MEDICAL BIOLOGY LABORATORY GUIDE

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STUDY II: USING THE MICROSCOPE AND PREPARING A MİCROSCOPE SLIDE

Practical/Laboratory Purposes: Learning the parts of the microscope, Learning how to use the microscope, Learning how to prepare the preparation directly, Obtaining a clear image in the microscope, Learning the cleaning of the microscope

Tools and materials: Newsprint, bottle cork, slide, coverslip, scalpel, razor, 2 glasses cloths (one for microscope cleaning and the other for glass-lamella cleaning), wet wipes

USING THE MICROSCOPE

1. Bring the smallest magnification lens (5x) to its socket by turning the turntable.

2. Place the preparation on the tray (the coverslip must always be on top), fix it on both sides with tongs.

3. Turn on the light source and move the preparation forward-backward or right-left with the carriage so that the object to be examined is on the light coming from the middle of the table.

4. Lift the table to the top with the help of a macroscrew.

5. With your eye on the eyepiece, slowly lower the tray with the macroscrew until you find the image.

6. Fine-tune the microscrew to sharpen the image.

7. Adjust the light with the diaphragm

8. Move the lens to 10X and 40x, respectively, to magnify the image. At these stages, macroscrew should not be played with (the location of the image is fixed), only sharpness adjustment with microscrew, light adjustment with diaphragm and the preparation can be moved with the carriage.

PREPARATION AND EXAMINATION OF DIRECT PREPERAT

1. The slide and lamella are cleaned with a spectacle cloth.

2 If the item to be examined is a section, make sure that the section is taken very thin and does not fold when placed on the slide. If it is a smear preparation, make sure that the material is well spread under the coverslip.

3. Place the slide on a smooth surface, drop a drop of water on it with a Pasteur pipette.

4. Drop the material to be examined in a drop of water and create a smooth surface by spreading it in the drop.

5. If the examination material is liquid, drop a drop of it onto the slide. Take care that the drop does not overflow the slide and does not wet the bottom of the slide.

6 .Place the two side edges of the coverslip with your thumb and forefinger on the handle, one edge at a 45 degree angle to the edge of the water droplet and close it slowly. Make sure that the water does not rise above the coverslip and that there are no air bubbles between the slide and the coverslip. If there are any air bubbles, gently tap the coverslip with the tip of your fingernail or the back of your pen so that the bubbles come out.

7. Put the preparation on the microscope stage and start image finding.

EXAMINATION WITH THE MICROSCOPE

Place one of the cut newspaper letters on the water dripped on the slide. Examine the letter in comparison with various magnifications. Pay particular attention to how much space the same letter occupies in the microscope's field of view at different magnifications. Take a thin section of the cork with a razor or scalpel. Examine the preparation you have prepared as described above at various magnifications.

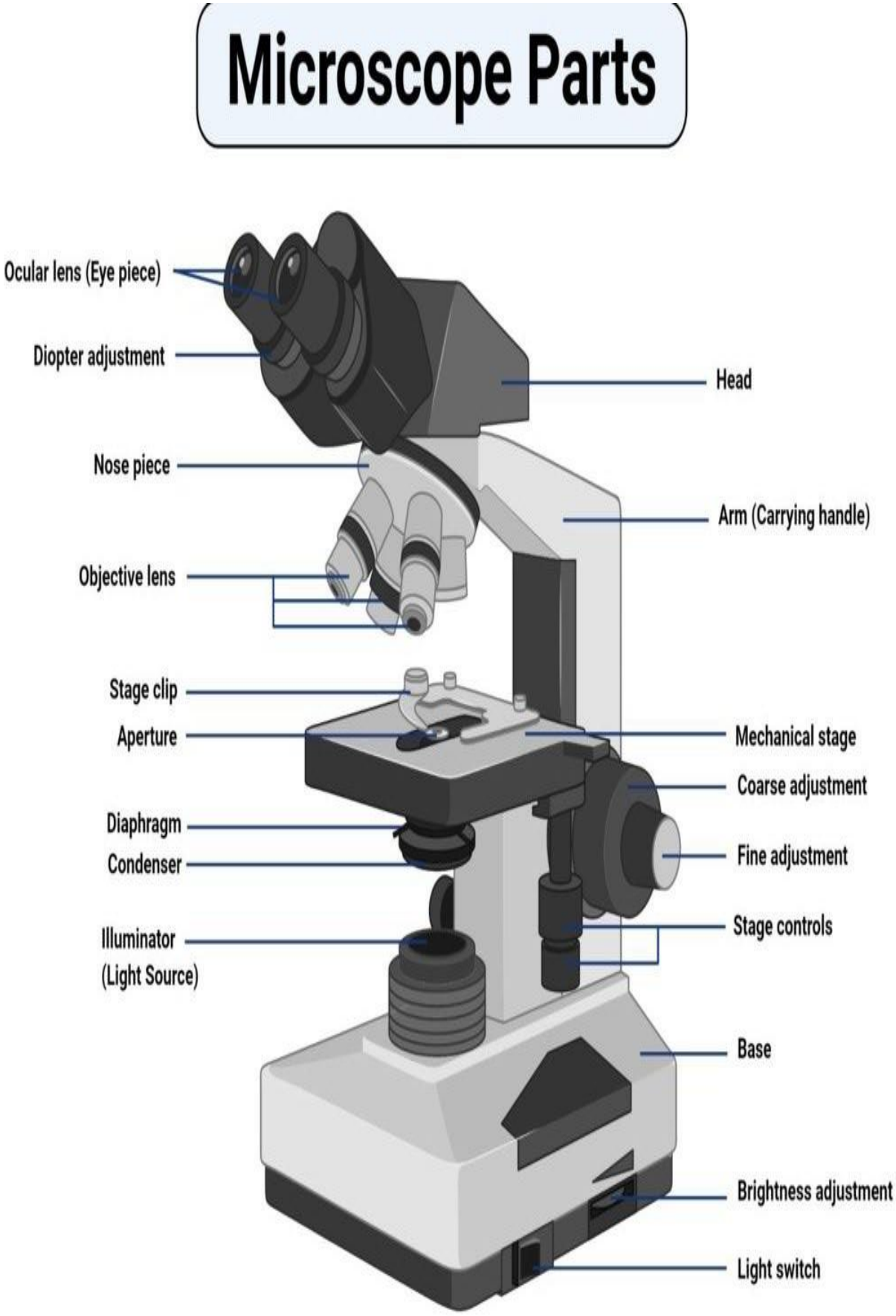


Figure 1 : Parts of Light Microscope

Generally, the microscope is divided into two parts, optical and mechanical parts.

OPTICAL PARTS

Ocular Lens: It is the part of the microscope that consists of a convergent lens that is viewed with the eye and placed in a tube. There are two (binocular microscope). It has different magnifications (5x, 10x, 20x).

Objective Lens: it is at the bottom of the eyepiece tube and is responsible for both total magnification of the specimen, as well as the resolving power of the microscope. The magnification power is written on it as 5x, 10x, 40x, 100x.

* Total magnification = Objective magnification X ocular magnification

Condenser: It is located under the stage, it is the lens part that directs the light to the slide by concentrating the light coming from the light source

Diaphragm: Increases or decreases the amount of light coming from the light source.

MECHANICAL PARTS

Tube: It is the moving part of the microscope with the objective at the lower end and the ocular at the top.

Arm: It is the part of the microscope that is held during transport from one place to another.

Base: it is the bottom of the microscope and the microscope stands on it.

Stage: It is the part where the slide is placed.

Stage clip: It is the mechanism for keeping the preparation on the table.

Stage Controls: It is placed on the stage, with the help of two screws on the side of the stage, they are the mechanisms that allow the slides to be easily moved to the right-left and front-back on the stage.

Coarse adjustment: It adjusts the distance between the object and the lens by moving the stage up and down.

Fine adjustment: it makes the image clear by moving the stage up and down at the micron level

STUDY III: EXAMINATION OF ANIMAL AND PLANT CELL UNDER THE MICROSCOPE

Practical/Laboratory Purposes: Study of animal and plant cells

Tools and materials: Onion, Potato, blunt-tipped toothpick, slide, coverslip, scalpel, razor blade, 2 glasses cloths (one for microscope cleaning and the other for slide-coverslip cleaning), Wet wipes

EXAMINATION OF TONGUE/CHECK EPITHELIUM CELLS

The best example of a living animal cell that can be studied in the laboratory is the tongue/cheek epithelial cell. A sample is taken by gently pressing on the tongue or cheek with a blunt toothpick. The toothpick is dipped into the prepared water on the slide and mixed. The coverslip is closed and examined first with small and then with large lenses. Since the tongue cells are transparent, it is necessary to partially close the diaphragm when examining it.

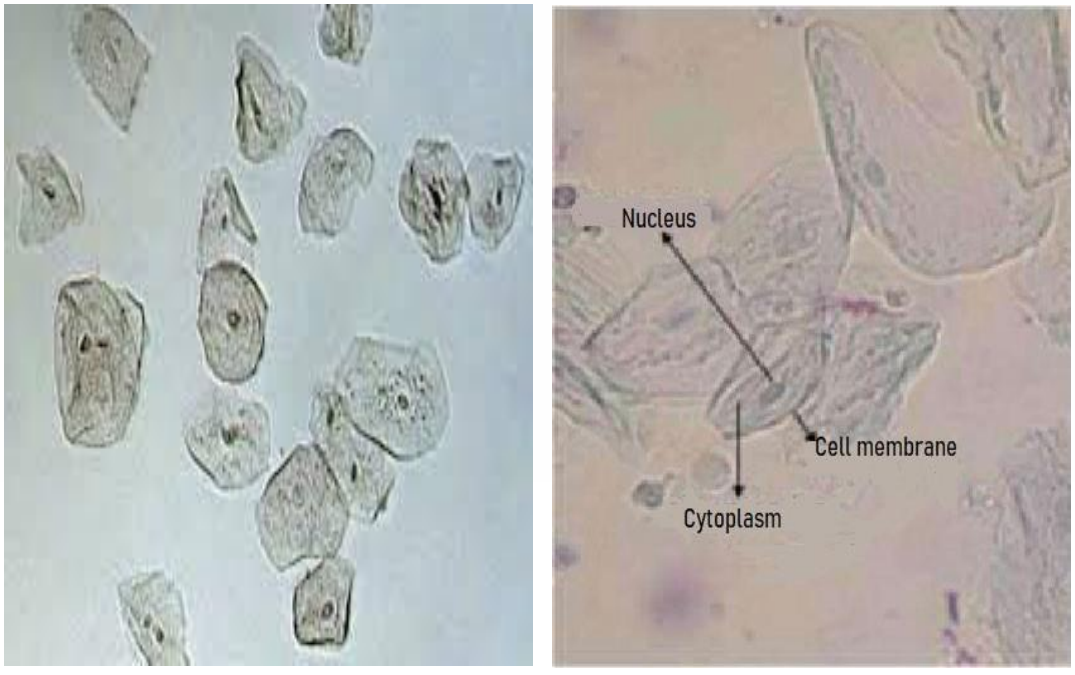


Figure 2. Tongue/Check Epithelium Cells

EXAMINATION OF PLANT CELLS

The most distinctive difference between plant cells and animal cells is the presence of a cellulose wall on the cell membrane. This wall gives shape to the cell, provides support, but does not have selective permeability. The vacuole is typical for these cells, the centrosome is absent..

EXAMINATION OF ONION MEMBRANE CELLS

One of the parts of the onion is taken, folded outward and broken, and a thin layer of membrane is peeled from the broken place. A small piece of the membrane is cut and placed on a clean slide. If there is a fold in the membrane, it should be corrected and a drop of water is dropped on it, the coverslip is closed and examined under the microscope.

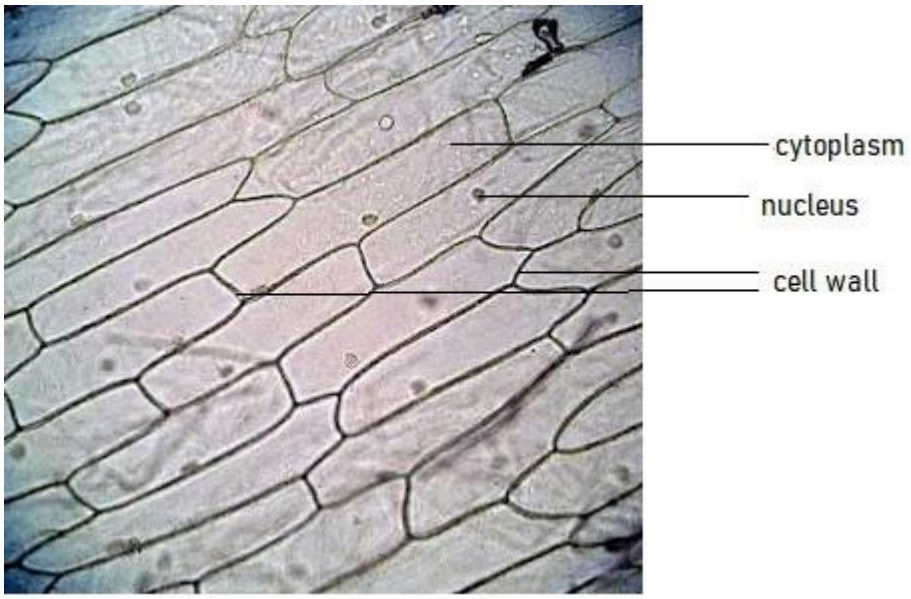


Figure 3. Onion membrane cells

EXAMINATION OF POTATO CELLS

A very thin section is taken from the potato pieces with a razor blade or scalpel. it is prepared with a drop of water. The thinnest part of the section is found and the cells are examined with a 40X objective.

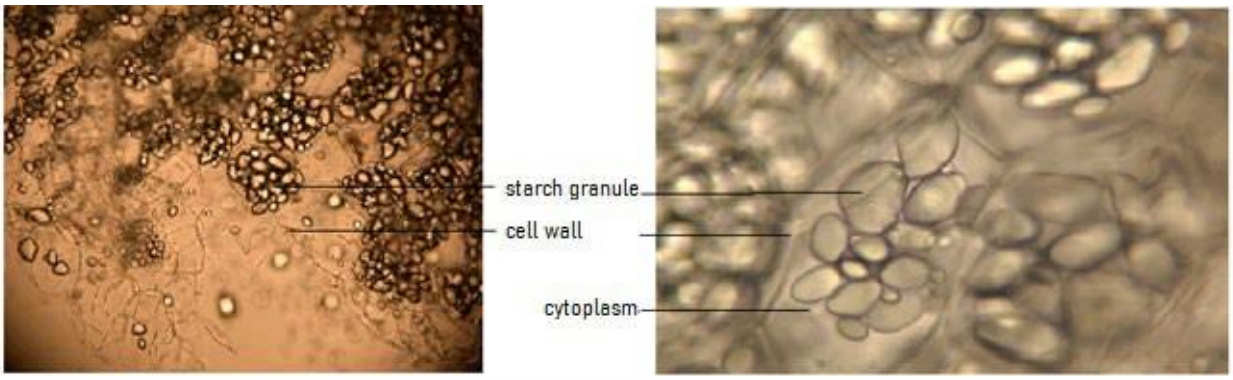


Figure 4. Cells of Potato

STUDY IV: MITOSIS EXPERIMENT

Practical/Laboratory Purposes: Observation of mitosis in the germinated root tips of onion plants

Tools and materials: Microscope, slide, coverslip, beaker, petri dish, acetic acid, carmine dye, filter, pasteur pipette, blotting paper, onion, forceps, scalpel, bunsen burner

Prepare aceto-carmine dye. For this, heat 100 ml of 50% acetic acid and 2 g of carmen dye slowly in a beaker and boil for 30 minutes. Shake well and filter after cooling. Cut 5-6 mm pieces from the ends of the onion roots that you germinate in water for 4-5 days and collect them in a petri dish. Apply aceto-carmen dye to cover them. Hold the Petri dish with the help of tongs and heat it on the flame for about 5 minutes. After the heated root tips have cooled, take them onto the slide with the help of a clean forceps. Drop 1-2 drops of aceto-carmen dye on it and close the coverslip. Press the coverslip firmly so that the root tips are crushed between the slide and the coverslip. Find the field of view at the X4/X5 lens and examine it at the X10, X40 and X100 lenses, respectively. Observe the stages of mitosis and draw with the X100 lens.

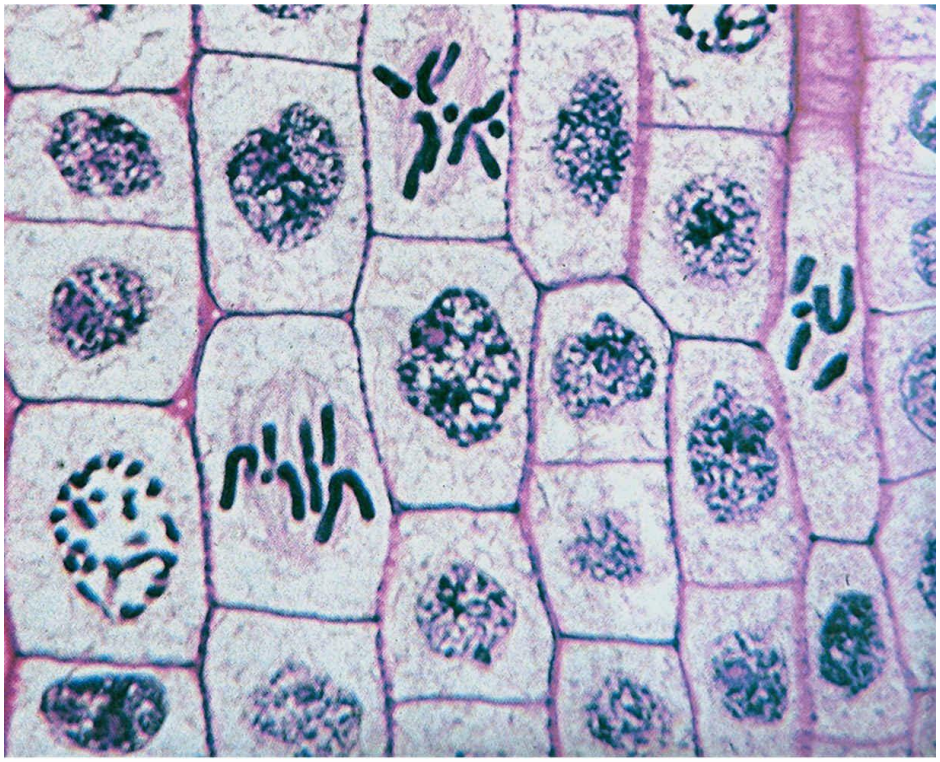


Figure 5. Microscopic view of onion root tip cells at different stages of mitosis

STUDY V: DNA EXTRACTION

DNA

The most basic feature of living things is their ability to reproduce themselves. All organisms inherit the genetic information that will determine their structure and functions from their parents. DNA (Deoxyribonucleic acid) is the molecule that carries genetic information in all organisms except some viruses. DNA consists of nucleotides formed by a 5-carbon sugar, a phosphate group, and an organic base (purine or pyrimidine). With the phosphodiester bond between sugar and phosphate, the nucleotides are linked to each other in the 5′→3' direction. Two strands in opposite directions are connected to each other by the formation of hydrogen bonds by the bases of the nucleotides coming together. Thus, there is the sugar-phosphate backbone on the outside and the bases on the inside that are connected by hydrogen bonds. The bonding of bases is specific and always a purine pairs with a pyrimidine (Purines are Adenine (A) and Guanine (G); Pyrimidines are the bases of Thymine (T) and cytosine (C)) (Fig. 6).

DNA is not found free in the cell. It has a certain organization. Most of the DNA is found in the nucleus, and some in the mitochondria and chloroplasts in plants. DNA molecules are not found free in the nucleus either. It is organized by proteins with and without histones.

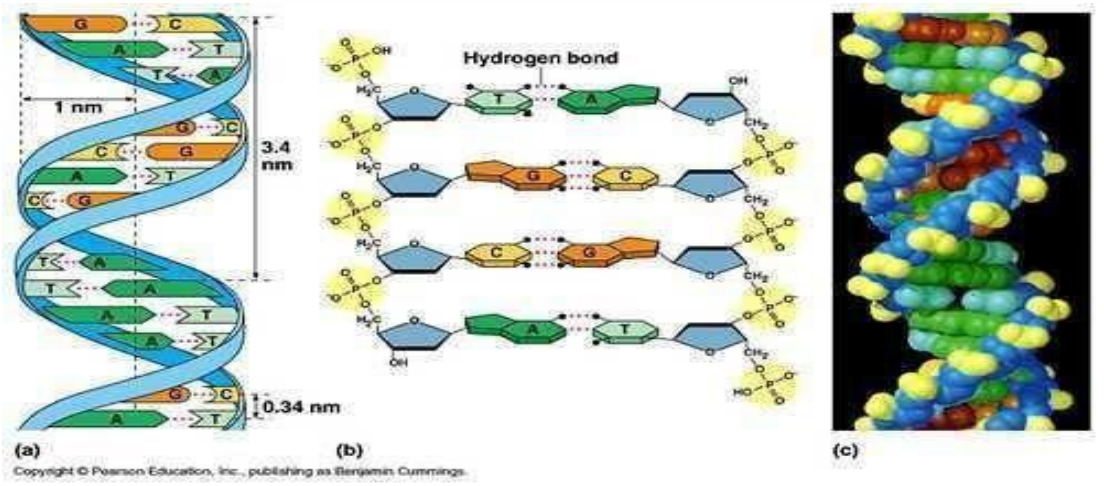


Figure 6: Schematic representation of the molecular structure of DNA

The destruction of the cell membrane or living cell wall of living cells with some chemicals and enzymes and revealing the DNA is called **'DNA isolation'**. In molecular studies, it is essential to obtain pure DNA, free from proteins. Although there are different isolation methods, there are generally 3 basic steps.

1. lysis of the cell and release of DNA

2. Separation of DNA from proteins and becoming soluble

3. Separation from protein, RNA and other macromolecules

It is possible to obtain DNA with short and easy methods that can be applied in student laboratories. Especially in this method, which is done without the need for many tools and equipment, one of the materials that can be easily obtained such as banana, onion, pea can be used. If it is desired to obtain DNA from animal cells, chicken liver, calf thymus gland, meat and eggs (chicken or fish) can be used as material.

DNA ISOLATION WITH THE SPIN COLUMN METHOD

1. Peripheral blood stored in a sterile centrifuge tube with 20µL Proteinase K and 200µL EDTA is added to 400µL lysis buffer and vortexed.

2. Samples are incubated for 10 minutes at 56 ºC.

3. 200 µl (96-100%) ethanol is added to the incubated samples. It is vortexed vigorously.

4. The prepared mixture is transferred to the column placed in a 2 ml collection tube and centrifuged at 8000 rpm for 1 minute, the collection tube is discarded and the column is placed in a new collection tube.

5. Pipette 500 µl Wash Buffer I onto the column and centrifuge at 8000 rpm for 1 minute. The liquid in the collection tube is drained and the column is placed again.

6. 500 µl Wash Buffer II is pipetted onto the column and centrifuged at 10000 rpm at maximum speed for 1 minute, the collection tube is discarded and the column is placed in a sterile 1.5 ml storage tube.

7. 1500 µl of Elution Buffer was pipetted onto the column. After waiting for 2 minutes at room temperature, it is centrifuged at 10000 rpm for 1 minute,

The column is discarded and the obtained DNA is stored at +4 ºC.

DNA EXTRACTION FROM BANANA

Purpose: Obtaining and monitoring DNA from bananas by salt precipitation method

Tools and equipment: Beaker (250 ml), beaker (50 ml), measuring tape (100 ml), falcon tube (15 ml), eppendorf tube, blender or porcelain mortar, filter or gauze, pasteur pipette, banana, detergent, salt , alcohol (96%)

Yöntem:

Banana thoroughly with 125 ml of water and turn it into a slurry.

into a 50 ml beaker; 2 teaspoons of detergent

1 teaspoon of salt

Add 20 ml of water and mix gently without foaming.

1.Put 4 teaspoons of banana slurry on the detergent mixture and slowly for 5-10 minutes, Mix without foaming.
2. Strain this mixture through a 50 ml beaker, filter or gauze.
3. Pour 10 ml of cold alcohol (96%) into the Falcon tube.
4. Add 5 ml of the filtered liquid onto the alcohol.
5. Wait 2-3 minutes without shaking.
6. Observe the white cloud of DNA.
7. Put about 0.5-1 ml of alcohol into the Eppendorf tube.
8. Take some of the DNA in the falcon tube with the help of a toothpick and drop it into the alcohol in the eppendorf tube.

Up to 2 ml of blood is taken into the heparin injector. Then, 5 drops and 3 drops of phytohemagglutinin from the blood in the injector are dropped into the tube containing 5 ml of medium next to the burner flame. The tube is slightly shaken and kept in an oven at 37 □C for 72 hours. 5 drops of colchicine are instilled 1.5 hours before the end of this period. At the end of the period, the tube is centrifuged at 1000 rpm for 10 minutes and the supernatant is discarded. About 12 ml of hypotonic solution is added to it and left for 13 minutes. The tube is centrifuged again at 1000 rpm for 10 minutes and the supernatant is discarded. The top is completed with a fixative (Methanol / Acetic acid 3:1) to 5 ml and centrifuged again at 1000 rpm for 10 minutes and the supernatant is discarded. This process is repeated until the precipitate turns white. After the precipitate is spread on the slides and dried, it is stained with 5% Giemsa dye and examined.

STUDY VI: KARYOTYPE ANALYSIS

Practical/Laboratory Purposes: Determining the size, size and number of chromosomes

Figure 7: Human Male G-bands

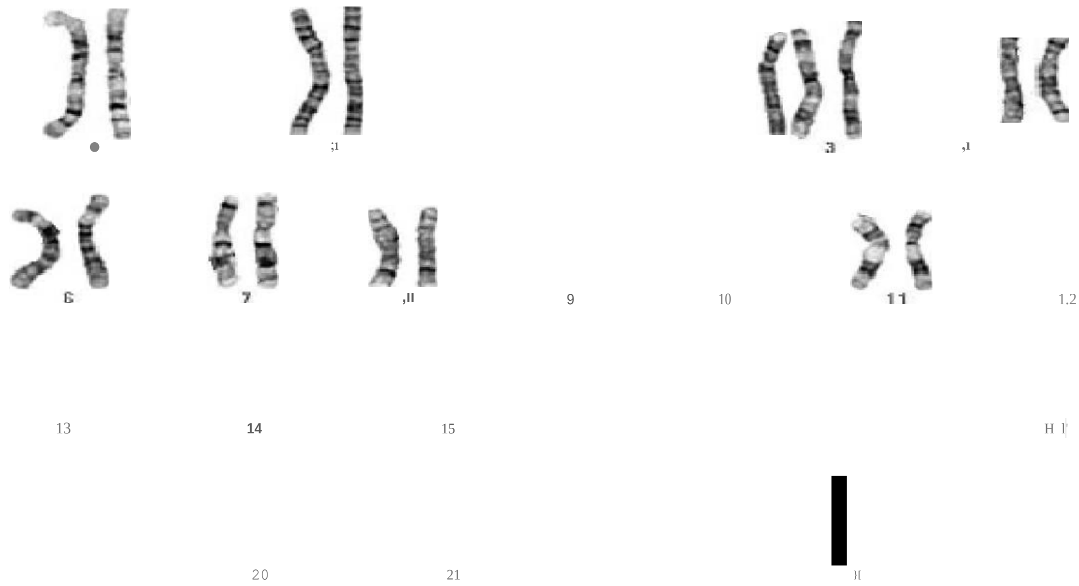
KARYOTYPE ANALYSIS AND IDIOGRAM

As it is known, the ordered chromosome table belonging to a human cell and according to Denver classification is called karyotype. The human karyotype consists of 23 pairs of chromosomes. Of these, 22 pairs are autosomes and 1 pair are gonosomes. In general, autosomes consist of two homologous chromosomes, gonosomes consist of a pair of homologous X chromosomes in the female, and two heterologous chromosomes in the male, one X and the other Y. These 46 chromosomes in humans are arranged in 8 groups from the largest to the smallest; these are the A, B, C, D, E, F, G and sex chromosomes.

Chromosomes are also separated according to the state of their centromeres.

1. Metacentric: The centromere is in the middle and the two arms are almost equal in length.
2. Submetacentric: Arm lengths are different from each other and the centromere is not in the middle.
3. Acrocentric: The centromere is much closer to one end of the chromosome than the other.

Examine the preparations given to you and group the chromosomes here, and if there are any changes you see in the preparation, discuss the reason; Draw a sample chromosome belonging to each group and name the parts of one.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STUDY VI: POLYMERASE CHAIN REACTION PCR (PCR)

DNA replicates naturally in cells by replication. Replication of desired DNA sequences by PCR is performed in-vitro in an accelerated manner. The basis of the method is the separation of two strands of DNA from each other (denaturation) by high temperature, then attachment of synthetic oligonucleotide (primer) sequences to the target DNA strand (annealing), elongation of the strand (polymerization) and repetition of these cycles a certain number of times (Figure 8).The following components make up a standard PCR environment:

Template DNA: Genomic DNAs, plasmid and phage DNAs, various genes and even any piece of DNA can be used as template in PCR.

Enzyme (Taq DNA polymerase): DNA polymerase enzymes are enzymes that catalyze the synthesis of a long polynucleotide chain with the help of deoxyribonucleotide triphosphates by using the base sequence in the original template strand to form a DNA chain complementary to the template strand. The thermostable Taq DNA polymerase purified from Thermus aquaticus is most commonly used in PCR.

Primers: Oligonucleotides are nucleotide chains that can be commercially obtained from primer synthesis laboratories and designed according to the target sequence to be amplified. Primers are synthesized to be complementary to the 3' and 5' ends of the region to be synthesized.

Deosiribonucleotide triphosphates (dNTP, dATP, dGTP, dCTP, dTTP): Taq Polymerase uses these nucleotides in the PCR medium while forming a complementary chain to the template chain.

PCR buffer that provides appropriate pH and ion conditions (Mg^{+2}): Mg^{+2} ions form soluble complexes with dNTPs, stimulate polymerase activity, and provide primer-die interaction. Therefore, $MgCl_2$ has a very important effect on the specificity and product yield of PCR.

PCR CONDITION

Distilled Water	12 µl
10XPCR Buffer.....	5 µl
dNTP Mix (2mM).....	5 µl
Primer Forward	1 µl
Primer Reverse.....	1 µl
$MgCl_2$ (25 mM).....	1-2 µl

Taq DNA Polymerase (2-5 Unit/µl) 0.5 µl

Genomic DNA .....3 µl

PCR ŞARTLARI

1- Initial Denaturation 1 Cycle

2- Denaturation

3- Primer attachment (Annealing)

4- Primary elongation (Extension)

5- Final Primary Elongation 1 Cycle

Stages 2, 3 and 4 25-35 Cycles

SAMPLE PCR CONDITION

96ºC→	2 min	1 Cycle
96ºC→	30 sec	
58ºC→	30 sec	25-35 Cycle
72ºC→	60 sec	
72ºC→	7 min	1 Cycle

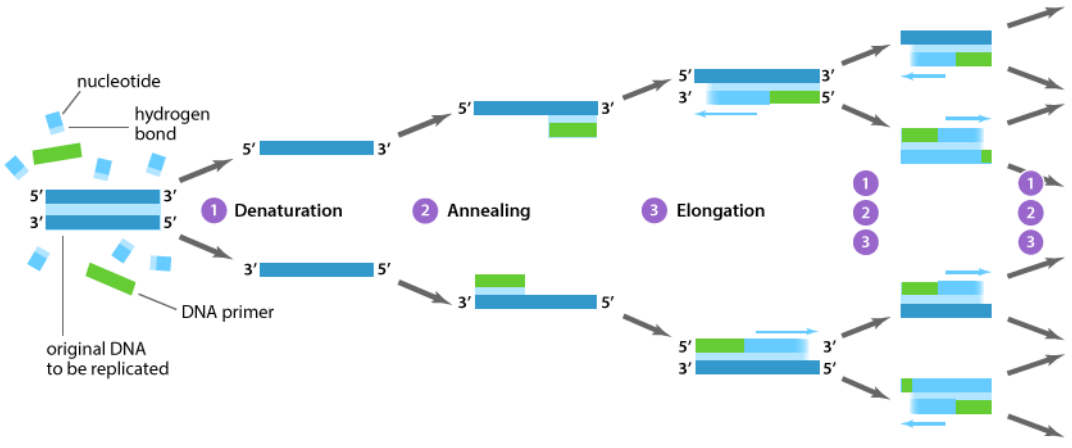

Figure 8. PCR Steps

3- NUCLEIC ACID ANALYSIS WITH AGAROSE GEL ELECTROPHORESIS METHOD

Although a wide variety of methods are used in the analysis of DNA molecules, agarose gel electrophoresis is the preferred method because it is simple and fast, which is frequently used in laboratories. Electrophoretic analysis is based on the migration of DNA on an agarose gel in an electrical field. Agarose is a linear polysaccharide isolated from Agar agar, a species of red algae. Agarose has the ability to dissolve in hot water and form a gel structure by polymerizing when cooled. Taking advantage of this feature, a gel is prepared by adding different concentrations of agarose in a buffer and heating it. The gel that is left to cool polymerizes and thus pores of different sizes are formed depending on the concentration of the agarose. In addition, for the DNA to be visible in the gel, ethidium bromide must be added to the gel when the gel is left to cool. Ethidium bromide has the feature of making the DNA visible in the gel by showing fluorescent effect as a result of absorbing light at 300 or 360 nm by entering between DNA bonds. This effect can be strong or weak depending on the DNA concentration. Ethidium bromide should never be inhaled as it is a carcinogen.

Electrophoresis Buffer

It is prepared by adding ethidium bromide (EtBr) at a concentration of 0.5 µg/ml into 1X TBE, which is prepared by diluting 10X TBE buffer with distilled water**.**

3% Agarose Gel Solution

It is prepared by adding EtBr at a concentration of 0.5 µg/ml after 4.2 g of agarose (Agarose plus) is melted in 140 ml of 1X TBE buffer in a microwave oven.

PCR-RFLP or just PCR products are loaded into the gel and run for 30-45 minutes, allowing the expected bands to be displayed.

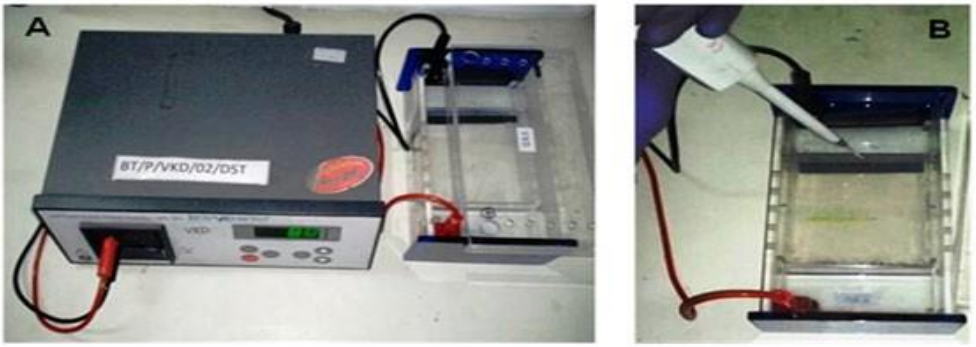

Figure 9. Preparation of agarose gel electrophoresis

4- RESTRICTION FRAGMENT LENGTH POLYMORPHISM, RFLP

There are certain polymorphic regions on the DNA helix that are recognized by specific restriction endonuclease enzymes (Restriction Endonuclease, RE). When treated with RE, these regions are cut and sequences of different lengths are formed. These sequences are called RFLP. These sequences are used as hereditary markers in many diseases. If a single nucleotide change in the DNA structure is desired to be examined, a region-specific RE is used and different alleles are detected. Polymorphisms resulting from insertion and deletion can be identified by many enzymes since they have two alleles. Alleles and genotypes are determined by taking the gel images of the resulting samples with the help of a UV transimilator.

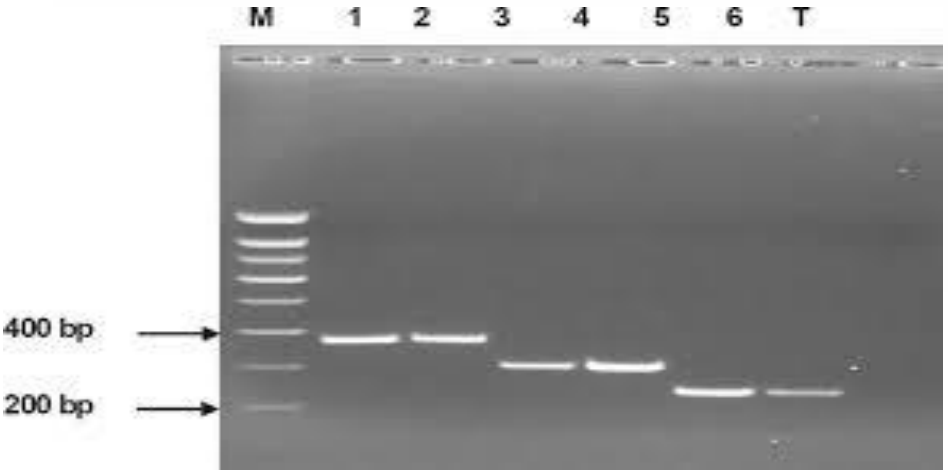

Figure 10. Imaging of the gel with a UV transmulator