Mustansiriyah University

College of Medicine

Department of Human Anatomy


# MEDICAL BIOLOGY

# LABORATORY MANUAL

## FOR

## MBIO1-102

BY

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# General Instructions

1. **No materials may be taken out of the laboratory!**

2. **Microscopes:**

a) Two students are assigned to one microscope; therefore, **cooperation is necessary!** Please keep your scope clean and in proper working condition. At the end of each laboratory session, return your microscope to its appropriate place.

b) Carefully read the instructions in this manual on proper care and handling of the microscope. If you don't remember anything else remember to **CHECK TO SEE THAT THE CONDENSER IS ALL THE WAY UP AND THAT THE LIGHT IS OFF.**

3. **Slide Trays:**

a) You are sharing slide Trays with other students. Please cooperate by leaving them in good order!

b) Before each lab, check for damage and/or lost slides. Report any damage or loss immediately. The instructors will check the microscopes and slide trays periodically, and **you will be charged (25000 IQD) per damaged/lost slide.**

c) At the end of each lab, return your slide trays to the instructor.

4. Each laboratory session is scheduled for TOW HOURS; it will be necessary for you to use this time efficiently. This will greatly decrease the time you will need for review.

5. Keep the lab tables clean.

6. The instructors are available any time during lab. Do not hesitate to ask for help when you need it. No question is "stupid" or "dumb".

7. **Students are strongly advised to bring both their ATLAS and their NOTEBOOK to the laboratory!**

8. **Do not place anything in your mouth or eyes while in the lab.** This includes pencils, pens, food, drink, and fingers.

A. Eating and drinking are **prohibited** in the lab at all times.

B. This includes gum, and candy.

9. **Never place book bags, purses, and/or carrying cases on bench tops.** These should be placed in the assigned cabinets at all times.

10. Immediately wash hands if contaminated with chemicals or microorganisms. **Wash hands before leaving the laboratory.**

11. **Know the location and operating procedures of safety equipment such as fire extinguishers, eyewash stations, first aid kit, broken glass containers, and sharps containers.**

## Clothing

1. **Dangling jewelry and loose or baggy clothing** are prohibited.

2. **Lab coats must be worn all the time. No exceptions!**

3. **Long hair must be tied back.**

## Chemicals

1. **All chemicals in a lab are to be considered dangerous.** Do not smell, touch, or taste any chemicals.

2. **Never return unused chemicals to their original containers.**

3. **Do NOT** dispose of any chemicals down the drain unless instructed to do so.

## Accidents

1. **Immediately report any accident to the instructor, no matter how small.** (broken slides, spills, cuts, burns, etc.)

2. **Broken glassware should never be handled with bare hands.** Use a dustpan and brush to collect broken glassware and dispose of in broken glassware box.

3. **If chemicals should splash in your eyes, immediately flush with running water from the eyewash station and notify the instructor.**

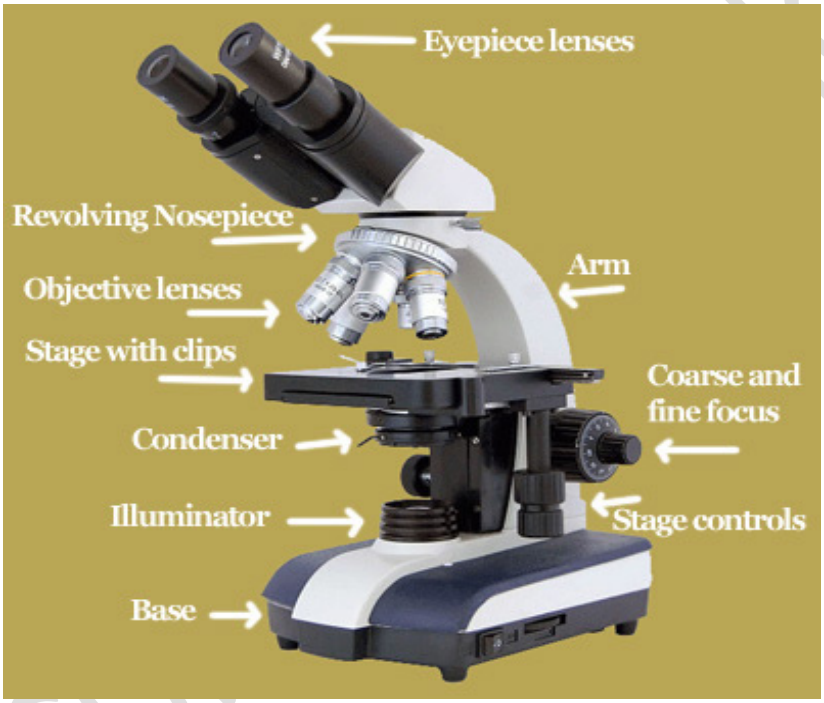
4. When removing an electrical plug from a socket, grasp the plug and pull. **Do NOT grab the cord and pull it out of the socket.**

# Lab. 1

# Compound Light Microscope

This is the type of microscope used in this lab. The major parts of the instrument will be named and a method for the effective use of the microscope will be outlined.
The **light microscope** uses lenses and light to enlarge the image and is also called **bright field** or **compound** microscope.
The light microscope has two systems of lenses for greater magnification:

1) The ocular, or eyepiece lens that one looks into and
2) The objective lens, or the lens closest to the object




## ☒ Parts of the microscope:

1. **Eyepiece (Ocular Lens)**: the lens at the top that you look through. They are usually 10X or 15X power.
2. **Tube:** Connects the eyepiece to the objective lenses
3. **Arm:** Supports the tube and connects it to the base. It is used along with the base to carry the microscope
4. **Base:** The bottom of the microscope, used for support
5. **Illuminator:** A steady light source (110 volts) used in place of a mirror.
6. **Stage:** The flat platform where you place your slides.
7. **Stage clips:** hold the slides in place.
8. **Stage controls:** two knobs that move the stage in 4 directions (to examine different areas of the slide).
9. **Revolving Nosepiece or Turret:** This is the part that holds the objective lenses and can be rotated to easily change power.
10. **Objective Lenses:** Usually you will find 3 or 4 objective lenses on a microscope.

They almost always consist of: **4X (scanning objective), 10X (low power objective), 40X (high power objective) and 100X (oil immersion objective)**.
They are changed by rotating the turret, usually in a clockwise direction
When coupled with a 10X (most common) eyepiece lens, we get total magnifications of 40X (4X * 10X), 100X, 400X and 1000X.
The shortest lens is the lowest power; the longest one is the lens with the greatest power.

> Total magnification power = magnification of ocular lenses multiplied by magnification of objective lenses

11. **Condenser:** It is a rotating disk under the stage. A lever projects from the condenser that is used to vary the size of the opening of the condenser (**iris** or **diaphragm)** and thus vary the intensity and size of the cone of light that is projected upward into the slide. There is no set rule regarding which setting to use for a particular power. Rather, the setting is a function of the transparency of the specimen, the degree of contrast you desire and the particular objective lens in use.
12. **Coarse adjustment knob:** This is used to focus the microscope. It is always used first, and it is used only with the scanning (4X).
13. **Fine adjustment knob:** This is used to focus the microscope. It is used with low power (10X) objectives and high power (40x) objective to bring the specimen into better focus.

☒ **Notes:**

The magnification power of the light microscope is limited to 1000-2000 X.
The resolving power (resolution) of the light microscope is 0.2 µm.

- ***The resolving power (resolution)*** *is defined as the smallest distance between two particles at which they can be seen as separated objects.*

The human eye has a resolution of **0.2 mm**.

## ☒ Rules for Microscope Use

1) Always carry the microscope from the arm with one hand and put your other hand under the base.
2) The lowest power objective 4x (scanning or very low) should be in position both at the beginning and at the end of microscope use.
3) Use only lens paper for cleaning lenses.
4) Do not tilt the microscope, as the eyepieces could fall out.
5) **Turn of** the microscope light if you are not using it!!!.
6) Keep the stage clean and dry to prevent rust and corrosion.
7) Do not remove parts of the microscope.
8) Keep the microscope dust free by covering it after use.
9) Report any malfunctions.
10) **Do not use coarse adjustment knob when viewing a specimen with the low-power (10x) or high-power (40x) objective.**

## ☒ The Proper Use of the Microscope

In examining a slide with the light microscope, the following sequence of steps should be used:

1. Turn on the microscope light from side switch (make sure the electric socket is on).
2. Lower the stage of the microscope to the lowest position using coarse adjustment knob
3. Place the slide on the stage and hold it in place with the stage clips.
4. Select the very low (scanning) objective (4x) first and while looking from the side, raise the stage with the coarse adjustment knob to make the slide as close to the lens as possible without touching it.
5. Now, look through the eyepiece lens and focus upward (using coarse adjustment knob only) until the image is sharp and clear. If you can't get it in focus, repeat steps 2-5 again.
6. Scan the entire section in organized way (*often tissue and organ identification can be made at this magnification*). Select an area or areas for study at higher magnification.
7. Rotate the revolving nosepiece to place the low-power objective (10x) in the optical axis (you will hear a click). When turning the nosepiece, grasp the nosepiece itself or the part of the objective adjacent to the nosepiece to avoid excess stress on the objective.
8. Look again through the eyepiece and do minor adjustments with the fine adjustment knob until the image is sharp and clear.
9. Proceed to the next step in magnification, if necessary, which is high dry (40x).

At the end of your work with the microscope do the following sequence of steps:

1. Turn of the light of the microscope from the side switch (NOT FROM ELECTRIC SOCKET).
2. Lower the stage of the microscope to the lowest position using coarse adjustment knob.
3. Rotate the revolving nosepiece to have the scanning 4x lens in place.
4. Open the stage clips and take slide out in proper way (holding it from sides).
5. Put the slide back in the slide tray.
6. Cover the microscope with its plastic covering.

**In this Lab, you will use only the 4X, 10X and 40X lenses.**

### ☒ Notes:

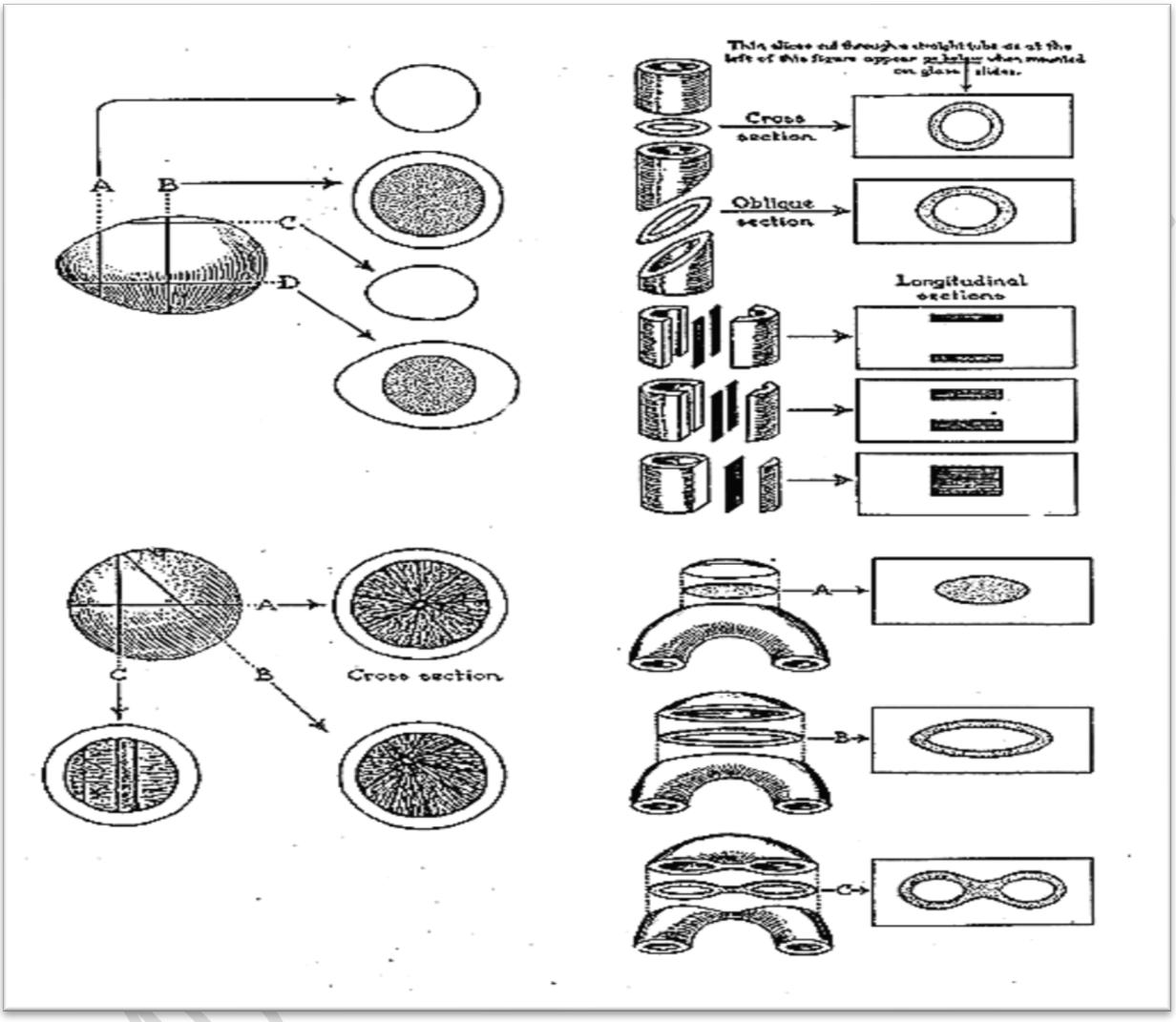
- ☺ Both eyes should be open when viewing through the microscope.
  This prevents eye fatigue, which occurs when the non-viewing eye is kept closed.
  Keeping both eyes open does take some practice, but it is highly recommended.
- ☺ Also, you should never let your eye touch the ocular lens.
  If your eyelashes touch the lens you are to close.
- ☺ Always remove eyeglasses when viewing through a microscope.
  If your eyeglass lens touches the microscope it may get scratched.
- ☺ Always be gentle and extra careful when handling and carrying the glass slides used in this lab

### Field of View

**A microscope's field of view is the circle visible through the ocular lenses.** The diameter of field is the length of the field from one edge to the other. Like any other measurement in science, the diameter of the field is measured using metric units.

## Tissue Sections

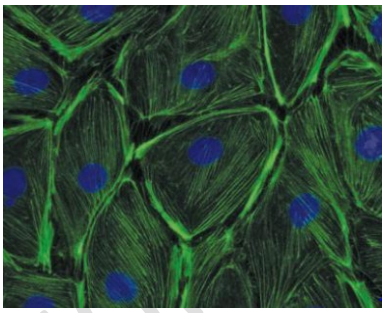
Most of the tissues you will be examining with the microscope are thin slices **(called sections)** of the tissues and organs being studied, such as **cross section**, **oblique section** and **longitudinal section**. The following image show you how to correlate between the section and the plane of sectioning through the tissue.

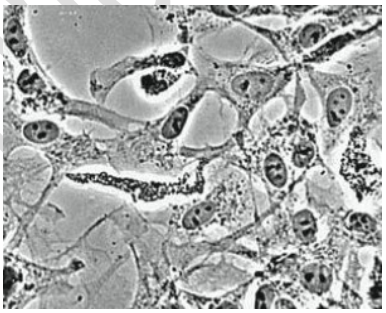


# Lab. 2

# Specialized Light Microscope & Electron Microscope

## Specialized Light Microscope





1. **Fluorescence Microscope:** When certain cellular substances are irradiated by light of a proper wavelength, they emit light with a longer wavelength— a phenomenon called **fluorescence**. In **fluorescence microscopy**, tissue sections are usually irradiated with ultraviolet (UV) light and the emission is in the visible portion of the spectrum. The fluorescent substances appear bright on a dark background.
2. **Phase-Contrast Microscope:** uses a lens system that produces visible images from unstained, transparent objects and, importantly, can be used with living, cultured cells. Cellular structures appear lighter or darker in relation to each other because of light refraction.
3. **Confocal Microscope:** it produces higher resolution and sharper focus than bright field microscope. It uses computer assisted lens system to produce many serial images that can be digitally reconstructed into 3D image.

## Electron Microscope:

An electron microscope is a type of microscope that produces an electronically-magnified image of a specimen for detailed observation. The electron microscope is an imaging system that permits high resolution (0.1 nm). In practice, however, a resolution of 1 nm in tissue sections is considered satisfactory. The electron microscope (EM) uses a particle beam of electrons to illuminate the specimen and create a magnified image of it. The microscope has a greater resolving power than a light-powered optical microscope, because it uses electrons that have wavelengths shorter than visible light, and can achieve magnifications of up to 2,000,000X, whereas light microscopes are limited to 2000X magnification.
The electron microscope uses electrostatic and electromagnetic "lenses" to control the electron beam and focus it to form an image. These lenses are analogous to, but different from the glass lenses of an optical microscope that form a magnified image by focusing light on or through the specimen.

**Types of Electron Microscope (EM):**

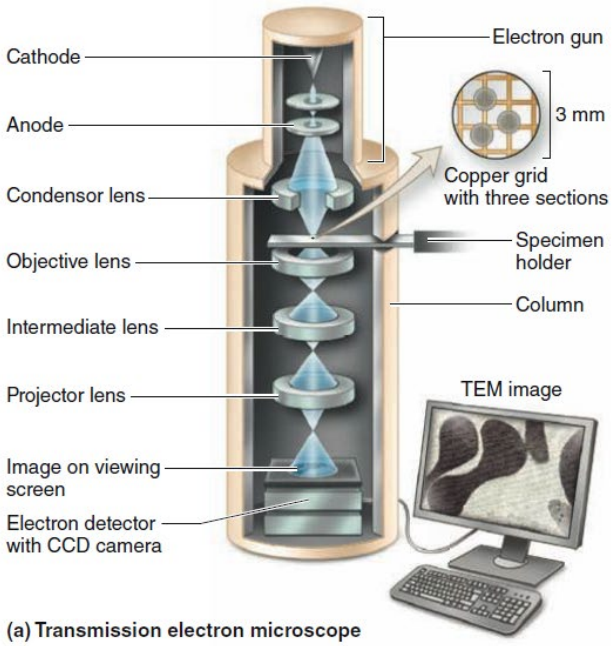
1. **Transmission Electron Microscope (TEM):**

The original form of electron microscope, the transmission electron microscope (TEM) uses a high voltage electron beam to create an image. The electron beam focused by electrostatic and electromagnetic lenses in a way roughly analogous to what occurs in the optical microscope (condenser, objective lens system), and transmitted through the specimen that is in part

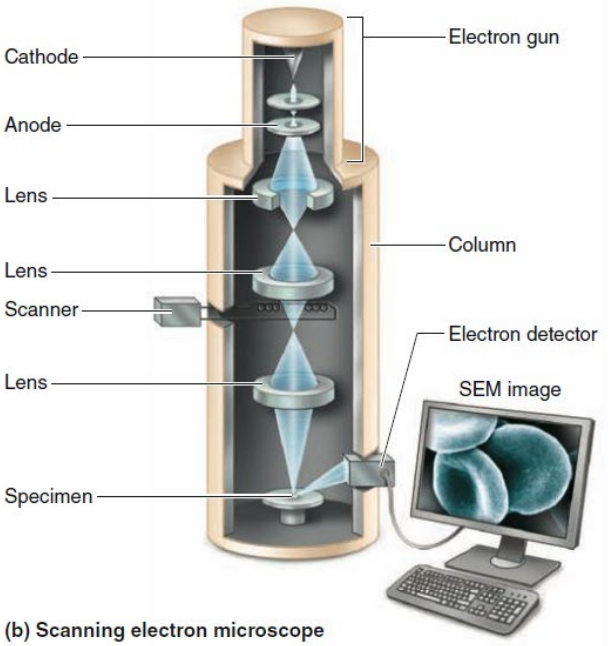
transparent to electrons and in part scatters them out of the beam. When it emerges from the specimen, the electron beam carries information about the structure of the specimen that is magnified by the objective lens system of the microscope. The spatial variation in this information (the "image") is viewed by projecting the magnified electron image onto a fluorescent viewing screen.

**2. Scanning Electron Microscope (SEM):**

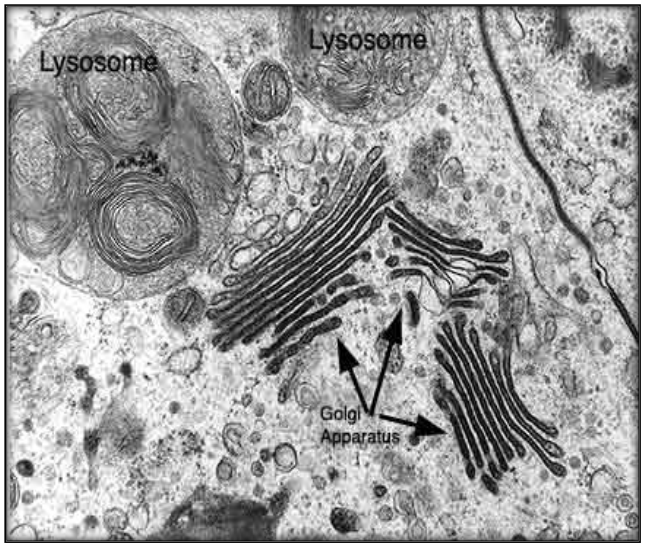
Is used to provide a three-dimensional image of cells. In SEM the electron beam does not pass through the specimen. Instead, the surface of the cell is coated with a heavy metal, and a beam of electrons is used to scan across the specimen. Because the resolution of scanning electron microscopy is only about 10 nm, its use is generally restricted to studying whole cells rather than subcellular organelles or macromolecules.

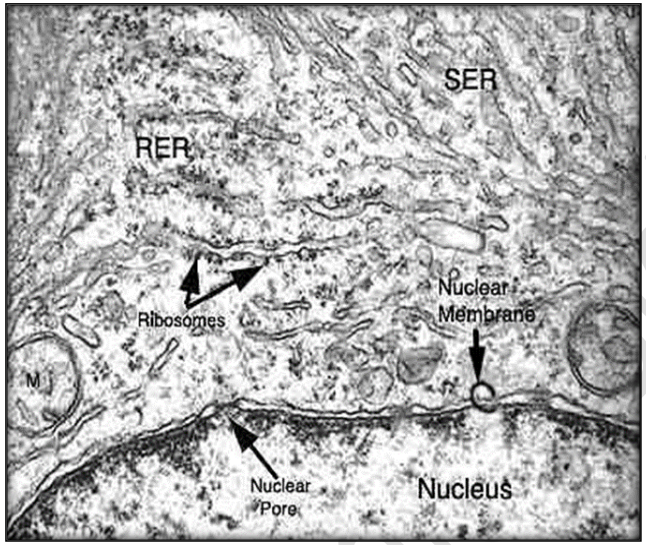


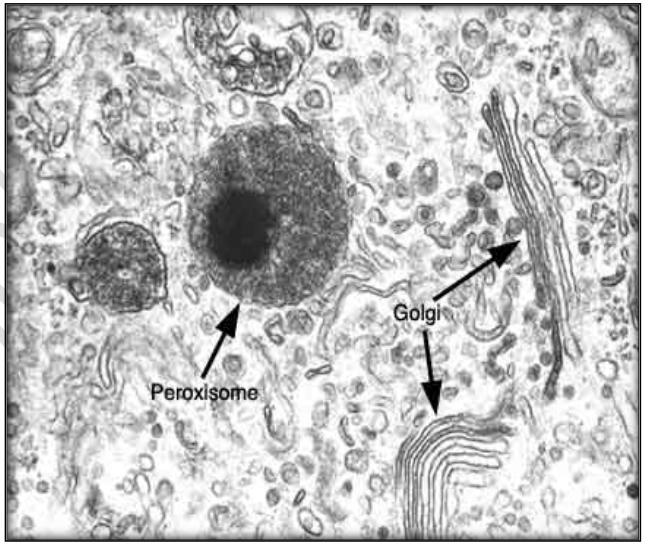

(a) Transmission electron microscope

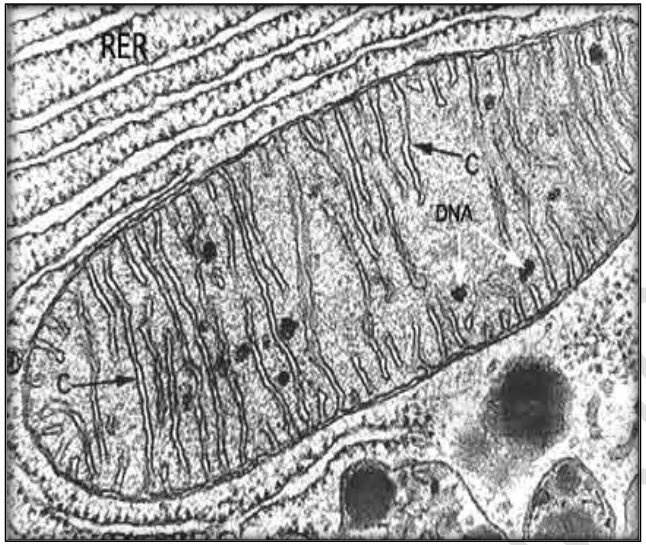


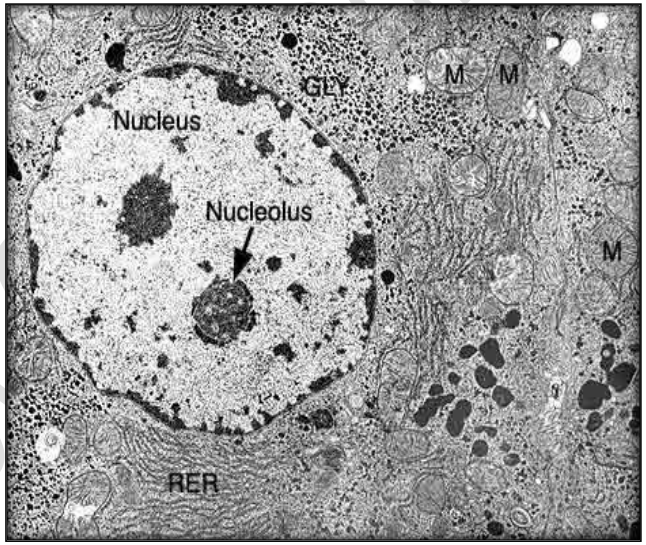

(b) Scanning electron microscope

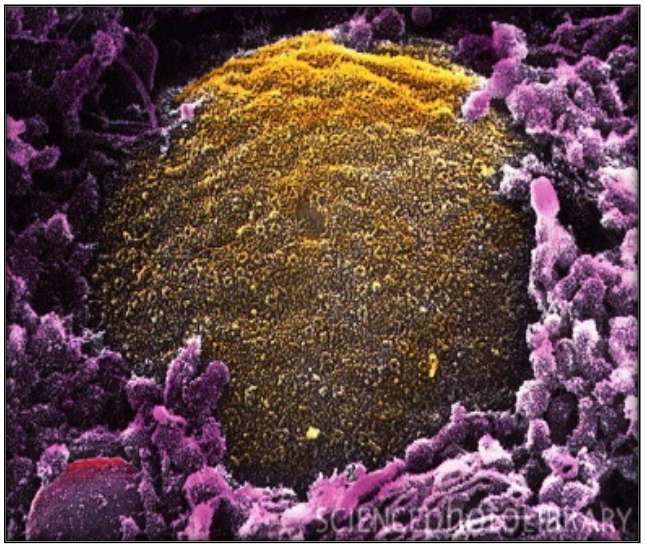


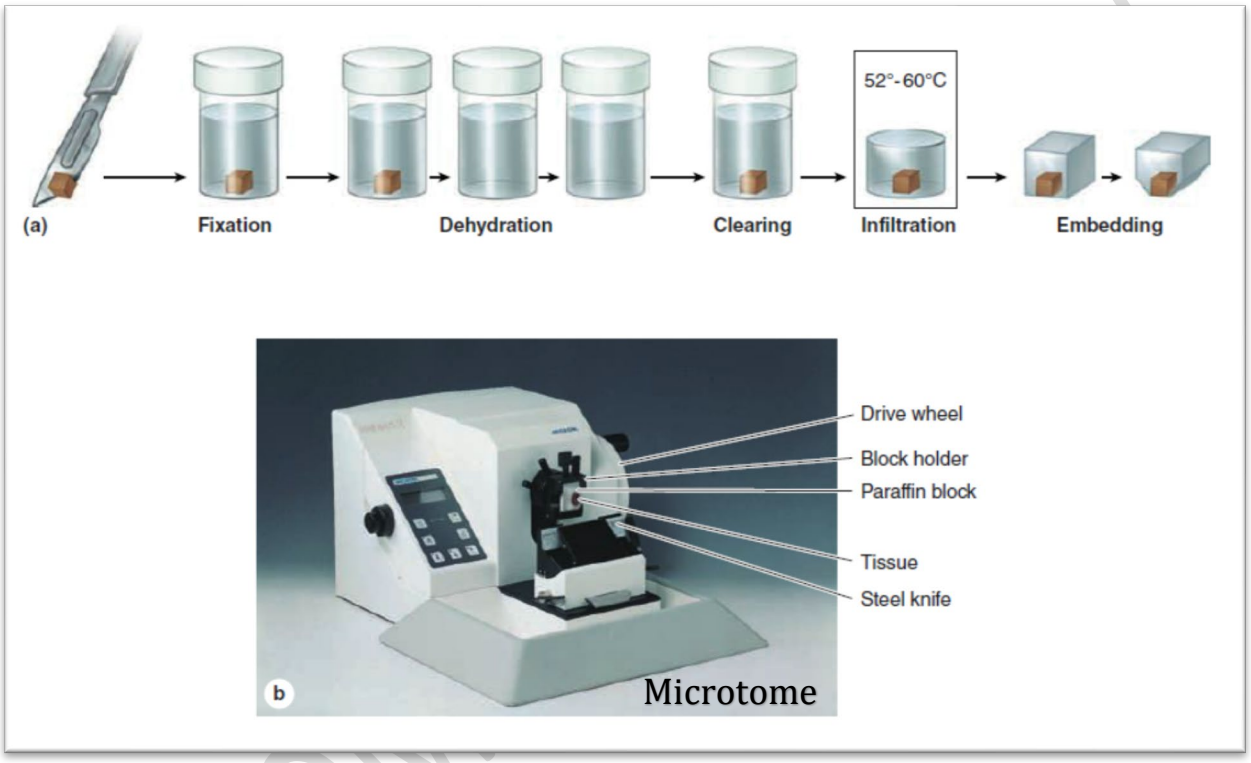



**The 2D images are from TEM of cell organelles and nucleus. The 3D image (colored) is from SEM of nuclear membrane**

# Lab. 3

## General Principles of Tissue Preparation and Staining

The study of tissue structure relies on the preparation of tissue samples in ways that allow their structural details to be viewed at light or electron microscopic levels. Because tissues and organs are usually too thick for light to pass through them, they must be sectioned to obtain thin, translucent sections and then attached to glass slides before they can be examined.




## The basic steps used in tissue preparation for histology are:

### ❖ Fixation

Fixation, usually done by a chemical or mixture of chemicals, permanently preserves the tissue structure for subsequent treatments. Fixation is used to:

1. Terminate cell metabolism.
2. Prevent enzymatic degradation of cells and tissues by autolysis (self-digestion).
3. Kill pathogenic microorganisms such as bacteria, fungi, and viruses.
4. Harden the tissue as a result of either cross-linking or denaturing protein molecules.
5. Transform the contents of the cell from a semifluid to a semisolid and prepare the cell contents for visualization with stains, dyes, or metallic salts.

➢ **Fixatives:** commonly used fixatives are

1. **Formalin:** (mixture of formaldehyde and alcohol). It penetrates the tissue rapidly, leaves no residues, and requires little or no washing of the tissue to remove.
2. **Aldehydes:** such as glutaraldehyde and paraformaldehyde, are excellent fixatives for light microscopic applications and are also widely used in electron microscopy.
3. **Other fixatives:** Picric acid, Alcohols, Mercuric chloride, and Acetic acid

➢ **Methods of Fixation:** Fixation is usually accomplished in one of two ways.

1. **Immersion fixation**. The fixative is prepared and a small piece of tissue removed and immersed in the fixative. The advantage of this method is that the fixative can penetrate from all sides of the tissue block. This method is useful when the tissue sample is small.
2. **Perfusion fixation**. In this method the fixative is perfused through the intact vascular system of the organism. After perfusion, the tissue samples are removed and placed in more of the same fixative used in the perfusion. This method provides superior fixation of large pieces of tissue.

> **FREEZING** is also used as a method of fixation, especially in the clinical setting when a rapid diagnosis is needed during a medical procedure. A fresh tissue sample is retrieved from the patient or organism and immersed in liquid carbon dioxide or in a substance cooled extremely rapidly by dry ice. The best results are obtained with small tissue samples that are rapidly cooled to very low temperatures (–40° to –60° C).

## ❖ Processing of Fixed Tissue

Once the tissue sample is satisfactorily fixed, it must be taken through a series of steps that result in a thin slice of tissue mounted on a glass slide (for LM) or an even thinner slice mounted on a copper grid (for EM). In general, this process requires three basic steps:

1. **Dehydration**: The goal of dehydration is to remove the water from the tissue. The most common method is to start with **alcohol** in a concentration of 70% to 80%. Recall that alcohol will mix with water; therefore, it can be used to remove water from the tissue. The tissue sample is passed, stepwise, through progressively higher concentrations (from 70%–80% up to 100%) of alcohol. Through this process, the water is completely removed from the tissue and replaced with alcohol.
2. **Clearing:** The clearing reagent is a substance that will mix both with alcohol and with the embedding medium. The most commonly used are **xylene**, **toluene**, and **chloroform**. The tissue is passed from100% alcohol through changes of the clearing reagent. This stepwise process progressively removes the alcohol from the tissue and replaces it with the clearing reagent.
3. **Impregnation and embedding:** Because the clearing reagent will mix with the embedding medium, the tissue sample is taken from the last step in this reagent and placed in melted embedding medium (**paraffin wax**). The sample is then progressively passed through several changes of the embedding material. This stepwise process progressively removes the clearing reagent and replaces it with the embedding medium that will harden when cooled.

   **Embedding** is generally done in two steps.

   - First, the tissue is removed from the last impregnation step and immediately placed in melted medium in a vacuum oven. The last traces of the clearing reagent and any minute bubbles are removed by vacuum.

- Second, the tissue sample is oriented in an **embedding mold**. The mold is then filled with melted medium and allowed to cool so the medium hardens. A similar set of steps are followed when a **polymer** is used as the embedding media for electron microscopy.

## ❖ Sectioning and Mounting

Sectioning of prepared tissues is done on **microtomes** that are designed to accommodate sections mounted in paraffin, frozen in ice, or embedded in plastic. The sections are cut using extremely sharp metal or glass knives, removed from the edge of the knife either as individual sections or as ribbons of sections, and floated in water (usually warmed).

For most applications in LM, the sections range in thickness from about **5** to **12 µm** (for paraffin embedded tissue) and from about **0.5** to **2.0 µm** (for plastic embedded tissue). For EM, glass or diamond knives are used to cut extremely thin sections ranges from 80 to110 nm.

After the sections are cut, they are mounted on slides using a mounting substance such as **DPX**.

## ❖ Staining

The goal of staining tissue slices is to use substances to impart color to various components of the section, making these components available for study.

The simplest way by which stains function is to exploit the electrostatic interactions between the stain molecules and components of the cell; positive charges on cellular structures attract negatively charged stain molecules and vice versa.

- **Basic dyes** carry positive charges and are, consequently, known as cationic dyes; they are attracted to negative charges within the tissue. **Hematoxylin** and **toluidine blue** are commonly used basic (cationic) dyes. They stain nuclear DNA, cytoplasmic RNA, sulfonated polysaccharides such as chondroitin sulfate, and polycarboxylic acids such as hyaluronic acid.
- **Acidic dyes** carry negative charges and are, consequently, known as anionic dyes; they are attracted to positive charges within the tissue. **Eosin Y** is a commonly used acidic (anionic) dye. It stains many proteins (and, therefore, stains many structures within the cell), and acid dyes also stain extracellular structures such as collagen.

## ➢ Commonly used stains

**Hematoxylin and Eosin (H&E)** is, by far, the most commonly used combination stain. This combination of cationic and anionic dyes results in most constituents of the cell (RNA, DNA, polysaccharides, and others) being stained with various tones of either blue (hematoxylin) or pink (eosin). This method also stains extracellular collagen.

Other stains: **Mallory trichrome stain; Wright stain; Silver stains; periodic acid-Schiff (PAS) reaction; Iron hematoxylin;** and **Giemsa stain**

# Lab. 4

## Immunocytochemistry and Immunohistochemistry (ICC & IHC)

While the term immunohistochemistry (IHC) is often used interchangeably with immunocytochemistry (ICC), significant differences exist between IHC and ICC in terms of the biological sample that is analysed. Simply put, **IHC is performed on samples derived from tissues that have been histologically processed into thin sections** and the staining process uses enzymes which catalyze the deposition of a colored staining product at antigenic sites within the sample**. ICC** relies on the same enzyme reactions as IHC, but it **is performed on samples consisting of cells grown in a monolayer or cells in suspension** which are deposited on a slide.

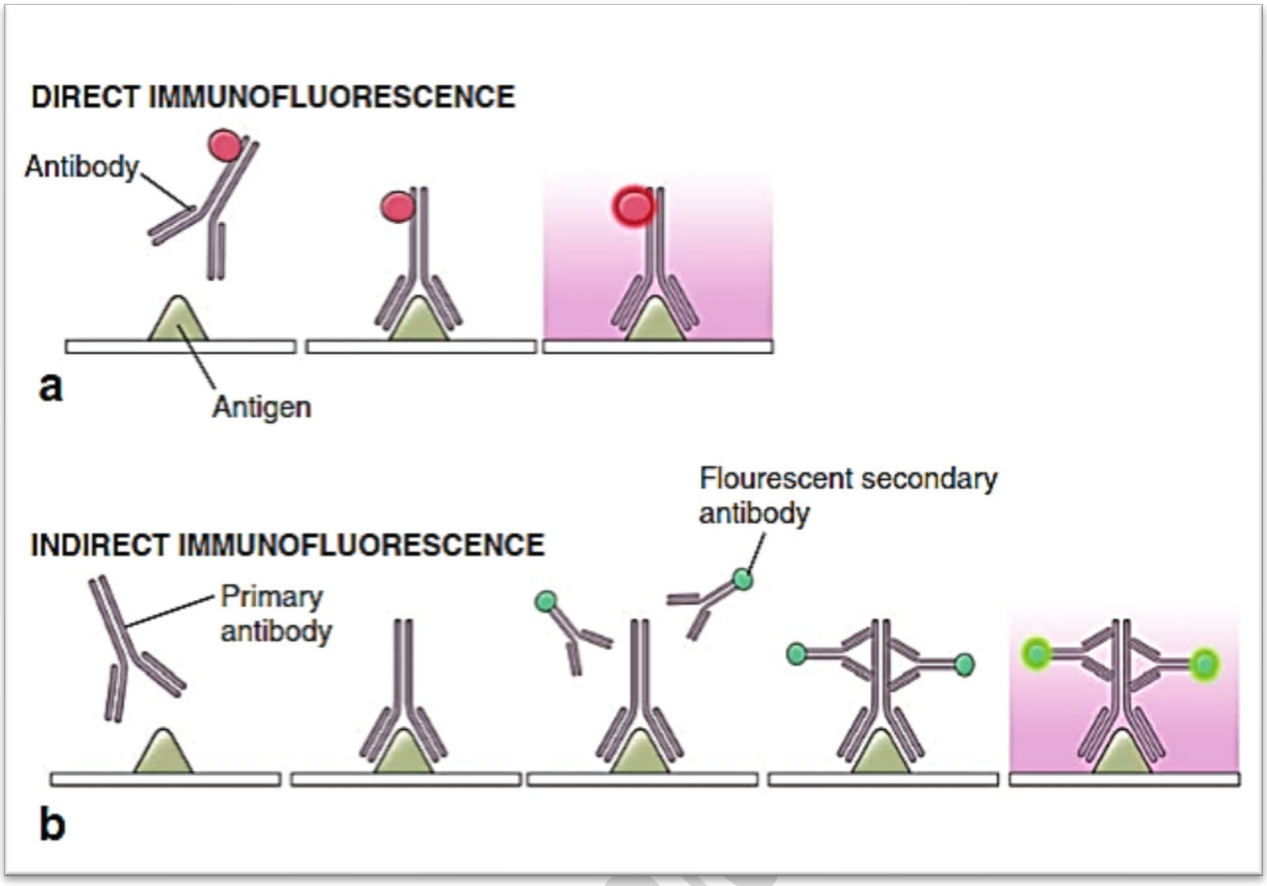
They are specialized methods that can be used to precisely localize enzymes or large molecules (macromolecules) within the cell or on its membrane. The immune system of the body is able to defend itself against foreign molecules (**antigens**) by producing specific types of proteins (**antibodies**). ICC & IHC methods use this feature of cells to visualize specific molecules.

For LM, an antibody is produced against a specified protein or molecule, and the antibody is coupled with a **fluorescent dye**, such as **fluorescein** or with an **enzyme** such as **peroxidase**. When this labeled antibody attaches to a specific antigen and is examined under microscope, it will show a color, thereby specifically identifying the location of that molecule.

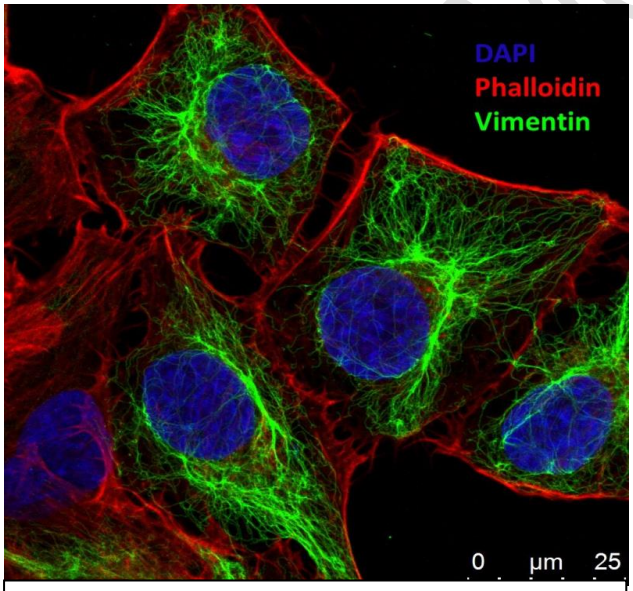
This is the **direct method**: An antibody is produced, coupled to a dye, attached to an antigen, and, thus, becomes visible.

In the **indirect method**, unlabeled antibodies (**primary antibodies**) are produced in one animal (e.g. mice or rabbit) against a specific antigen and then applied to a tissue to which they attach. The unlabeled antibodies are visualized by exposing them to labeled antibodies (**secondary antibodies**) that are made in another species (e.g. goat) and that are directed against the immunoglobulins from the first species.

One way to visualize this is as follows: An antibody (primary antibody) is produced, not labeled, and attached to an antigen; a second antibody (secondary antibody) is produced, coupled with a dye, attached to the first unlabeled antibody, and, thus, becomes visible.




**Example of ICC**

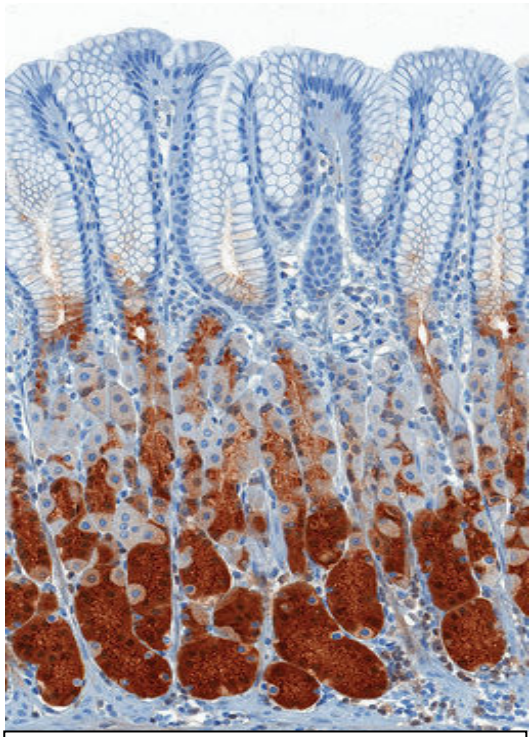



**DAPI** stain the nucleus

**Phalloidin** stain actin filaments

**Vimentin** stain intermediate filaments

**Example of IHC**

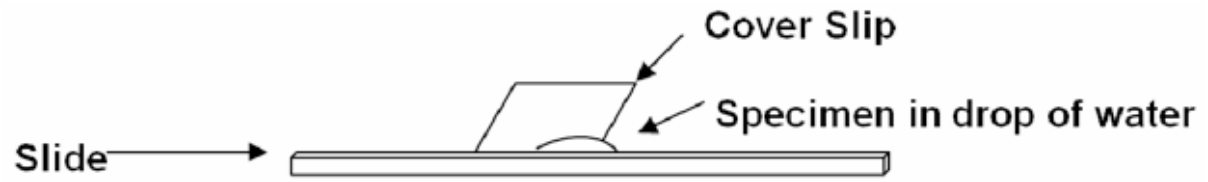


Brown stain represent antibody staining of pepsinogen enzyme in stomach glands

# Lab.5

# Wet Mount

If you want to look at something small under the microscope, you must know how to prepare a wet mount of the specimen.




To create a wet mount, the specimen of interest (skin cells, hair, pond water, etc.) is suspended in liquid between the microscope slide and a thin cover slip. Since the specimen is suspended in liquid, it will not dry out during observation. If the specimen contains translucent cells, then a liquid stain (example: methylene blue) may also be used to increase contrast.

## Observing Hair with Light Microscopy

1. Collect a hair strand from your head or arm.

2. Place a small drop of water on the center of a clean microscope slide.

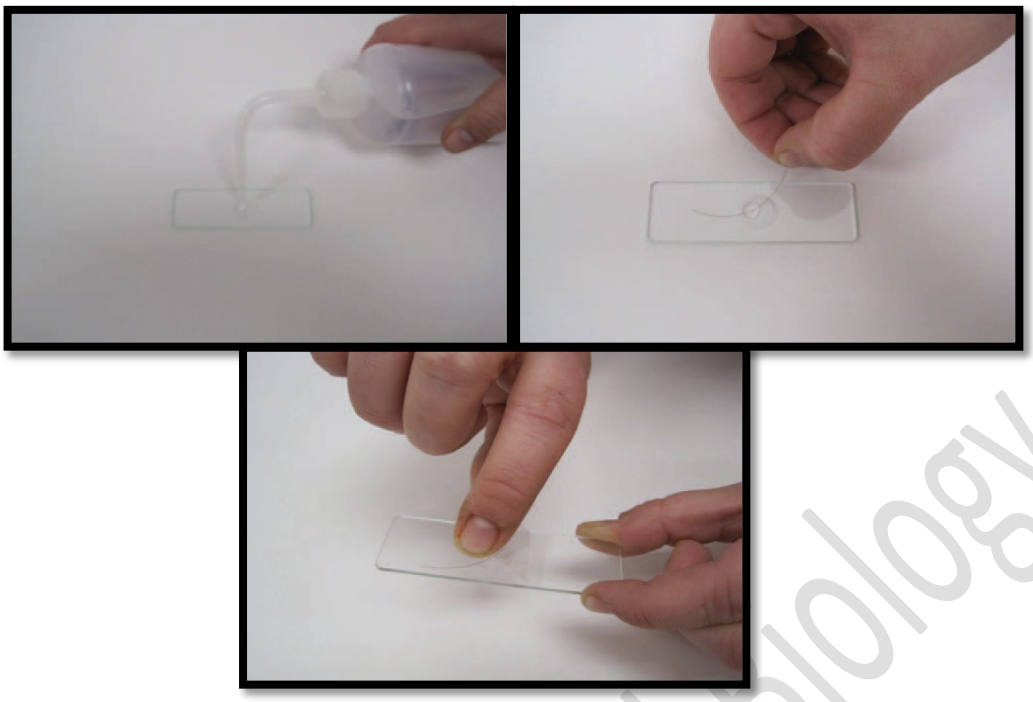
5. Place your hair strand into the water drop.

6. Starting with a 45° angle, gently lower a cover slip onto the microscope slide. ***Your hair strand should be sandwiched between the cover slip and slide by the end of this step***.

7. View your wet mount through a compound light microscope.

8. Draw a sketch of your hair strand in the provided Table at each level of magnification.

9. Compare your hair strand with that of students who have other colors or textures of hair.



| Compound Microscope<br>Low-Power Objective Lens<br>Total Magnification = _____x | Compound Microscope<br>Medium-Power Objective Lens<br>Total Magnification = _____x | Compound Microscope<br>High-Power Objective Lens<br>Total Magnification = _____x |
|---|---|---|
| | | |

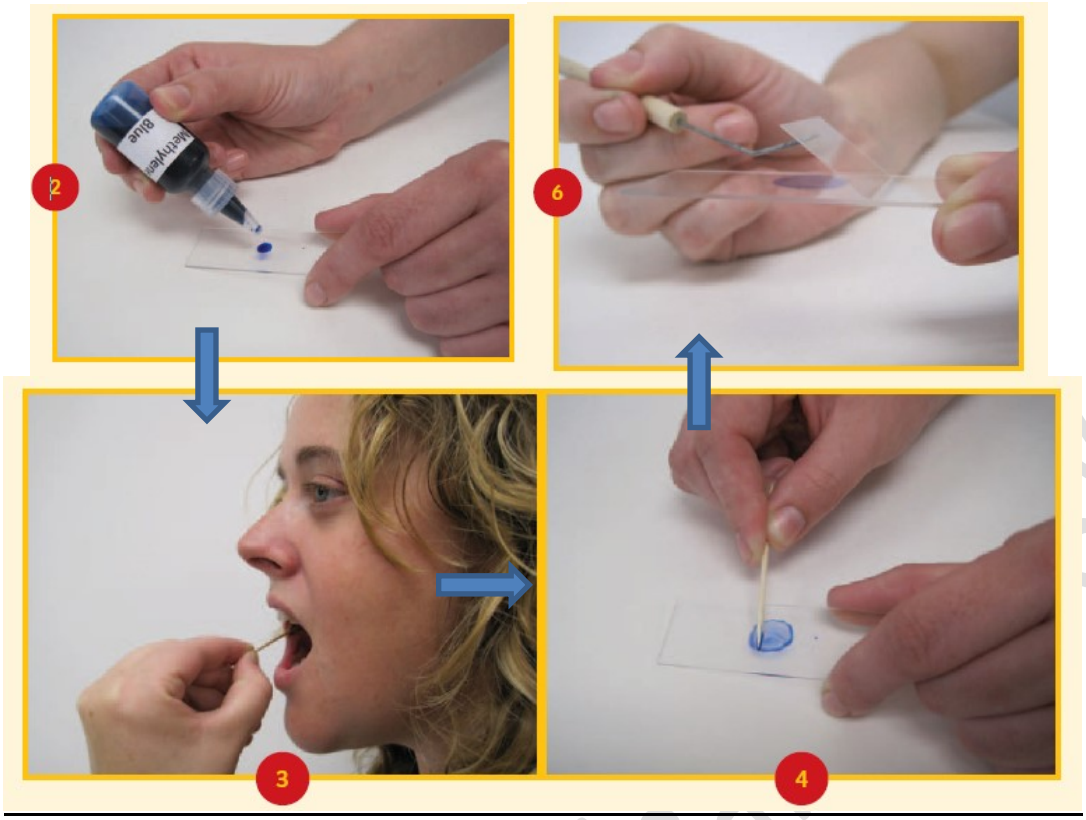
# Lab. 6

# Buccal Smear

The cells, which line the inside of your cheeks, form a mucous membrane and are classified as a stratified squamous epithelium tissue. These flat, scale-like buccal cells (pronounced, "buckle") resist friction and are shed constantly as the tissue is renewed. By gently scraping the inside of your cheek, these cells can be collected, and when smeared and stained, may be used to illustrate a number of important biological phenomena including cell and tissue structure.

## Slide Preparation:

1. Collect a clean microscope slide from the instructor bench.
2. Place a small drop of methylene blue stain onto the center of the slide.
3. Use a toothpick to gently scrape the inside of your cheek.
4. Swirl the toothpick into the methylene blue stain on your microscope slide.
5. dispose your used toothpick in a medical waste bin.
6. Starting with a 45° angle, gently lower a cover slip onto the microscope slide. By the end of this process, the stain should be sandwiched between the cover slip and the slide.
7. Examine your prepared slide under microscope, first with the 4x objective, scanning the entire field to find a well-distributed region with individual cells (no big clumps). Then view with the 10x and 40x objectives. Note the nucleus, nuclear membrane, cytoplasm and cell membrane.
8. Draw a sketch of your cheek cells in Table below at each level of magnification.




| Compound Microscope<br>Low-Power Objective Lens<br>Total Magnification = _____x | Compound Microscope<br>Medium-Power Objective Lens<br>Total Magnification = _____x | Compound Microscope<br>High-Power Objective Lens<br>Total Magnification = _____x |
|---|---|---|
| | | |

# Lab. 7

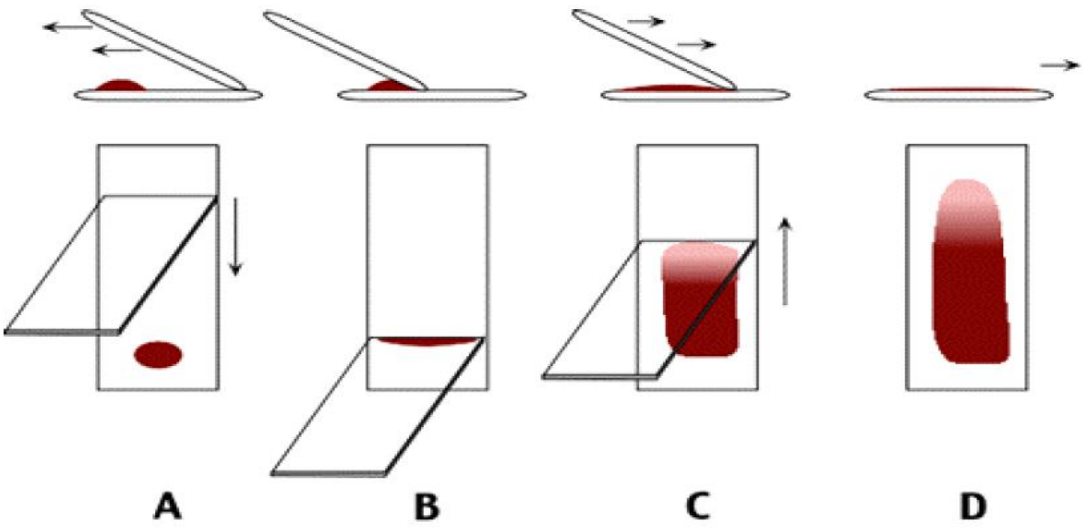
# Blood Smear

## Slide Preparation

1. Take a clean slide.
2. Sterilize the tip of your finger by washing with water, then in 95% alcohol.
3. Prick the tip of your finger with a lancet, and then discard the first drop of blood.
4. Let the second drop of blood touch one end (distal 1/3) of your slide.
5. Place the slide on a flat surface, and hold the narrow side of the other edge of the slide between your left thumb and forefinger
6. With your right hand, place the smooth clean edge of a second (spreader) slide on the specimen slide, just in front of the blood drop.
7. Hold the spreader slide at a 30-45' angle and draw it back against the drop of blood.
8. Allow the blood to spread almost to the edges of the slide.
9. Push the spreader slide forward with one light, smooth, and fluid motion. A thin film of blood in the shape of a bullet with a feathered edge will remain on the slide.
10. Dry the smear slide by waving in air.

## Lieshman's Stain

1. Add sufficient amount of Lieshman's stain with buffer to cover the blood smear, leave to fix for 1 minute.
2. Add distilled water; allow dilute stain to act for 10 minutes.
3. Tip of the staining solution and rinse the slide with distilled water until the smear appear pink.
4. Blot dry with a filter paper.
5. Examine under microscope with **4x**, **10x**, and then **40x** to identify blood cells (RBCs and WBCs).



# Lab. 7

# Mitosis

The period of cell division, or **mitosis** (Gr. *mitos*, a thread), is the only cell cycle phase that can be routinely distinguished with the light microscope. During this process, the parent cell divides, and each of the daughter cells receives a chromosomal set identical to that of the parent cell. The period between mitoses is called **interphase,** during which the DNA is replicated, centrosomes and centrioles are duplicated, and the nucleus appears as it is most commonly seen in histological preparations. The process of mitosis is subdivided into four phases.

1. **Prophase**: during which:-

   a. The nucleolus disappears, and the replicated chromatin condenses into discrete rod-shaped bodies, **the chromosomes**, each consisting of duplicate sister chromatids closely associated longitudinally.
   b. Outside the nucleus, the centrosomes with their centrioles separate and migrate to opposite poles of the cell and organize the microtubules of the mitotic spindle.
   c. Late in prophase, the nuclear envelope breaks down when proteins of the nuclear lamina and inner membrane are phosphorylated.

2. **Metaphase :** during which **:-**

   **a.** The condensed chromosomes attach to microtubules of the mitotic spindle at large electron-dense protein complexes called **kinetochores**, which are located at a constricted region of each chromatid called the **centromere**.
   **b.** The chromosomes are moved to the equatorial plane of the now more spherical cell. Kinetochore microtubules bound to sister chromatids are continuous with centrosomes at opposite poles of the mitotic spindle.

3. **Anaphase** : during which:-

   **a.** The sister chromatids separate from each other and are slowly pulled at their kinetochores toward opposite spindle poles by kinesin motors moving along the microtubules.
   **b.** Also, dynamic changes in the lengths of the microtubules occur as the spindle poles move farther apart.

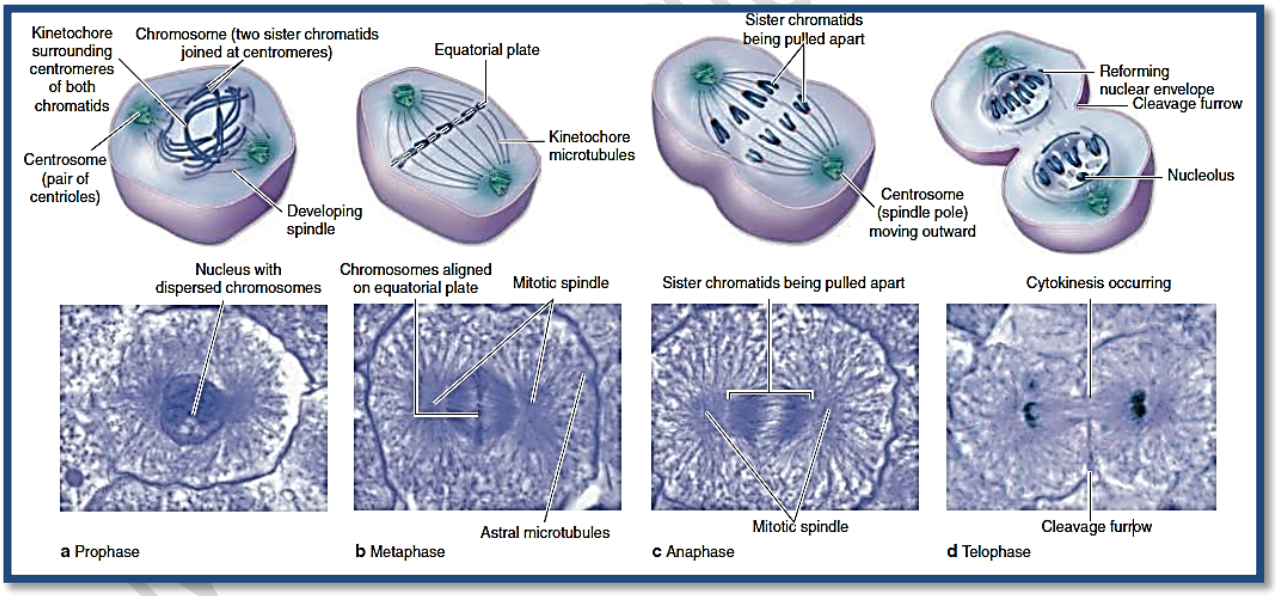
4. **Telophase** : during which:-

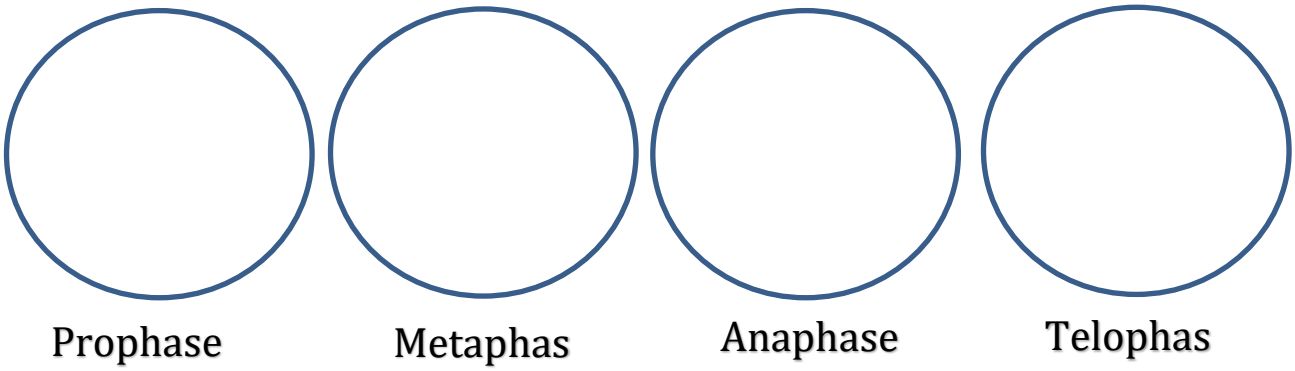
   **a.** The two sets of chromosomes are at the spindle poles and begin reverting to their decondensed state (chromatin threads).
   **b.** Microtubules of the spindle depolymerize and disappear.
   **c.** The nuclear envelope begins to reassemble around each set of daughter chromosomes.
   **d.** Reappearance of the nucleolus.

**Cytokinesis**

A belt-like **contractile ring**, containing actin filaments associated with myosins, develops in the peripheral cytoplasm at the equator of the parent cell. Then constriction of this ring produces a **cleavage furrow** and progresses until the cytoplasm and its organelles are divided in two daughter cells, each with one nucleus.

Mitotic cells are often difficult to identify conclusively in sectioned adult organs but can be recognized in rapidly growing tissues by their condensed chromatin (dark nuclei).



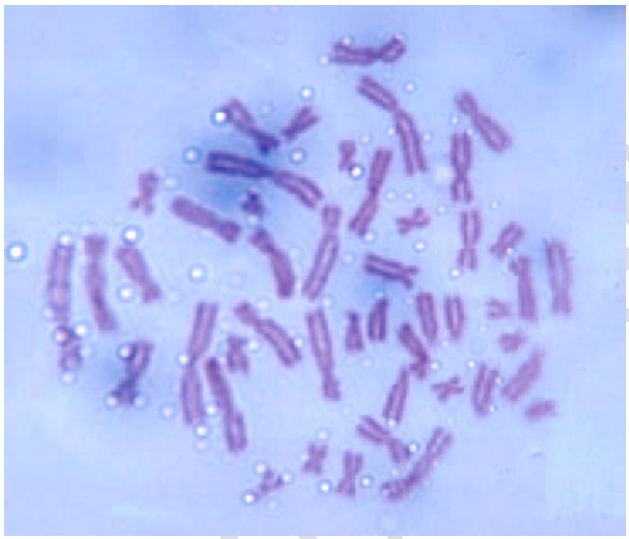
# Lab.8

# Chromosomes & Karyotyping

## Examining Chromosomes on a Microscope Slide

When a cell is not actively dividing, its DNA loosens up and fills the interior of the nucleus. When a cell is preparing to divide, though, each DNA molecule coils into a compact chromosome; the nuclear membrane also dissolves around the same time. During this lab, you will examine chromosomes on a prepared microscope slide. The cells on this slide originated from a human being, and the chromosomes were stained to increase their visibility. Compare your focused microscope slide to the photomicrograph shown below and answer the provided questions.

### Stained Chromosomes in a White Blood Cell



**Label a chromosome on the photomicrograph. How would you describe the shape of a human chromosome? Do they all share the same basic shape, or do they vary in shape?**

---

---

**Do human chromosomes vary in size, or do they all share a similar size?**

---

**Do you see a nucleus present in the cell you are viewing? What does the presence (or absence) of a nucleus tell you about this cell?**

---

---

---

**How many chromosomes are visible in the cell you are viewing? Is this the number you expect to find in a human cell?**

---

---

## KARYOTYPE ANALYSIS

Did you know that some genetic syndromes, such as Down syndrome, can be diagnosed by examining a person's chromosomes? After the chromosomes are harvested from a patient's cell, they are organized into a profile called a **karyotype** for analysis. (The prefix *karyo* refers to the *nucleus* of a cell.) The Figure below shows a typical human karyotype. As you can see, **46 chromosomes** are present per cell, and each chromosome belongs to a pair. Since two X chromosomes are present—*and the Y chromosome is absent*—this karyotype belongs to a female. With the exception of the sex chromosomes (X and Y), each chromosome in the human genome is numbered based on its relative size. Chromosome **1** is the largest chromosome, and chromosome **22** is the smallest chromosome.

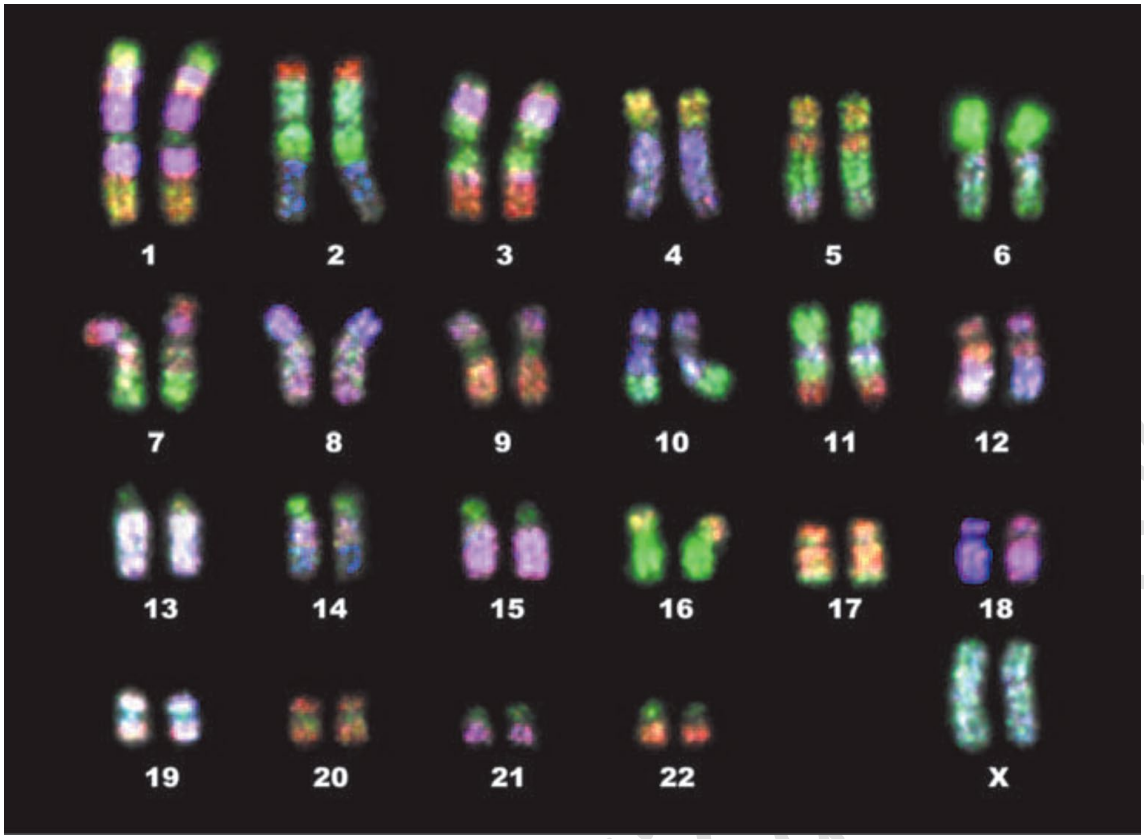
**While analyzing karyotypes, geneticists ask themselves the following questions:**

• How many chromosomes are present in each cell?

• If an abnormal number of chromosomes is present, which chromosome (1-22, X, or Y) is involved?

• Do any chromosomes have structural abnormalities, such as missing parts or additional parts?

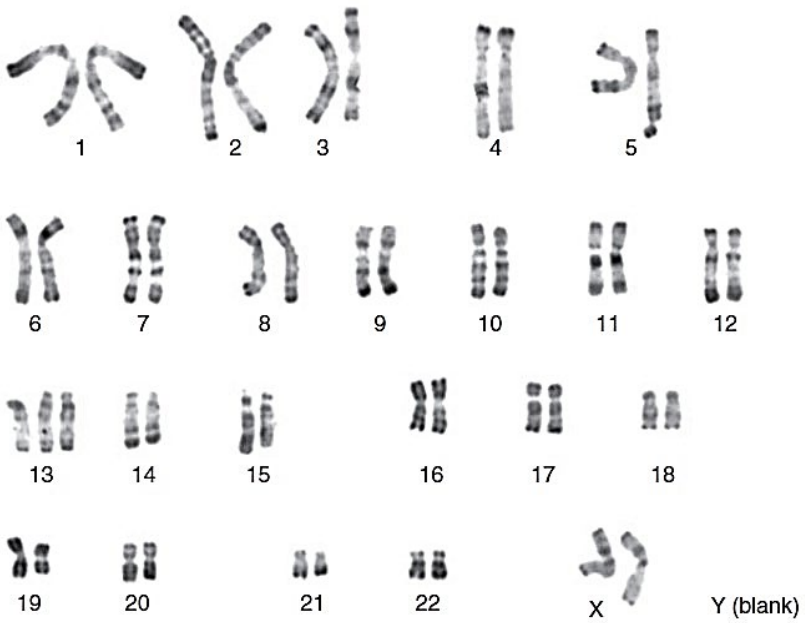
• If a structural abnormality is identified, which chromosome (1-22, X, or Y) is involved?




Typically, somatic cells carry two copies of each chromosome in the genome. Serious complications may arise during development if cells inherit an abnormal number of chromosomes. Some chromosomal abnormalities cannot lead to a viable pregnancy, while others may lead to genetic syndromes with severe birth defects. If a particular chromosome is present in triplicate, then this condition is referred to as a **trisomy**. A person with Trisomy 21, for instance, carries three copies of chromosome 21 per cell. Although most trisomies do not lead to viable births, several trisomies have been identified and characterized in humans: Trisomy X (also known as Triple X syndrome), Trisomy 21 (also known as Down syndrome), Trisomy 18 (also known as Edwards syndrome), Trisomy 16, and Trisomy 13 (also known as Patau syndrome). If a single copy of a particular chromosome is present, then this condition is referred to as a **monosomy**. With the exception of the sex chromosomes in males, the only viable monosomy in humans is Monosomy X (also known as Turner syndrome).

- **You will see 4 examples of abnormal karyotype and you are required to analyze each one according to the provided criteria**

## KARYOTYPE 1




**Examine the karyotype shown above. Does this karyotype belong to a male or a female?**

_____

**Are any chromosomal abnormalities present on this karyotype? If so, describe the nature of this chromosomal abnormality.**

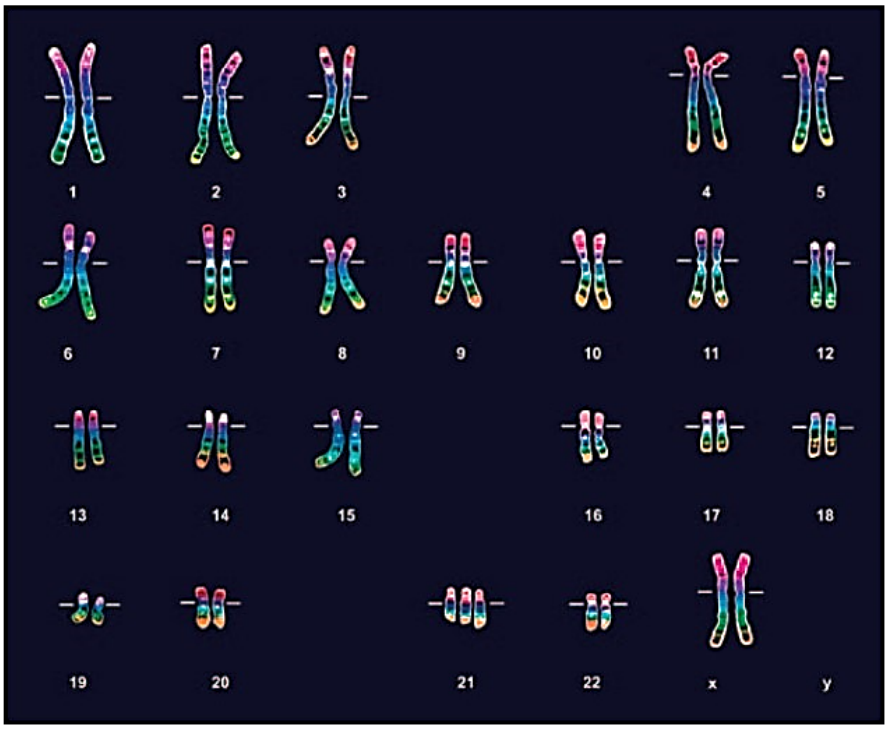
- Is a monosomy or trisomy present?
- Which chromosome is involved?

_____

_____

**Does Karyotype 1 belong to a person with Turner syndrome, Patau syndrome, Down syndrome, or Klinefelter syndrome?** _____

# KARYOTYPE 2




**Examine the karyotype shown above. Does this karyotype belong to a male or a female?**

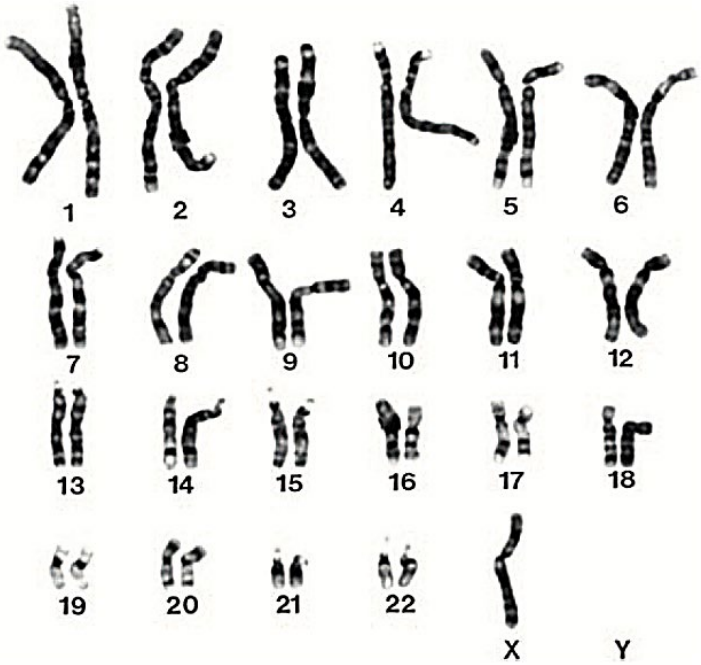
**Are any chromosomal abnormalities present on this karyotype? If so, describe the nature of this chromosomal abnormality.**

- Is a monosomy or trisomy present?
- Which chromosome is involved?

**Does Karyotype 2 belong to a person with Turner syndrome, Patau syndrome, Down syndrome, or Klinefelter syndrome?**

**Name two common features of this genetic syndrome.**

## KARYOTYPE 3




**Examine the karyotype shown above. Does this karyotype belong to a male or a female?**

_____

**Are any chromosomal abnormalities present on this karyotype? If so, describe the nature of this chromosomal abnormality.**

- Is a monosomy or trisomy present?
- Which chromosome is involved?

_____

_____

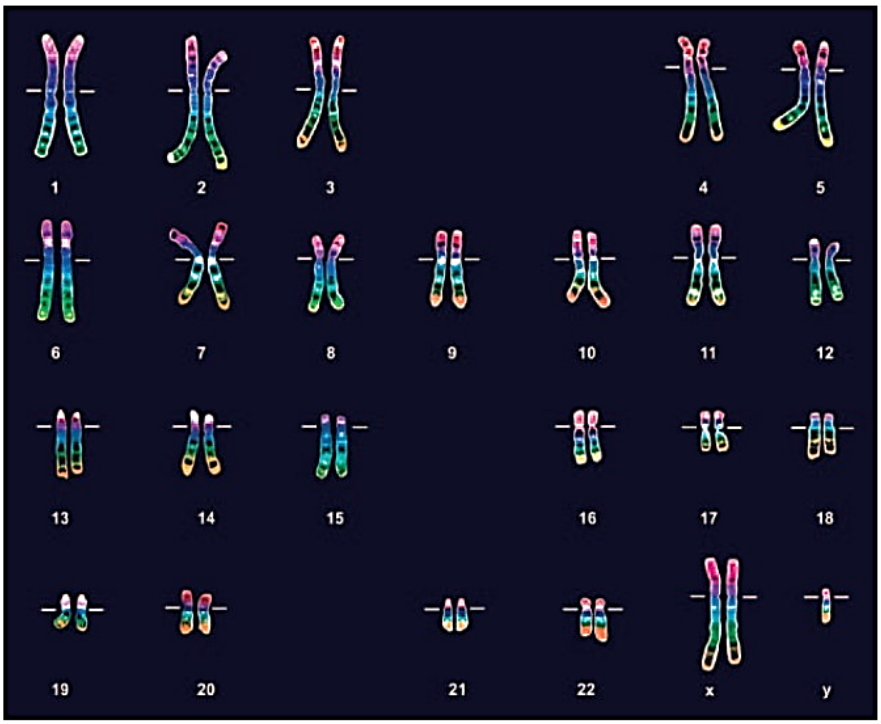
**Does Karyotype 3 belong to a person with Turner syndrome, Patau syndrome, Down syndrome, or Klinefelter syndrome?** _____

**Name two common features of this genetic syndrome.**

_____

_____

## KARYOTYPE 4




**Examine the karyotype shown above. Does this karyotype belong to a male or a female?**

_____

**Are any chromosomal abnormalities present on this karyotype? If so, describe the nature of this chromosomal abnormality.**

- Is a monosomy or trisomy present?
- Which chromosome is involved?

_____

_____

**Does Karyotype 4 belong to a person with Turner syndrome, Patau syndrome, Down Syndrome, or Klinefelter syndrome?** _____

**Name two common features of this genetic syndrome.**

_____

_____

# Lab.9

# DNA, RNA & Gene Expression

## DNA structure

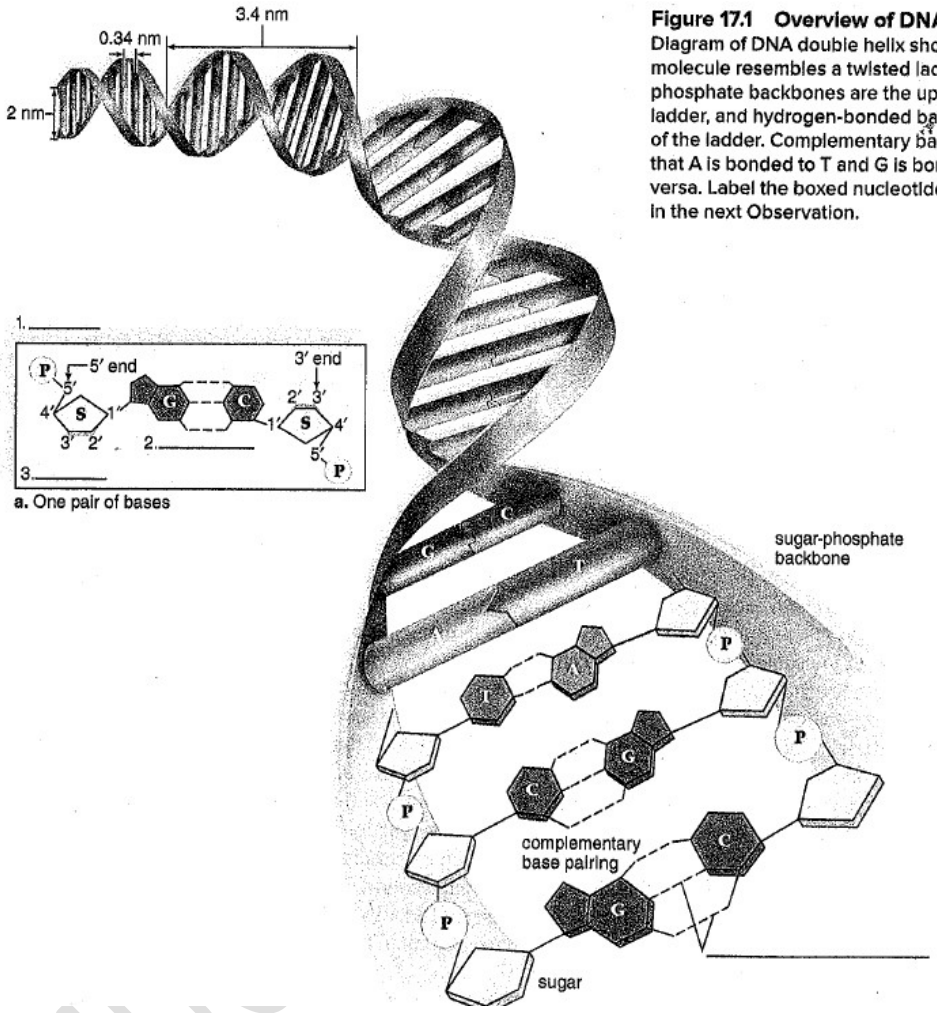


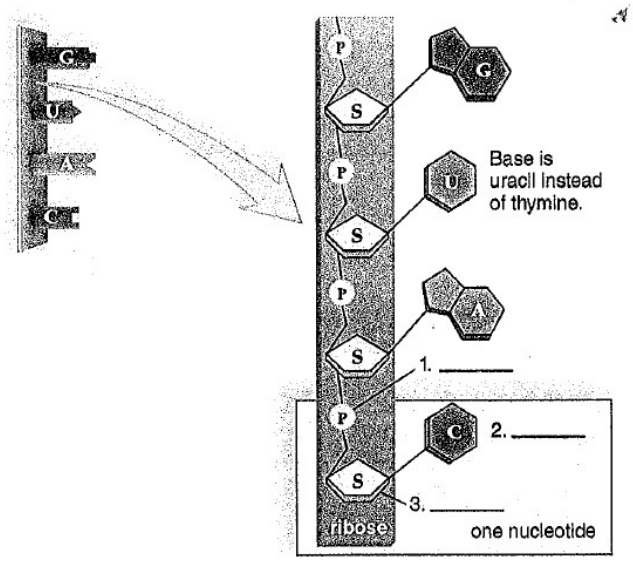

**Figure 17.1 Overview of DNA structure.** Diagram of DNA double helix shows that the molecule resembles a twisted ladder. Sugar–phosphate backbones are the uprights of the ladder, and hydrogen-bonded bases are the rungs of the ladder. Complementary base pairing dictates that A is bonded to T and G is bonded to C and vice versa. Label the boxed nucleotide pair as directed in the next Observation.

☒ **Draw a representation of nucleotide base pair. Label *phosphate, base pair and deoxyribose* in your drawing.**

☒ The base **A** (known as ---------- ) is always paired with base ---------- (known as ----------), and the base **C** (known as ----------) is always paired with the base ------- (known as ----------). This is called ---------- base pairing.

| DNA replication | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Old strand | **G** | **G** | **G** | **T** | **T** | **C** | **C** | **A** | **T** | **T** | **A** | **A** | **A** | **A** | **T** | **T** | **C** | **C** | **A** | **G** | **A** | **A** | **A** | **T** | **C** | **A** | **T** | **A** |
| New strand | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

## RNA structure




☒ **Draw a nucleotide for construction of RNA. Label the *ribose, the phosphate and the base* in your drawing.**

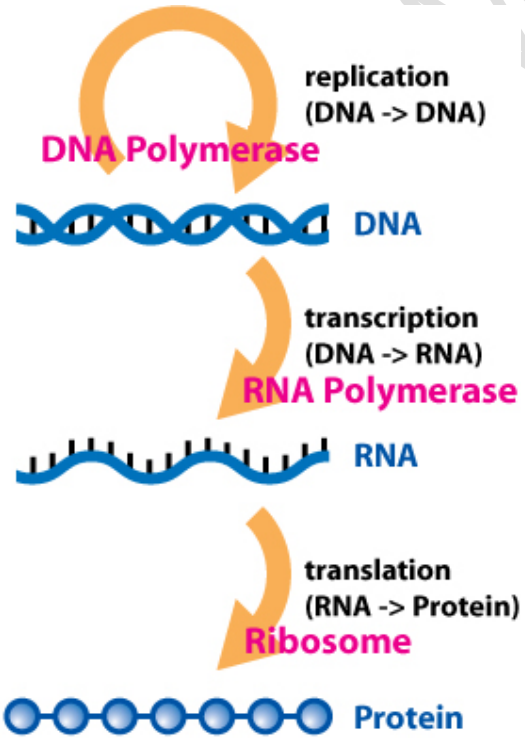
| DNA and RNA Bases | | | | |
|---|---|---|---|---|
| RNA bases | **C** | **U** | **A** | **G** |
| DNA bases | | | | |

| DNA structure compared with RNA structure | | |
|---|---|---|
| | **DNA** | **RNA** |
| **Sugar** | Deoxyribose | |
| **Bases** | Adenine, Guanine, Thymine, Cytosine | |
| **Strands** | Double stranded with base pairing | |
| **Helix** | Yes | |

## Gene Expression (Protein Synthesis)

| Transcription | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DNA | **T** | **A** | **C** | **A** | **C** | **G** | **A** | **G** | **C** | **A** | **A** | **C** | **T** | **A** | **A** | **C** | **A** | **T** |
| mRNA | | | | | | | | | | | | | | | | | |

| Translation | | | | | | | |
|---|---|---|---|---|---|---|---|
| mRNA codons | **AUG** | **CCC** | **GAU** | **GUU** | **GAG** | **UUG** | **UCU** |
| tRNA anticodons | | | | | | | |
| Amino acid | | | | | | | |



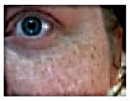
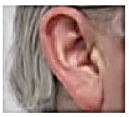
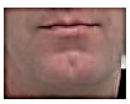

| First Base | Second Base U | Second Base C | Second Base A | Second Base G | Third Base |
|---|---|---|---|---|---|
| U | UUU phenylalanine | UCU serine | UAU tyrosine | UGU cysteine | U |
| | UUC phenylalanine | UCC serine | UAC tyrosine | UGC cysteine | C |
| | UUA leucine | UCA serine | UAA *stop* | UGA *stop* | A |
| | UUG leucine | UCG serine | UAG *stop* | UGG tryptophan | G |
| C | CUU leucine | CCU proline | CAU histidine | CGU arginine | U |
| | CUC leucine | CCC proline | CAC histidine | CGC arginine | C |
| | CUA leucine | CCA proline | CAA glutamine | CGA arginine | A |
| | CUG leucine | CCG proline | CAG glutamine | CGG arginine | G |
| A | AUU isoleucine | ACU threonine | AAU asparagine | AGU serine | U |
| | AUC isoleucine | ACC threonine | AAC asparagine | AGC serine | C |
| | AUA isoleucine | ACA threonine | AAA lysine | AGA arginine | A |
| | AUG *(start)* methionine | ACG threonine | AAG lysine | AGG arginine | G |
| G | GUU valine | GCU alanine | GAU aspartic acid | GGU glycine | U |
| | GUC valine | GCC alanine | GAC aspartic acid | GGC glycine | C |
| | GUA valine | GCA alanine | GAA glutamic acid | GGA glycine | A |
| | GUG valine | GCG alanine | GAG glutamic acid | GGG glycine | G |

# Lab.10

# Genetic Inheritance & Pedigree

## Examining Traits with Simple Inheritance Patterns

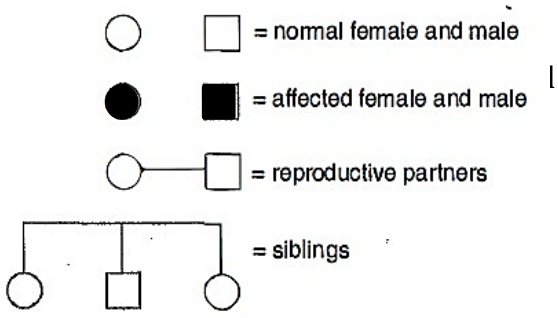
To begin this lab, determine your phenotype for each trait listed in the Table below. To accurately assess these traits, you may need to work in pairs or examine yourself in the mirror. Once the class results have been pooled, you will then analyze the prevalence of dominant traits (example: presence of dimples) and recessive traits (example: absence of dimples) in the class. Assign a genotype for each phenotype by choosing a capital letter for the dominant trait and small letter for the recessive trait, then add the genotype to the table.

| Trait | Dominant Phenotype | Recessive Phenotype | My Phenotype | Percentage of Class with Dominant Phenotype |
|---|---|---|---|---|
| Dimples (+ or –) |  Dimples |  No Dimples | | |
| Freckles (+ or –) |  Freckles |  No Freckles | | |
| Hairline (straight or Widow's peak) |  Widow's Peak |  Straight Hairline | | |
| Earlobes (attached or detached) |  Unattached Earlobes |  Attached Earlobes | | |
| Cleft chin (+ or –) |  Presence of Cleft |  Absence of Cleft | | |
| Tongue Rolling (+ or –) |  Ability to Roll Tongue |  Inability to Roll Tongue | | |
| Mid-Digital Finger Hair (+ or –) |  Presence of Any Hair |  Absence of Hair | | |

## Determining the Pedigree

A pedigree shows the inheritance of a genetic disorder within a family. A pedigree can help determine the inheritance pattern and whether any particular individual has an allele for that disorder. Then a Punnett square can be done to determine the chances of a couple producing an affected child.

In a pedigree, roman numerals indicate the gener
particular individuals in that generation. The sym
males and females, reproductive partners, and sil

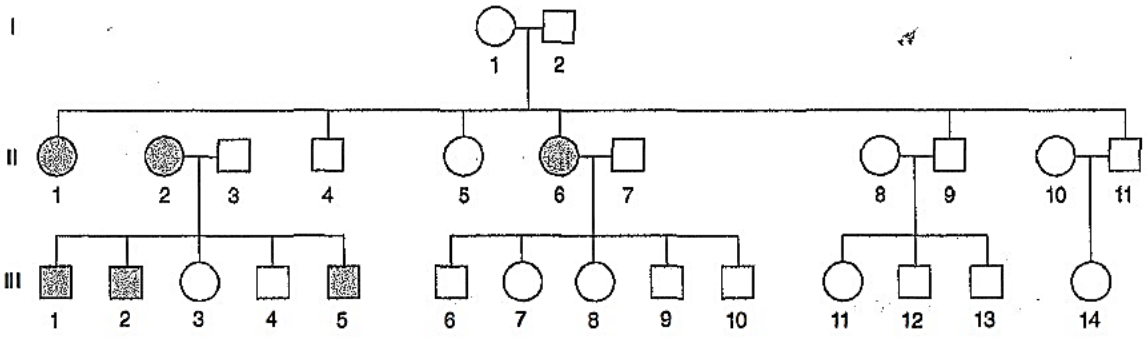



**Pedigree symbols.**

## Pedigree Analyses

For each of the following pedigrees, determine how a genetic disorder is inherited. Is the inheritance pattern autosomal dominant, autosomal recessive, or X-linked recessive? Also, decide the genotype of particular individuals in the pedigree. A pedigree indicates the phenotype, and you can reason out the genotype.

1. **Study the following pedigree:**




a. What is the inheritance pattern for this genetic disorder? _____

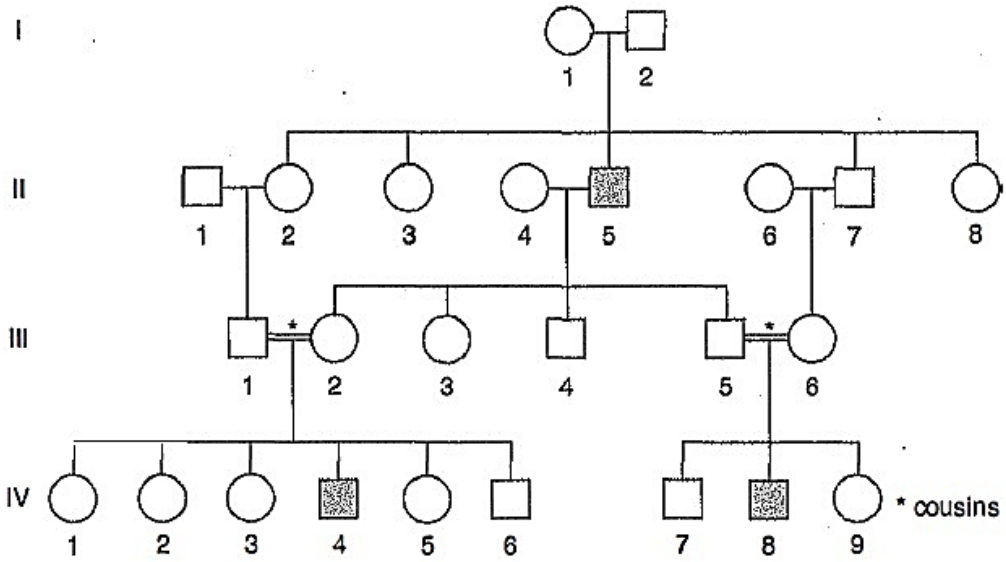
b. What is the genotype of the following individuals? Use A for the dominant allele and a for the recessive allele.

Generation I, individual 1: _____

Generation II, individual 1: _____

Generation III, individual 8: _____

**2. Study the following pedigree:**




a. What is the inheritance pattern for this genetic disorder? _____

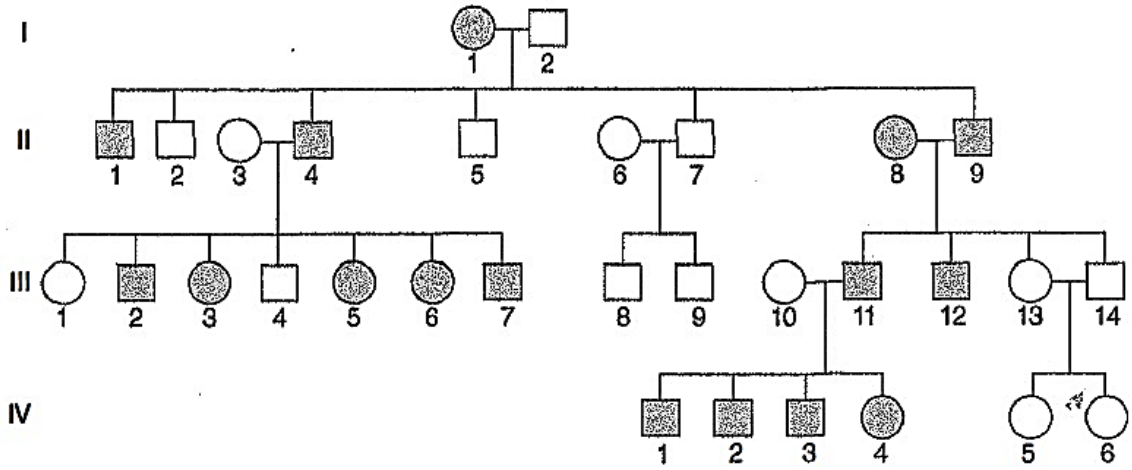
b. What is the genotype of the following individuals?

Generation I, individual 1: _____

Generation II, individual 8: _____

Generation III, individual 1: _____

**3. Study the following pedigree:**



a. What is the inheritance pattern for this genetic disorder? _____

b. What is the genotype of the following individuals?

Generation I, individual 1: _____

Generation II, individual 7: _____

Generation III, individual 11: _____

## Construction of a Pedigree

You are a genetic counsellor who has been given the following information from which you will construct a pedigree.

Your data: Henry's maternal grandfather and his mother have double eyelashes. Their spouses do not. Henry who has double eyelashes is married to Isabella who has normal eyelashes, and their first child Polly has normal eyelashes. The couple wants to know the chances of any child having a double row of eyelashes.

1. Using pedigree symbols only, construct two pedigrees. Begin with the maternal grandfather and grandmother, and end with Polly. Place it on both the left and right.

**Pedigree 1** **Pedigree 2**

2. What is your key for this trait?

Key:

_____ normal eyelashes

_____ double row of eyelashes

3. Try out a pattern of autosomal dominant inheritance by assigning appropriate genotypes for the dominant pattern of inheritance to each person in pedigree 1. Then try out a pattern of X-linked dominant inheritance by assigning appropriate genotypes for the x-linked pattern of inheritance to each person in pedigree 2. Which pattern is correct?

4. Use correct genotypes to show the cross between Henry and Isabella and perform a Punnett square for this cross.

Henry Isabella

---------- X ----------

# REFERENCES

1. Junqueira's Basic Histology: text and atlas, 15th edition, 2018.
2. Human Biology, 15th edition, 2017.
3. Laboratory Manual for Human Biology, 14th edition, 2015.
4. Visualizing Human Biology Lab Manual, 1st edition, 2011