DNA samples, which we isolated in previous lesson, will be used as a template, and a gene region will be amplified by PCR reaction.**



- Template DNA: 5µl
- Buffer: 2,5 µl
- dNTP: 1 µl
- MgCl<sub>2</sub>: 3,6 µl
- Primer F: 1 µl
- Primer R: 1 µl
- Ultra distilled water: 10,40 µl
- Taq polymerase: 0,5 µl

These contents are put into a PCR tube, mixed, and load in the thermal cycler instrument.

**Table 7.1** Thermal Cycler program

<b>Initiation 1x</b>	<b>30 x</b>	<b>1x</b>	$\infty$
95°C for 5 minutes	95 °C for 15 seconds 58 °C for 50 seconds 72 °C for 30 seconds	72 °C for 10 minutes	+4 °C

We obtain a number of amplicon copies of the target region at the end of this procedure.



**PART 7.1 QUESTIONS**  
**(The questions will be given at the end of the lesson)**

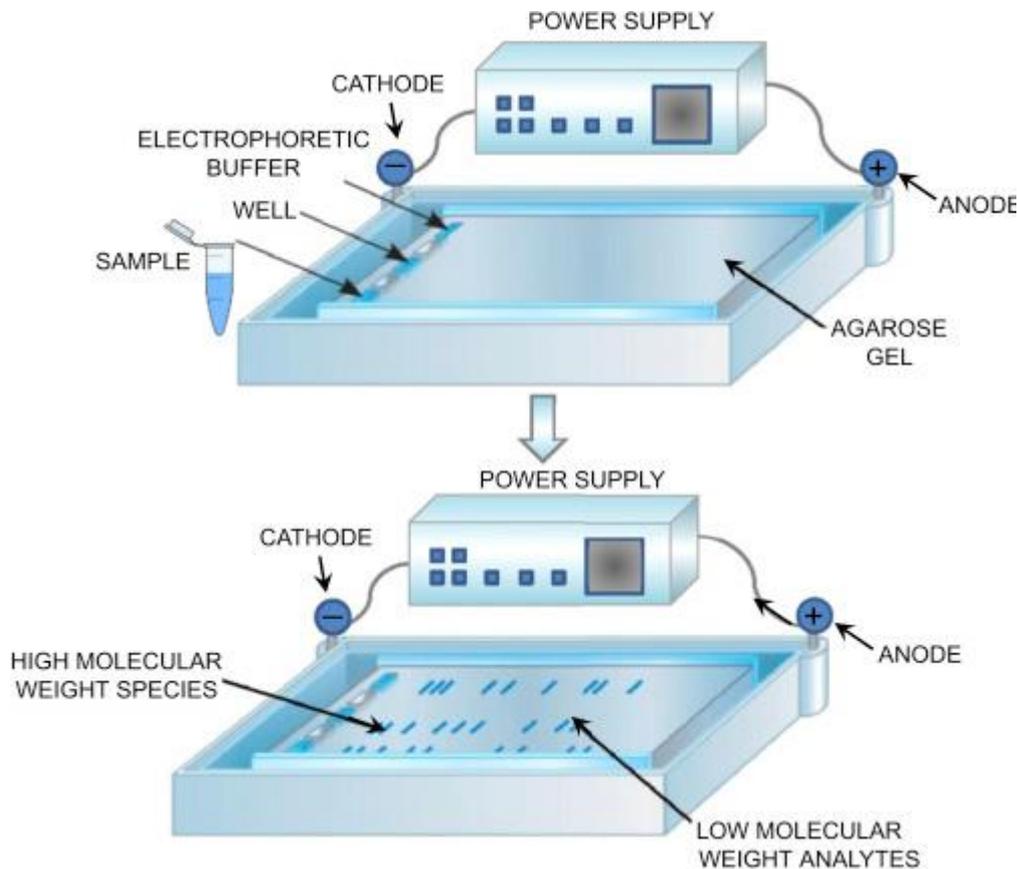
**Prepared by:**  
**Comments:**

**Reviewed by:**



## LAB.7.2 AGAROSE GEL ELECTROPHORESIS

The net electrical charges of molecules affect the movement of these molecules in an electrical field. In the electrophoresis technique, the movement of DNA molecules on the gel is provided by an electric current.



**Figure 7.8** General principle of electrophoresis

(<https://laboratoryinfo.com/wp-content/uploads/2020/01/image-above-shows-how-an-agarose-gel-electrophoresis-is-performed.jpg>)

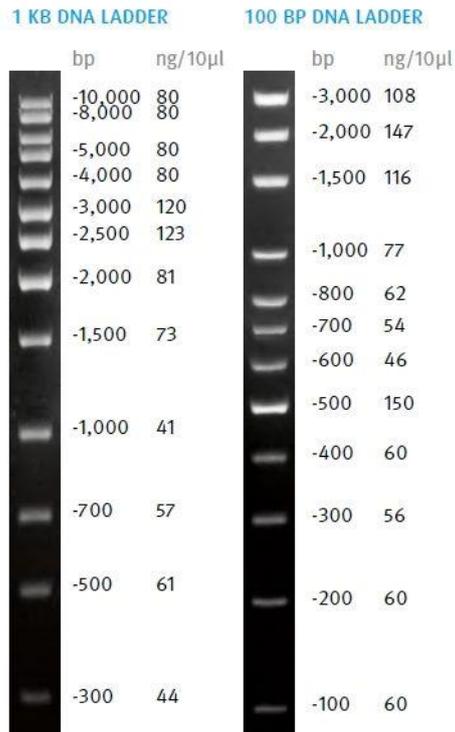
The most common method used for the identification, purification and separation of nucleic acid fragments is agarose gel electrophoresis. For this reason, this technique is an important experimental technique in the field of molecular genetics in terms of identifying DNAs isolated for various purposes, controlling their cleanliness, determining which form they are in, determining their size, and investigating new forms obtained after changes in DNA structure, especially by genetic engineering techniques.

Although the separation power of agarose gels is lower than polyacrylamide gels, the lengths of the DNA particles they can separate can range from 200 base pairs (bp) to 50 kilo base (kb). Samples in agarose gel are generally run in a horizontal position, in an electrical field of constant power and direction.

Agarose is an unbranched chain polymer derived from seaweed. Commercially available agarose is not completely pure and can be found with other polysaccharides, salts and proteins. The rate of these impurities affects both the movement of the DNA in the gel and the ability of the DNA to be obtained from the gel to be the substrate in enzymatic reactions.

## The Factors Affecting the Movement Rate of DNA in an Agarose Gel:

**Molecular size of DNA:** The speed of double-stranded linear DNA molecules in the gel is inversely proportional to the logarithm of the number of base pairs (bp). Large molecules move slower because of the greater friction and the more difficult path they find between pores in the gel.



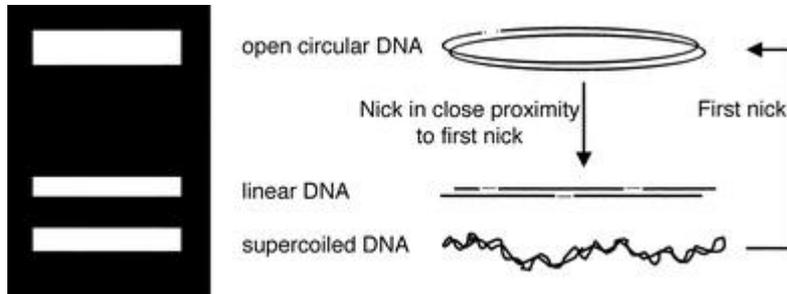
**Figure 7.9** DNA molecules according to their molecular weight (<https://laborimpex.be/07-11-00050-100-bp-dna-ladder-ready-to-load-50-g-for-100-applications.html>)

**Agarose Concentration:** A linear DNA molecule of a certain size travels at different speeds in gels of different agarose concentrations. There is a relationship between the logarithm of the electrophoretic mobility of DNA and gel concentration. Therefore, it is possible to separate DNA molecules of very different sizes by using gels of different concentrations.

**Table 7.2** Agarose concentration according to DNA size

Percentage of Agarose gel (weight/volume)	DNA size
%0.5	1 - 30 kb
%0.7	800 bp - 12 kb
%1.0	500 bp – 10 kb
%1.2	400 bp – 7 kb
%1.5	200 bp – 3 kb
%2.0	50 bp – 2 kb

**DNA Conformation:** Supercurved circular, circular with single chain fracture and linear DNA molecules with the same molecular weight move at different speeds in agarose gels. Although the relative velocities of these three forms are mainly dependent on the agarose concentration of the gel, the strength of the applied current, the ionic strength of the buffer, and the number of supercoils in the supercoiled circular DNA are also among the factors affecting the velocity.



**Figure 7.10** DNA conformation on an agarose gel

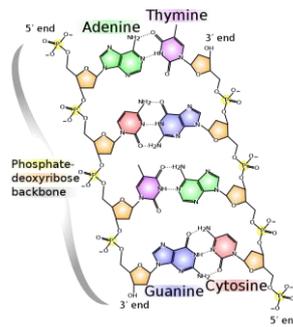
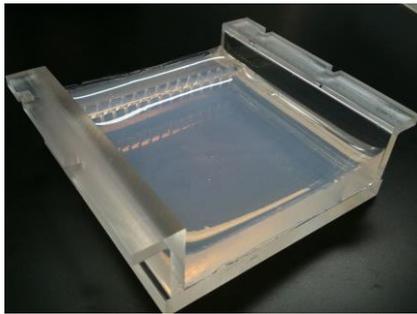
(<https://pubs.rsc.org/en/content/articlelanding/2004/dt/b313634e/unauth#!divAbstract>)

**Voltage:** The motion speed of linear DNA segments at low voltages is directly proportional to the applied voltage. However, as the strength of the electrical field increases, the motion of large molecular weight DNA fragments increases to varying degrees. Therefore, the effective separation range in the agarose gel decreases with increasing voltage. To best separate DNA fragments larger than 2 kb in size, agarose gels should be run with a current of no more than 5 volts/cm.



**Figure 7.11** Voltage source (<https://www.fishersci.com/shop/products/fisherbiotech-electrophoresis-power-supplies-120v-maximum-voltage-3000v/fb3000q>)

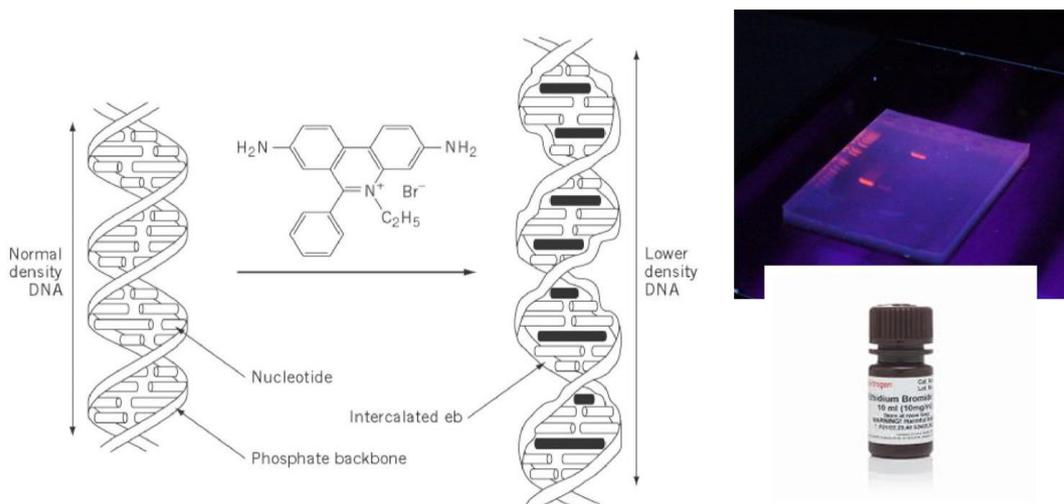
**Base composition and temperature:** The behavior of DNA molecules in agarose gels is not much affected by the base composition and the temperature at which the gel is run. Therefore, the relative motion of DNA molecules of different lengths in agarose gel does not change between 4-30 °C. Gels are generally run at room temperature. However, it is more convenient to run gels containing less than 0.5% agarose at 4 °C.



**Figure 7.12** Agarose gel at room temperature (<https://en.wikipedia.org/wiki/Agarose>) and DNA base composition ([https://en.wikipedia.org/wiki/Complementarity\\_\(molecular\\_biology\)](https://en.wikipedia.org/wiki/Complementarity_(molecular_biology)))

### The Presence of Intercalating Agents:

The fluorescent ethidium bromide dye used to observe DNA in agarose and polyacrylamide gels reduces the electrophoretic action of linear DNA molecules by 15%.



**Figure 7.13** Principle of ethidium bromide fluorescence radiation (<http://what-when-how.com/molecular-biology/ethidium-bromide-molecular-biology/>, [https://en.wikipedia.org/wiki/Ethidium\\_bromide](https://en.wikipedia.org/wiki/Ethidium_bromide), <https://www.fishersci.com/shop/products/ethidium-bromide/15585011>)

**Composition of Electrophoresis Buffer:** The electrophoretic action of DNA is affected by the composition and ionic strength of the electrophoresis buffer. In the absence of ions (in example; if buffer is not added to the gel mistakenly) the electrical conductivity is minimal and the movement of DNA is very slow. If very high ionic strength buffer is used (in example; when 10x electrophoresis buffer is used accidentally), the electrical conduction is too high and too much heat is released. The worst case is when the gel melts and the DNA is denatured. Different buffers can be used for natural double stranded DNA. These include EDTA (pH 8.0), and Tris-acetate (TAE), Tris-borate (TBE) or Tris-phosphate (TPE) at a concentration of approximately 50 mM pH 7.5-8.5. The buffers are usually prepared as concentrated solutions and stored at room temperature.

Electrophoresis buffers with low ion capacity are less heated in long-term (overnight) electrophoresis processes because their electrical conductivity is also lower. For this reason,

low current is preferred for long-term electrophoresis processes. Electrophoresis buffers with high ion capacity are used in short-term electrophoresis processes as they heat up quickly.



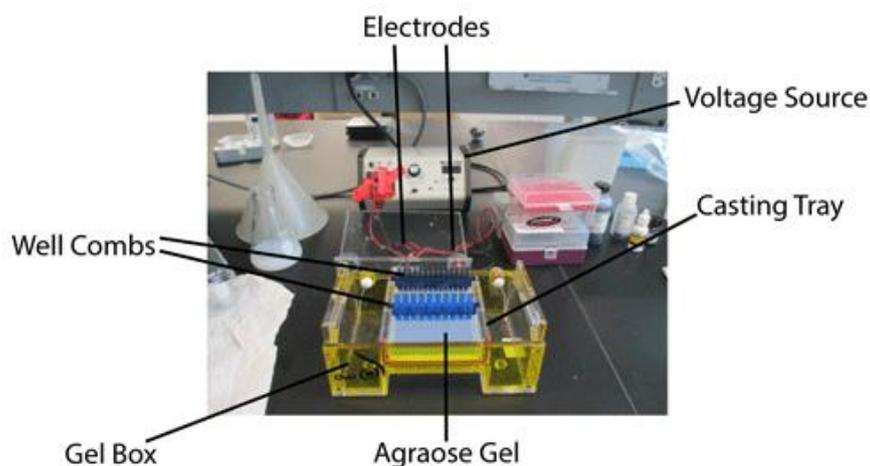
**Figure 7.14** TAE (<https://www.gbiosciences.com/Buffers-Reagents-Chemicals/Electrophoresis-Related-Buffers-Chemicals/TAE-Running-Buffer-50X>) and TBE buffers (<https://www.amazon.com/10x-TBE-Buffer-Electrophoresis-1L-10L/dp/B074RCYWV5>)

### Loading Dye:

It is used to monitor how fast DNA is traveling on agarose gel. Contains two different dyes (bromophenol blue and xylene cyanol FF). It allows the DNA to settle in the well in the gel. It binds to the EDTA metal ions it contains and inhibits metal-dependent nucleases.



**Figure 7.15** DNA loading dye used in our laboratory (<https://www.thermofisher.com/order/catalog/product/R0611>)



**Figure 7.16** Parts of electrophoresis equipments (<https://www.addgene.org/protocols/gel-electrophoresis/>)



**Figure 7.17** UV transilluminator (<https://www.industrybuying.com/ultraviolet-transilluminator-tarson-LA.UL.UL.1443645/>, <https://www.coleparmer.in/i/cole-parmer-uv-transilluminator-25w-254nm-21x26cm-filter-230v/9762210>)

### The Protocol

1. The agarose, which is weighed according to the size of the gel to be used, is dissolved by boiling in TBE buffer. (For this study; 2 g of agarose is weighed and placed in a small beaker, 100 mL 0.5X TBE is added on it and boiled in the microwave for 2 minutes to homogeneously melt.)
2. Add 1 $\mu$ l ethidium bromide into the gel.
3. Place the combs into molds with closed edges, pour the gel and allow it to freeze for approximately 15-20 minutes.
4. After the gel is frozen, place the mold in the electrophoresis tank and remove the combs.
5. Fill the gel box with the same buffer that you used for the gel preparation.
6. Load the amplicons into the wells in the gel via micropipette.
7. Connect the box to the power source and an electric current is passed through the gel (Executed for 105 v 10 minutes).
8. DNA carrying a charge at neutral pH (-) moves towards the anode. Smaller molecules move faster than large ones towards the (+) electrode.
9. After the electrophoresis is completed, the gel is placed on a transilluminator and the DNA is observed under UV light.

**Table 7.3** Possible problems, reasons, and solutions

Problem	Possible Reason	Solution
DNA sweep in the gel road	High DNA concentration	Decrease the DNA concentration
Background bands Non-specific PCR products	Unpurified DNA Wrong amplification conditions	Prepare DNA again and measure at OD 260/280. The average ratio should be 1.8. Control the thermal cycler. Thermal cycler should be validated for the process.
Weak B bands or inhibition of the reaction	Low DNA concentration Wrong amplification conditions Not properly closed wells Insufficient mixing of the samples	Increase the DNA concentration. Control the thermal cycler. The thermal cycler should be validated for this process. Control and be sure o the strip caps are closed. Vortex sufficiently the master mix.



**PART 7.2 QUESTIONS**  
**(The questions will be given at the end of the lesson)**

**Prepared by:**  
**Comments:**

**Reviewed by:**



## LAB. 8-DNA SEQUENCING

DNA sequencing is the determination of the order of nucleotide bases (adenine, guanine, cytosine, and thymine) in a DNA molecule.

The first DNA sequence was obtained by two-dimensional chromatographic methods in 1970s. Later, with the development of dye-based automatic sequencing methods, DNA sequencing became easier and faster. In biological research, it is necessary to know the sequence of the gene or other part of the genome in order to make a diagnosis in disease or forensic cases.

### The principle of DNA Sequencing:

It is a chemical procedure that creates DNA fragments of different sizes by breaking the labeled DNA molecule at each repeat of a base. The length of each labeled fragment determines the position of the base. As a result, when these products are dissolved by electrophoresis on polyacrylamide gel according to their size, DNA sequences can be observed as radioactive bands. This technique can read a region of at least 100 bases from the marking point. The reactive dimethyl sulfate is specific for purines and hydrazine is specific for pyrimidines.

### The first studies:

1973 – Gilbert and Maxam explained 24 bp sequence.

1975 – Sanger revealed chain termination method.

1975 – The whole genome of  $\phi$ X174 bacteriophage was sequenced.

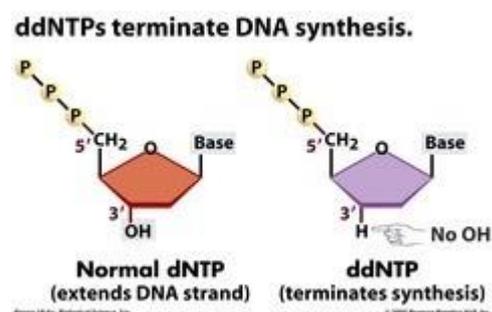
The knowledge of DNA sequence explains the reasons of various diseases and enables the treatment for the diseases.

### SANGER SEQUENCING:

It is more effective than Maxam-Gilbert method and less toxic materials are used.

**The principle:** The usage of dideoxynucleotide triphosphates (ddNTPs) as a DNA chain terminator.

The difference between ddNTPs and dNTPs is the lack of OH group on 3' end of ddNTPs.



**Figure 8.1** The difference between dNTP and ddNTP (<https://www.quora.com/In-terms-of-biology-what-is-the-main-and-most-important-difference-between-ddNTP-and-dNTP>)

### Necessary components:

1. Template DNA – single stranded DNA
2. DNA primer
3. DNA polymerase
4. Radioactive or fluorescence-labeled nucleotides - dNTP
5. Radioactive or fluorescence-labeled modified nucleotides - ddNTP
6. Buffer mix

### Protocol:

- DNA sample and the other components are added into four different tubes, each containing one standard deoxynucleotide (dATP, dGTP, dCTP, dTTP).
- Their own ddNTPs are added into each tube.
- Newly produced and labeled DNA fragments are denatured and separated by gel electrophoresis. The samples from each tube is loaded on different coloumn on the gel.
  - DNA bands can be observed directly under UV light.

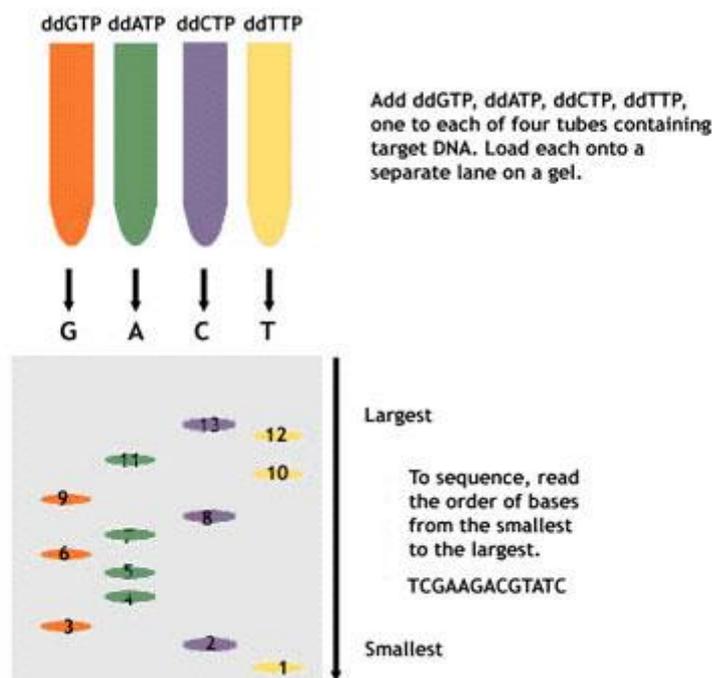


Figure 8.2 Main principle of Sanger sequencing method (<https://askabiologist.asu.edu/sequencing>)

### Limitations:

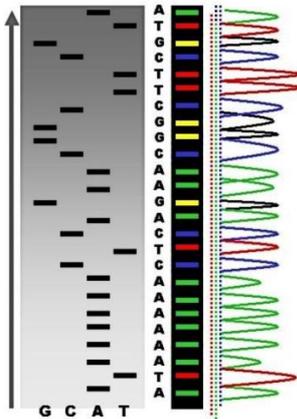
1. Non-specific primer-DNA binding
2. Lack of DNA sequence accuracy due to manual reading
3. Requires experience

## Dye-terminator sequencing:

It is more advanced version of Sanger sequencing. The reaction that occurs in four different tube above occur in one tube.

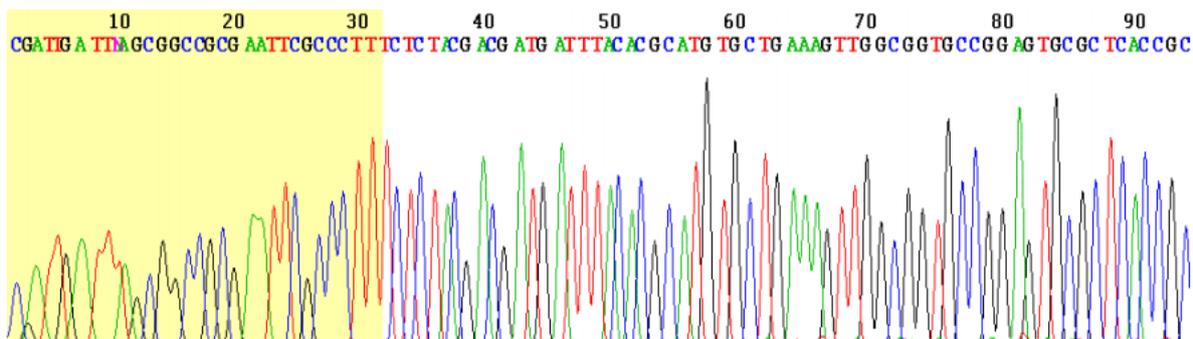
Each ddNTP is labeled with a different fluorescent dye. Each of these dyes realizes different sizes of radiation and emission. Depending on this difference, all of them can be analyzed separately in a single tube. Today, this method takes place in automated devices and gives different sized peaks according to the radiation it gives.

The limiting factor is that the first 15-40 reads some in low quality, then corrected, and degraded again after 700-900 bases.



**Figure 8.3** The comparison of gel and the fluorescent peaks in Sanger sequencing ([https://en.wikipedia.org/wiki/Sanger\\_sequencing](https://en.wikipedia.org/wiki/Sanger_sequencing))

With the newly developed automatized devices, 384 samples can be obtained in 24 hours in a single study. These devices separate the fragments by capillary electrophoresis according to their size. Analysis of the results is carried out by the analysis of fluorescent peaks.



**Figure 8.4** Fluorescent peaks obtained as a result of Sanger sequencing method ([https://en.wikibooks.org/wiki/Methods\\_and\\_Concepts\\_in\\_the\\_Life\\_Sciences/DNA\\_Sequencing](https://en.wikibooks.org/wiki/Methods_and_Concepts_in_the_Life_Sciences/DNA_Sequencing))

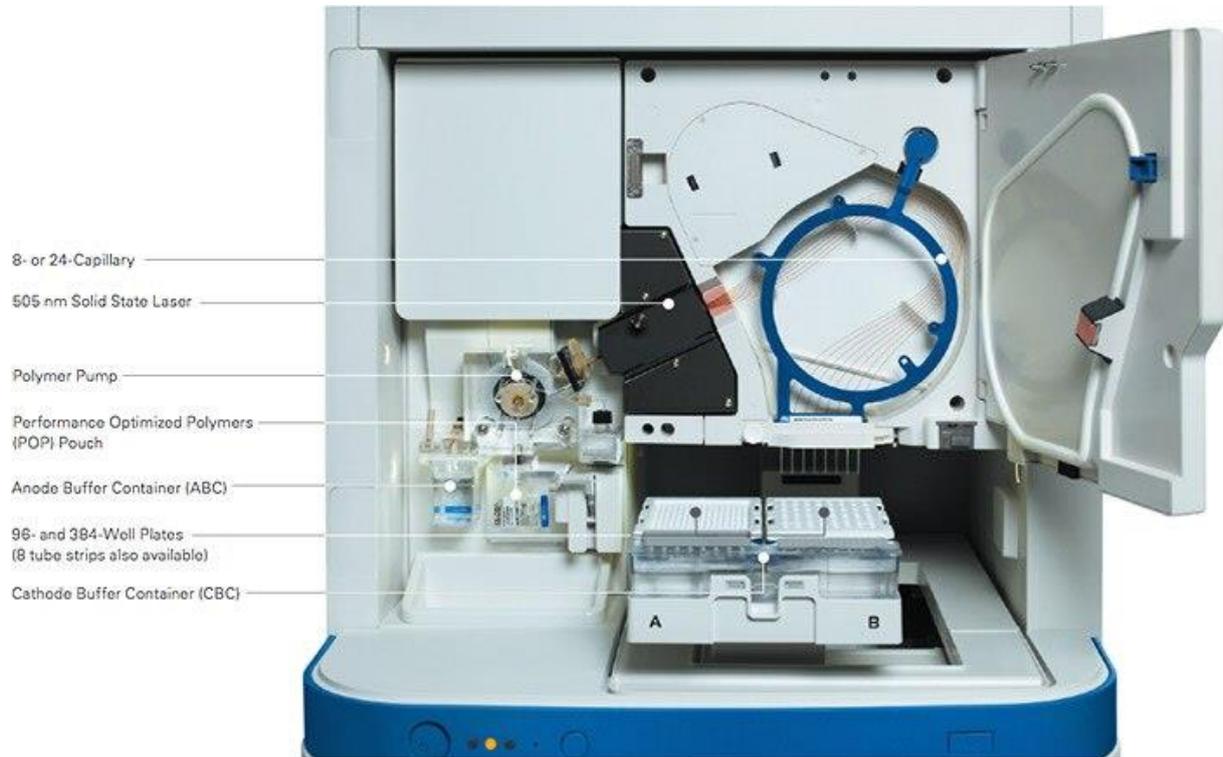
## Automatized system application:

### Necessary components:

1. Template DNA – single stranded DNA
2. DNA primer
3. DNA polymerase
4. Radioactive or fluorescence-labeled nucleotides – dNTP
5. Radioactive or fluorescence-labeled modified nucleotides - ddNTP
6. Buffer mix

**Protocol:**

- For one patient, all of the required components are added into a tube, mixed via pipettes, and loaded on thermal cycler for the reaction.
- After PCR step, the samples are filtrated and put into the wells on the plates, and the plates are loaded on sequencing instrument.
- The results are analyzed on the Software Program.



**Figure 8.5** ABI 3500 Genetic analyzer (<https://www.thermofisher.com/tr/en/home/life-science/sequencing/sanger-sequencing/sanger-sequencing-technology-accessories/applied-biosystems-sanger-sequencing-3500-series-genetic-analyzers/3500-series-genetic-analyzer.html>)

**In this lesson, the videos of automatized Sanger sequencing method will be watched.**



**PART 8 QUESTIONS**  
(The questions will be given at the end of the lesson)

**Prepared by:**  
**Comments:**

**Reviewed by:**



## **LAB.9-KARYOTYPE ANALYSIS**

Karyotyping is the matching and sequencing of all chromosome pairs of an organism. In this way, a general picture of an individual's chromosomes is presented. In the clinic, cytogenetics specialists use karyotyping method to determine large genetic changes. Changes in chromosome number (such as aneuploidy, trisomy 21) or structural changes (deletions, duplications, translocations and inversions) can also be detected by karyotyping. Today, it is used in the diagnosis of birth anomalies, genetic disorders, and even cancer.

The samples used for karyotype analysis:

- Peripheral blood
- Tumor biopsies
- Bone marrow samples
- Amnion fluid
- Chorionic villus

Samples for karyotype analysis are prepared from cells that undergo mitosis, are in the metaphase stage, and are examined under a microscope by staining with various banding techniques.

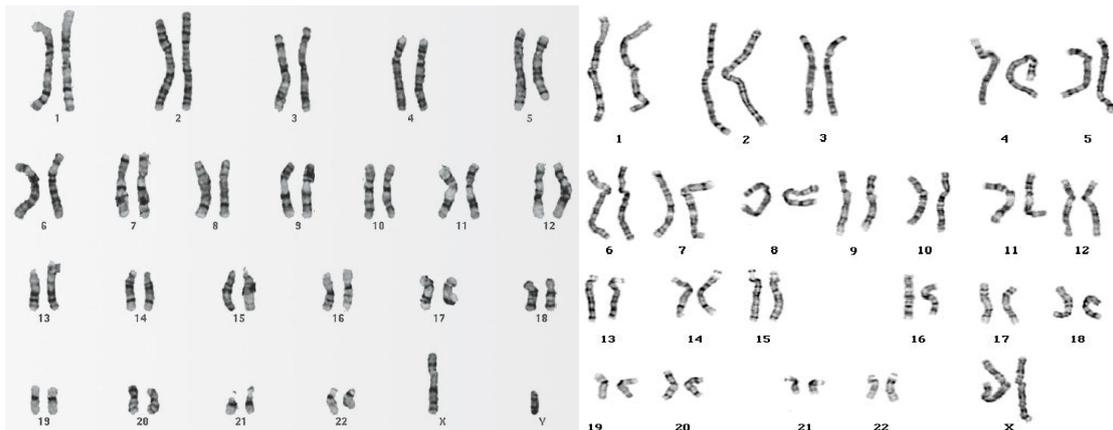
### **Sample Preparation Method for Karyotype Analysis**

- Many methods have been developed to ensure the identification and classification of chromosomes. The simplest of these is to identify chromosomes at a certain stage, then obtain them, and examine them by painting.
- Chromosomes are obtained from directly bone marrow or by short-term 72-hour tissue culture method. The sample material obtained from the bone marrow is either put into the nutrient medium and directly studied or kept in a culture medium for 24 hours.
- In the karyotype analysis of the peripheral blood sample, the blood taken from the vein with a heparinized injector is placed in tissue culture tubes with sterile medium, and phytohemagglutinin is added and the cells are expected to proliferate for 72 hours at 37 degrees. After this stage, the method for bone marrow and peripheral blood follows a common series of procedures.
- In both methods, colchicine is added for a certain time and dose to stop the lymphocytes in the metaphase phase.
- Afterwards, the cells are expected to swell by adding hypotonic solution to the cells that are separated from the nutrient medium after centrifugation.
- Then, the cells are centrifuged again, the supernatant part is discarded and the fixative is added. Cells washed three times with this solution are purified from erythrocytes and fixed.
- The cells are spread on clean slides and prepared for staining.
- Chromosomes are stained with DNA dyes. With some techniques developed after the 1970s, today each chromosome can be identified one by one. With these new methods, metaphases spread on the slide are first treated with some enzymes (trypsin, papain) or buffers (sörensen phosphate buffer) or some other chemical substances (SSC) and then stained with Giemsa. As a result, stained and non-stained parts of each chromosome are formed.

- In addition, the same bands can be obtained with fluorescent dye. There is also radioactive marking method which is not used very often in identification because it is an expensive method. It is used in the separation of X chromosomes.

### Giemsa Banding Technique (G-Banding):

- It is the most common banding technique.
- After the preparations are aged, histone and non-histone proteins are denatured with trypsin enzyme. The resulting DNA is stained with Giemsa.
- 400-700 bands are evaluated.
- The GC-rich regions are seen as light bands.



**Figure 9.1** The bands after G-banding (<http://bio3400.nicerweb.com/Locked/media/ch12/G-banding.html>, <http://labmed.hallym.ac.kr/cytogenetics/methodology/G-banding.htm>)

In this lesson, chromosomes will be classified on ready-to-use slides.



**PART 9 QUESTIONS**  
**(The questions will be given at the end of the lesson)**

**Prepared by:**  
**Comments:**

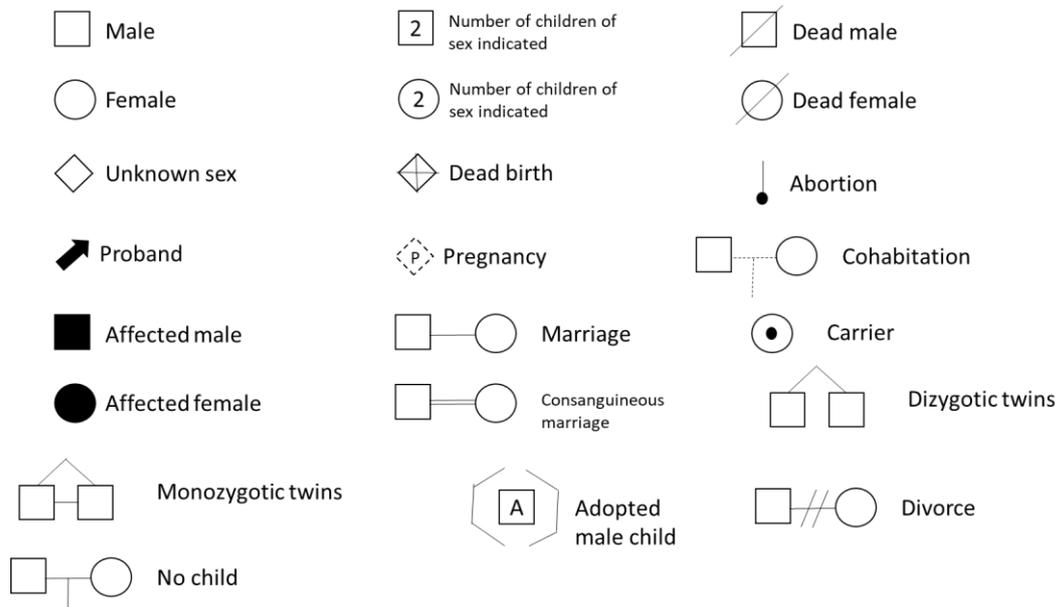
**Reviewed by:**



## LAB.10-HEREDITY PATTERNS and PEDIGREE ANALYSIS

### Terms

Single gene disorders are characterized by inheritance in families. In order to fully reveal this transition, the individual's pedigree is drawn by taking information from the family history. Pedigree is the chart where the family tree is created using standard symbols.



**Figure 10.1** The symbols used in pedigree analysis

The person who applied to the geneticist first and whose family history was taken is called the **proband**. Relatives are in three groups: **first degree** (mother, father, sibling and child), **second degree** (grandmother, grandmother, grandparents, grandchildren, uncles, uncles, aunts, aunts, nephews, stepbrothers) and **third degree** (cousins). If the proband is the only affected person in that family, it is called an **isolated case**. If the isolated case is proven to be caused by a new mutation in the proband, it is called a **sporadic case**. The first important step in the examination of pedigree is to determine the inheritance pattern of a genetic disorder in a family.

Heredity patterns depends on two factors:

- Is the related gene locus on autosomal chromosome? (chromosome 1-22)?, or sex chromosome (X ve Y kromozomları)? or mitochondrial genome?
- Is its phenotype **dominant**? (The disease is observed even if only one chromosome carries the mutant allele) or **recessive**? (The disease is observed only both chromosome carries the mutant allele at the same time)



**PART 10 QUESTIONS**  
**(The questions will be given at the end of the lesson)**

**Prepared by:**  
**Comments:**

**Reviewed by:**

