PRACTICAL MANUAL

CELL & MOLECULAR BIOLOGY

4(3-1)

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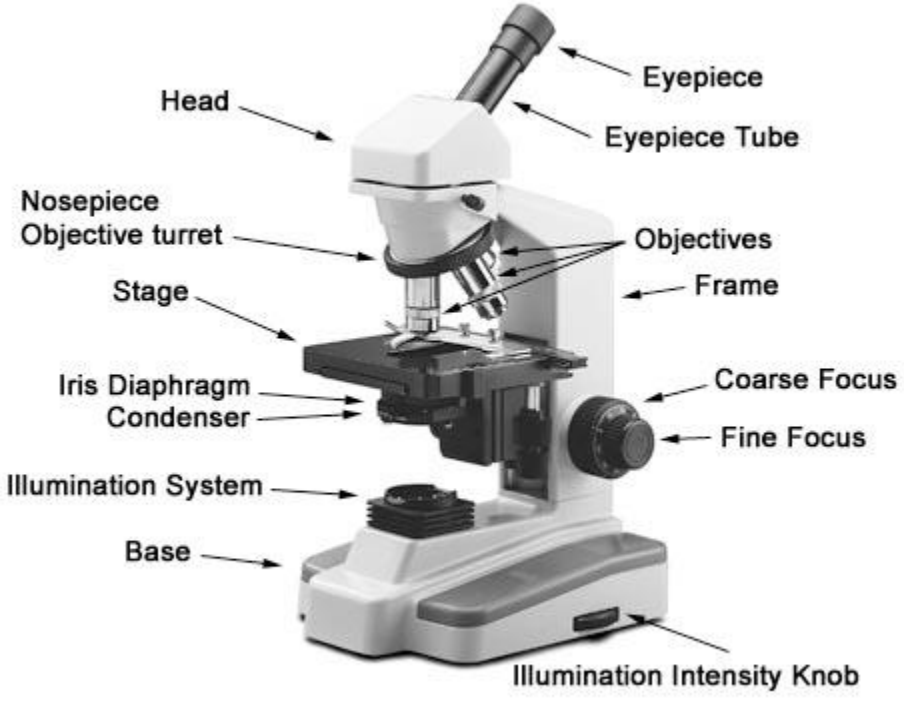
Contents

PRACTICAL NO 1

Study of compound microscope (Light)

Material:

- Microscope



Parts of a microscope:

The compound microscope is a delicate instrument composed of many parts that are accurately filled together in (Figure)

1. Ocular of eyepiece lens.

The ocular lens is the lens you look through, it is inserted at the top of the body tube. If your microscope has one ocular, it is a monocular microscope, if it has two, it is binocular. Its magnification is written on it.

2. Body tube.

Body tube is the optical housing for the objective lenses.

3. Objective lenses.

The objective lenses are a set of three to four lenses mounted on a rotating turret at the bottom of the body tube. The four objective lenses of your microscope and their magnifications are:

Scanning lens 4X magnification
Low power lens 10X magnification
High power lens 40-45X magnification
Oil immersion lens 100X magnification

The magnification of the objective lens is written on the lens.
Note: with the exception of the oil immersion lens all the objective lens is used dry.
The magnification of oil immersion lens requires using the lens with special immersion oil for proper resolution.

4. Stage

The horizontal surface on which the slide is placed is called the stage. It may be equipped with simple clips for holding the slide in place or with a mechanical stage, a geared device for precisely moving the slide. Two knobs, either on top of or under the stage, move the mechanical stage.

5. Condenser lens.

Condenser lens system, located immediately under the stage, contains a system of lenses that focuses light on your specimen. The condenser may be raised or lowered using the condenser knob. An older microscope may have a concave mirror instead.

6. Iris diaphragm

Iris diaphragm is located below the condenser or immediately below the stage in microscopes without a condenser. It functions in regulating the light intensity passing through to the stage. More light is required at higher magnification.

7. Light source

The light source has an (ON/Off) switch & may have adjustable lamp intensities
& color filters.

8. Base

Base – also called the supporting stand, rests on the bench.

9. Body Arm

The body arm is used when carrying the instrument.

10. Nose piece

Nosepiece is the mounting for the objective lenses which rotates to bring the desired objective into position.

11. Coarse adjustment

Coarse adjustment knob is a large knob located at either side of the microscope which functions in controlling the distance between the objectives and the stage.
Use the coarse adjustment only with the scanning (4X) & low- power (10X) objectives.

12. Fine adjustment

Fine adjustment is a small knob located at either side of the microscope. This is used for the control of the object, precise focusing you should use just the fine adjustment knob with the higher magnification objective lenses; because using the coarse adjustment knob with the higher objective lenses may damage the lens
&/or the slide you are observing.

13. Magnification:

Compound microscopes consist of two lens system: the objective lens, which magnifies, & projects a "virtual image" into the body tube and the ocular lens, which magnifies the image further and projects the enlarged image into the eye.
The total magnification of a microscope is the product of the magnification of the objective and the ocular. If the objective lens has a magnification of 5X and the ocular
12X, then the image produced by these two lenses is 60 times larger than the specimen.

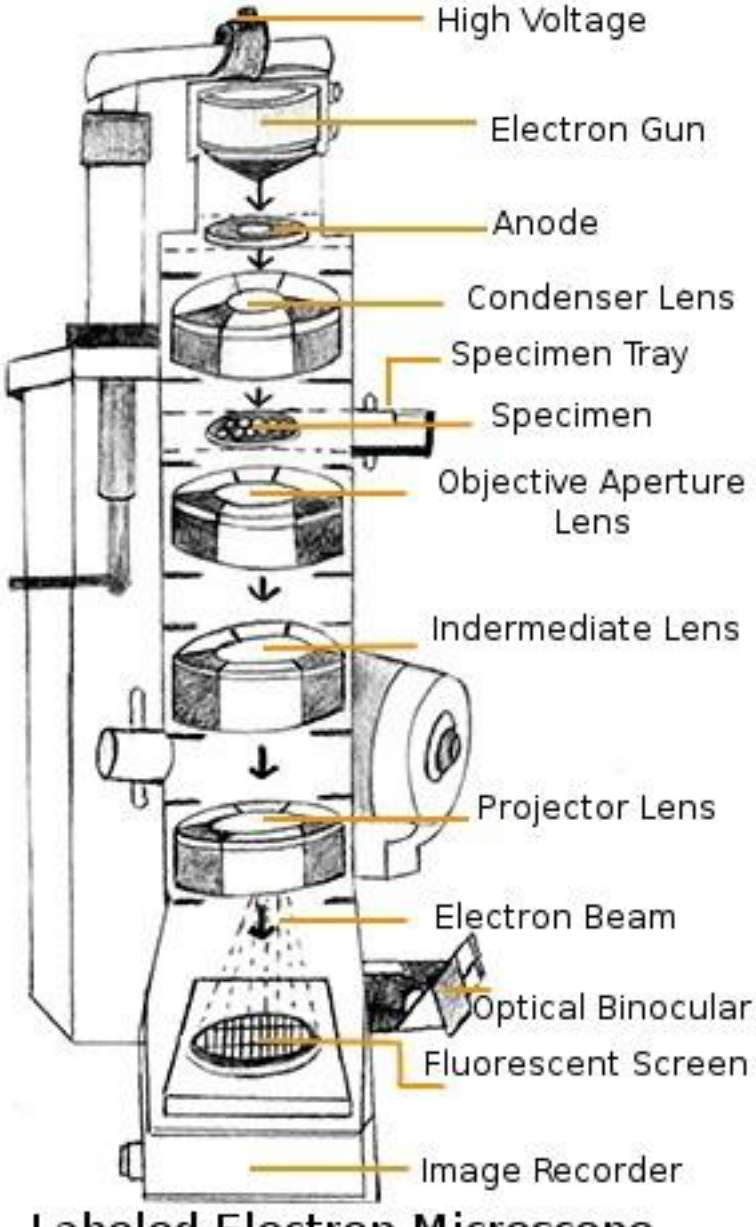
PRACTICAL NO 2

Study of compound microscope (Electron Microscope)

Material:

- Electron microscope

As the name suggests, electron microscopes focus electron beams upon the specimen instead of using light. The use of electron helps in viewing the specimen at an extremely high resolution. The camera present within the microscope captures these images to reveal the finer details of the specimen. This microscope can zoom and view the density of a specimen until it is only a micrometer thick and has a magnification ranging between 1,000 - 250,000X on the fluorescent screen. This type of microscope requires computer software in order to function accurately and yield precise results. To have a better understanding of the function and basic structure of an electron microscope, let us follow the labeled electron microscope diagram that has been provided here.



Labeled Electron Microscope Diagram

Electron Microscope Parts and Functions

Electron Gun:

The electron gun contains the cathode and control shield or grid. The cathode is the negative electrode through which electrons are generated. These electrons travel as cathode rays or electron beams within the electron microscope. The cathode and the control grid are placed above the electron gun, in a separate compartment away from the lenses. This part must be placed accurately for the microscope to generate accurate results.

Anode:

Anode is the second electrode of the electron gun and is shaped like a disk. This disk contains an axial opening for the electron beams to pass through it. A high voltage is made to pass towards the anode, which transmits through it at a constant rate.

Nature of lenses:

Electromagnetic lenses are used in electron microscope.

Condenser Lens:

Condenser lens collects the electron beam, and is responsible for controlling the aperture and focus of the rays. The intensity and diameter of the electron beam is also curtailed and directed through the diaphragm or aperture, onto the specimen and thereafter to the objective lens. The diameter of the beam can be tweaked in order to get the optimal contrast and brightness.

Objective Aperture Lens:

Objective Aperture Lens is placed beneath the specimen tray. The focal length of this lens is short and ranges from 1-5 mm. The objective lens helps to generate a real image of the specimen, which is magnified further by the intermediate lens along with the projector lens. The concentration of beams and its spatial resolution (ability to view details of the specimen) are dependent on the aperture or diameter of the objective lens.

Intermediate Lens:

Intermediate Lens is actually the first projector lens and is placed above the second projector lens. This magnification is further enlarged through the use of the actual projector lens. The projector lens magnifies the image by five times and thus has a 5:1 ratio or range of magnification. The combination of two lenses allows for greater zooming of the image without having to change the actual length of the electron microscope.

Fluorescent Screen:

Fluorescent Screen is a transparent sheet which is coated with a phosphoric substance. The coat is applied on the side of the sheet which is facing towards the rays. When the electron rays hit this sheet, it causes the phosphor to fluoresce or illuminate the image of the specimen. This image is simultaneously captured by the image recording device that is placed beneath the fluorescent screen.

PRACTICAL 3

SAFETY RULES AND REGULATIONS IN LABORATORY OF MOLECULAR BIOLOGY

At the beginning of the work in the laboratory, students have to know laboratory Safety rules and regulations in Laboratory of molecular biology.

- It is forbidden to eat, drink and smoke in the laboratory.
- Students have to wear laboratory coats, long boots and rubber gloves.
- Unauthorized experiments are strictly forbidden
- The laboratory must be kept clean and organized.
- Check the proper installation of the equipment. If there is any problem with equipment, not use it and inform the supervisor.
- Do not work with UV light on.
- If a piece of equipment fails while being used, report it immediately to your laboratory assistant or tutor. Never try to fix the problem yourself because you could harm yourself or the others.
- Clean up your work area before leaving the laboratory.
- Turn off all electric devices before leaving the laboratory.
- Before leaving the laboratory, wash your hands.
- Ask the supervisor if you are in doubt.
- Read labels carefully.
- Never "smell" a solvent directly! Read label on the solvent bottle to identify its contents. Chemicals must never be tasted!
- Check where the laboratory fire extinguisher and wash station are located and how to use them.
- The staff and students are obliged to manipulate with poisonous, volatile and smelly substances exclusively in Fume hood.
- Always give the chemicals and reagents you used back to the place where you had taken them from.
- Special care should be taken while working with open fire, combustibles, corrosives and toxic substances.
- Always inform the teacher about any accident or injury and provide the first aid if necessary.
- The reagent solutions are always casted from the reagent bottle on the unlabeled side to avoid the damage of the label. Illegible inscription and incidental substitution linked with it can cause dangerous consequences.
- Concentrated acids, especially sulphuric acid, are diluted by infusion of acid into the water. Acid is infused in the thin stream to the solution which is mixed up by the glass stick throughout the whole dilution.

- Manipulation with irritating, smelling and toxic substances (i. e. chlorine, chloroform, carbon disulphide, etc.) and easily flammable substances (i. e. gasoline, acetone, etc.) is allowed only in well aired and functional hood.
- Throw toxic and nontoxic waste into the appropriate containers.
- Everybody who work in the laboratory, have to respect all the rules mentioned above

PRACTICAL NO 4

Study of prokaryotic cell through gram staining

Gram Staining of Bacteria:

The Gram staining method is named after the Danish (Denmark) bacteriologist Hans Christian Gram (1853 –1938) who originally devised it in 1882 (but published in 1884), to discriminate between pneumococci and *Klebsiella pneumoniae* bacteria in lung tissue. It is a differential staining method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative) based on the chemical and physical properties of their cell walls. This reaction divides the eubacteria into two fundamental groups according to their stain ability and is one of the basic foundations on which bacterial identification is built.

Material:

Glass Slides
Microscope
Burner
Sample
Dropper
Pipette

Reagents:

1. Crystal violet (primary stain)
2. Iodine solution/Gram's Iodine (mordant that fixes crystal violet to cell wall)
3. Decolorizer (e.g. ethanol)
4. Safranin (secondary stain)
5. Water (preferably in a squirt bottle)

Procedure:

Take a clean glass slide

Take a drop of bacterial sample on the slide (near the edge)

Make a smear of the sample on the slide by just moving another slide

Make sure the smear is feather like that would be the perfectly made

Air dries the smear or heat fixes the smear

Rinse the smear with crystal violet for 30 secs

Gently wash the smear with tap or distilled water to remove excess stain

Rinse the smear with gram iodine for 30 sec

Gently wash the smear with tap or distilled water to remove excess stain

Rinse the smear with decolorizer for 15 sec

Gently wash the smear with tap or distilled water to remove excess stain

Rinse the smear with secondary stain for 30 sec to counter stain the smear

Gently wash the smear with tap or distilled water to remove excess stain

Air dries the smear

Observe it under microscope

Examine the slide under the light microscope. Gram-positive bacteria appear purple as stained by crystal violet, which is trapped within their thick cell walls. Gram-negative bacteria appear pink as stained by the safranin counter-stain, as their thin cell walls allow the crystals violet to wash out during decolonization. Bacteria are further classified by their shape under the microscope, most commonly as cocci (spherical) or rods (cylindrical). The most common bacterial species in each of the four groups thus classified are as follows:

Gram Positive Bacteria:

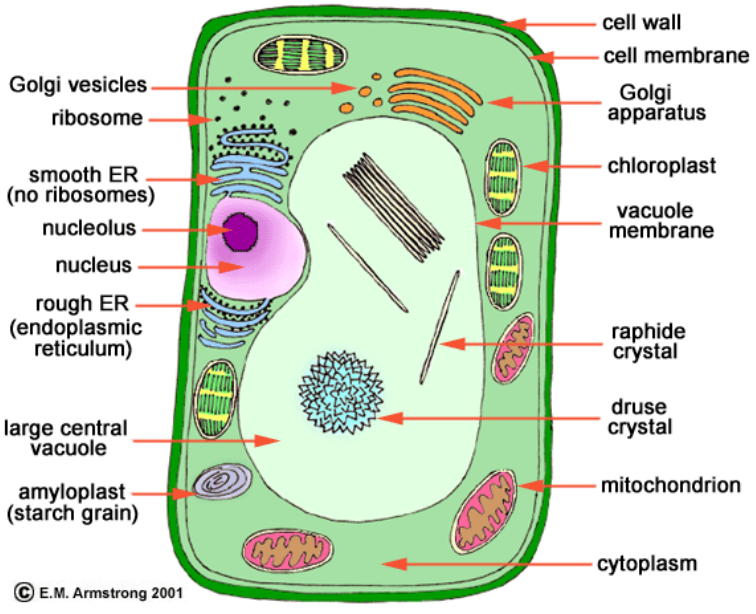
- Gram positive cocci are generally either Staphylococci (meaning cocci in clusters) or Streptococci (meaning cocci in chains).
- Gram positive rods include Bacillus, Clostridium, Corynebacterium, and *Listeria.*

Gram Negative Bacteria:

- Gram negative cocci are most commonly Neisseria spp.
- Gram negative rods are subclassified as follows:
    - Gram negative "coccoid" rods (or coccobacilli) include *Bordetella, Brucella, Haemophilus, Pasteurella.*
    - Other Gram negative rods (not coccobacilli) include *E. coli,Enterobacter, Klebsiella, Citrobacter, Serratia, Proteus, Salmonella,Shigella, Pseudomonas,* and many others.

PRACTICAL NO 5

Study of eukaryotic cells



Plant Cell

Material:

Onion

Blade

Iodine solution

Microscope

Glass slide

Coverslip

Tissue

Procedure:

Take an onion.

Removed transparent skin from inside of onion

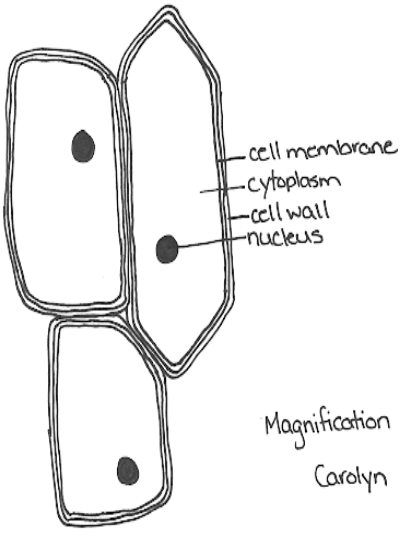
Take small piece of transparent skin & placed it on slide.

Add iodine sol. And place cover slip on the slide.

Used tissue to remove excess stain

Place it on microscope and observe it.

Onion Epithelial Tissue



Animal Cell

Material:

Ice cream stick

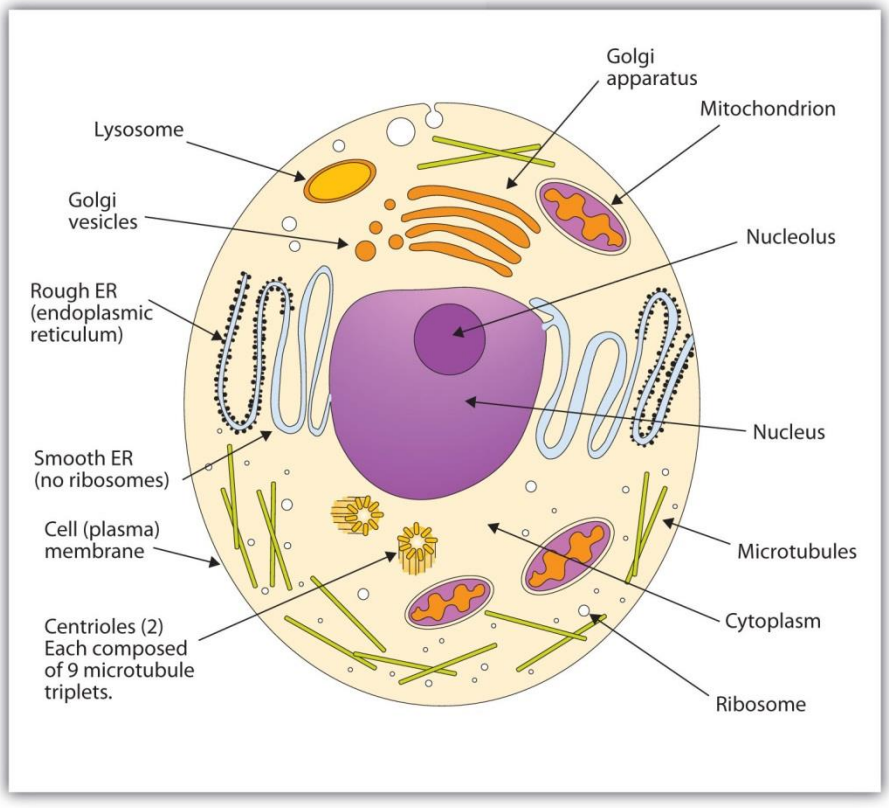
Cheek cell

Methylene Blue

Microscope

Glass slide

Cover slip



Procedure:

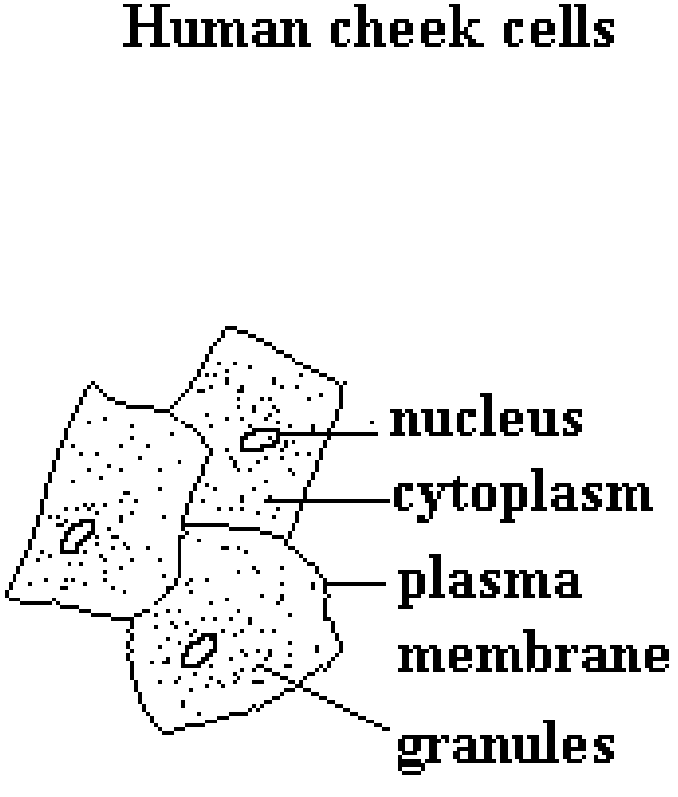
Take clean ice cream stick

Rub it on inner side of your cheek to remove some cheek cell

Place it on slide and add 2 drops of methylene blue and place cover slip on it.

Clean excess stain with tissue and place it on microscope.

Observe it.



Yeast cell

Material:

Yeast Powder

Glucose

Petri dish

Microscope

Glass slide

Distilled Water

Cover slip

Procedure:

Take some water in petri dish

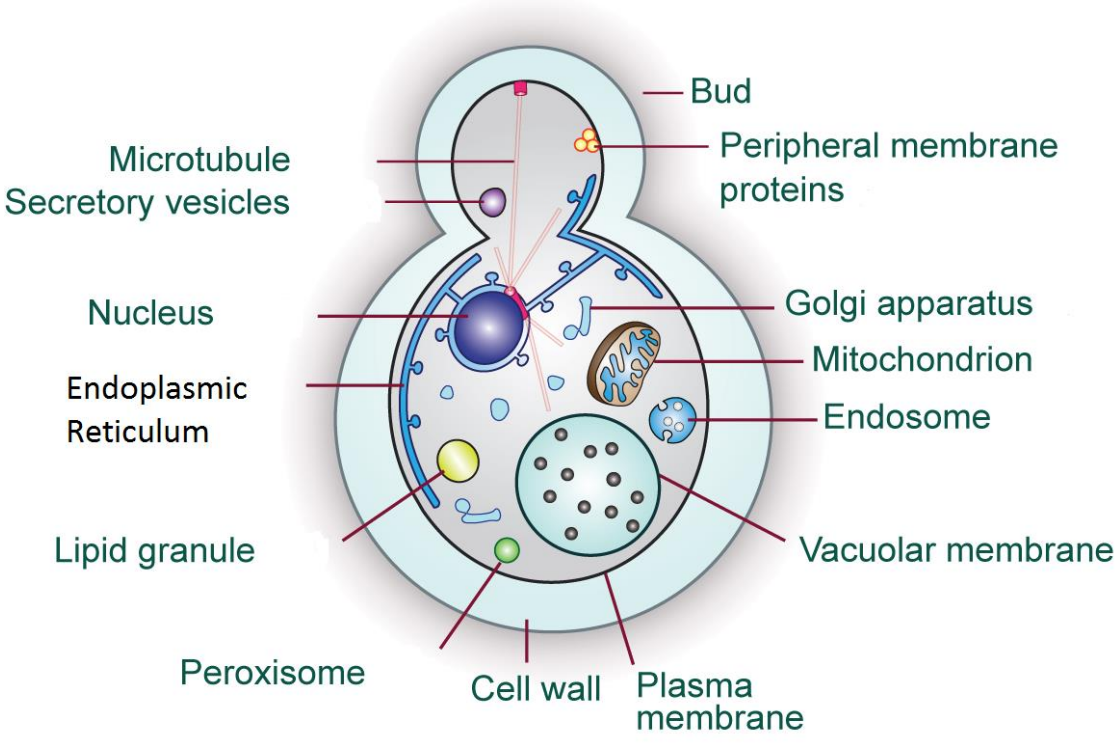
Add some glucose and mix it in water

Pour some yeast powder in petri dish

Place it for 1 hour

Take a drop from petri dish and place it on slide

Put covers slip on it and observe under microscope



PRACTICAL NO 6

Study of human blood cells through smear technique

Aim:

To identify the blood cell types in human blood smear.

Materials:

Microscope

Glass slides

Spirit lamp

Sterilized needle

A drop of blood

Giemsa stain

Distilled water

Procedure:

Preparation of Blood smears:

1. Obtain 2 clean microscope slides, alcohol wipes, and lancet
2. Clean a finger with an alcohol and puncture with lancet
3. Place a small drop of blood at the end of one slide.
4. Use the second slide to make a thin blood film as directed below:

a. Place the second slide at a 30 degree angle and touch the slide with the blood drop

b. Move the spreader slide to touch the blood drop allowing the drop to spread by capillary action along the edge of the slide

c. Immediately pull/push the slide away from the blood drop, making a thin smear that should dry quickly as you move away from the drop.

d. A perfect smear will have a "feathered" edge and separated RBCs when you view it with the microscope.

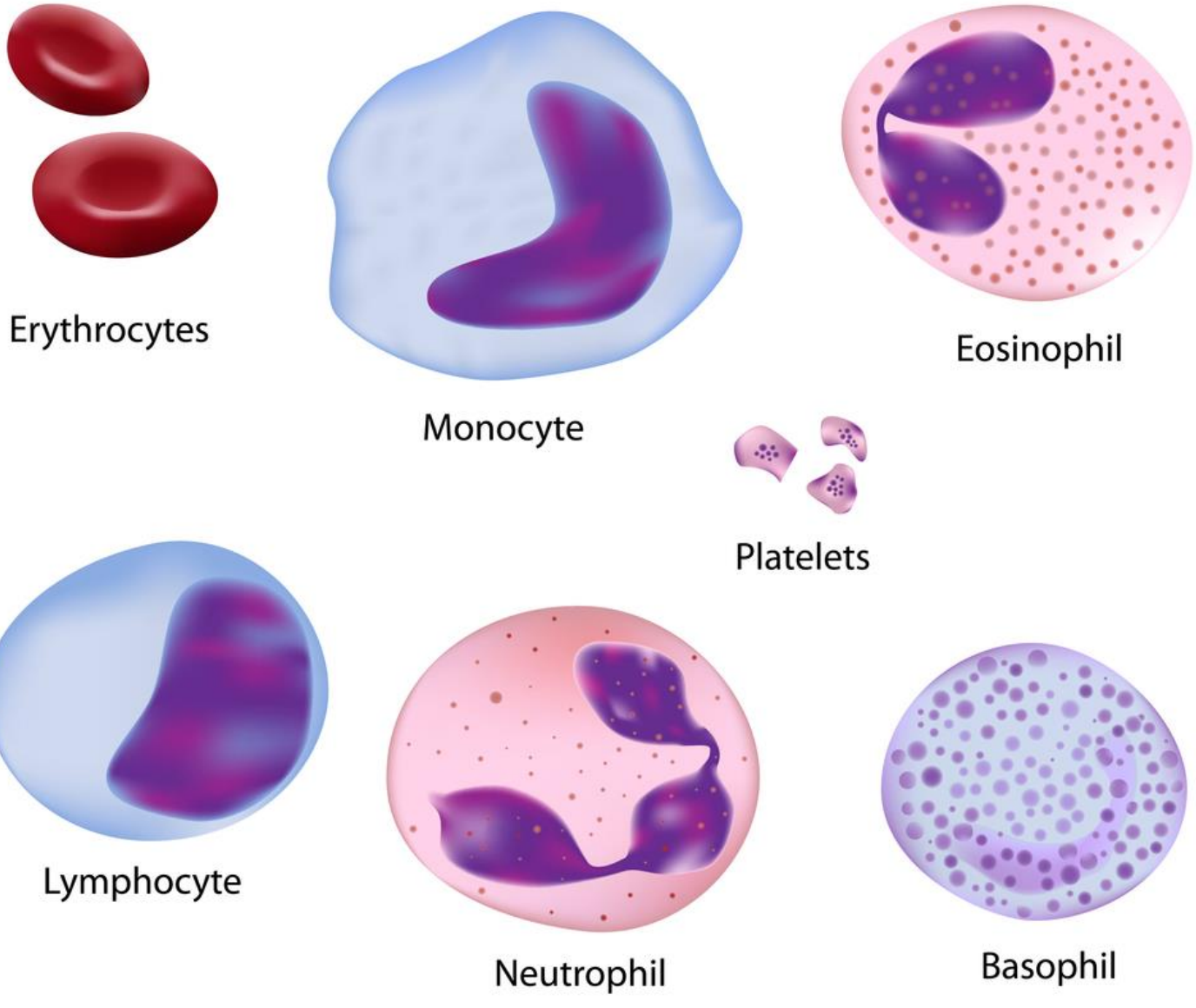
Staining the blood smear (Horizontal staining procedure):

1. Place thoroughly dried smear on horizontal staining rack
2. Flood smear with Fixative for 10 seconds, (fixes cells to slide/prepares cells for dyes) drain
3. Flood smear with Dye for 10 seconds, drain
4. Rinse the smear with distilled water for 1 minute
5. Air dry and examine under the microscope, using low power first, then high power.

6. Observe as many different types of blood cells as possible. Pay close attention to size, frequency, and nuclear features.

Observation:

Different types of blood cells are visible (Neutrophil, Basophil, Eosinophil, Lymphocyte, Erythrocyte, Platelets and Monocytes).



PRACTICAL NO 7

Counting of erythrocytes through improved neuabur haemocytometer

Material:

Microscope

Improved nubaur haemocytometer

Fresh blood

Syringe

Vacutainer

Diluent (RBCs)

Bulb pipette (with red bead)

Cover slip

Ethanol 70%

Spirit

Procedure:

Take improved naubaur haemocytometer and wash it with water.

Disinfect the haemocytometer and coverslip with 70% ethanol.

Put cover slip on haemocytometer.

Clean your arms vein with spirit and get blood with syringe. (If have to save for later work put it in vacutainer otherwise use it from syringe).

Take blood in bulb pipette up to 0.5 mark.

Suck diluent in bulb pipette and make the volume up to 101 mark.

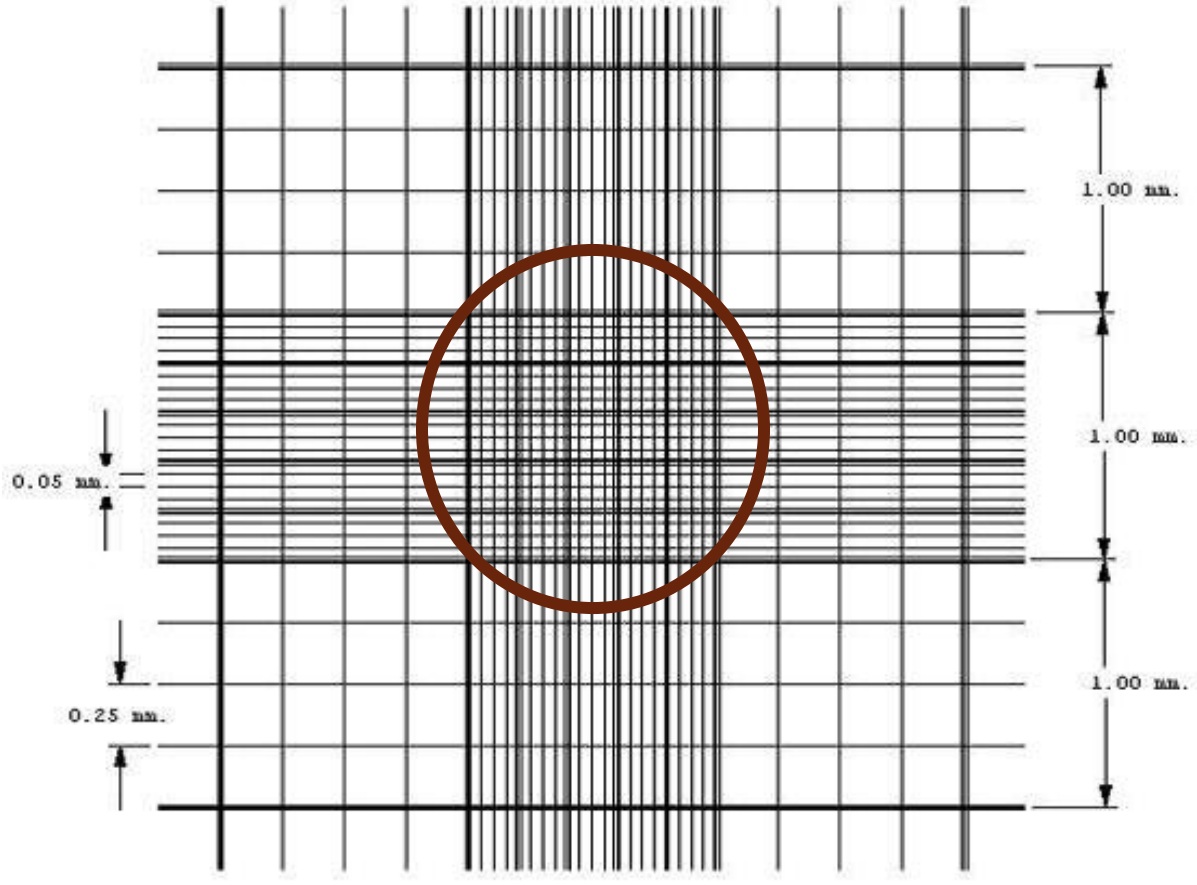
Then rotate gently to mix the blood and diluent for 1-2 mins.

Then discard 1^{st} two drops and put 3^{rd} drop on the haemocyometer by slightly moving the coverslip from haemocyometer and after that properly cover the haemocyometer.

Put it on microscope, observe and count the RBCs from the central box of haemocyometer in this pattern.

Observation:

Count the blood cells in central box of the haemocytometer



Viability

1. The trypan blue is used to stain any dead cells. Cells looking faint or dark blue within the grid being counted are counted as dead cells. To check the viability of the cells requires:

Live cell count (not including trypan blue cells)

Total cell count including those stained with trypan blue.

Live cell count

Total cell count = percentage viability

Example:

45 x 104 / ml

46 x 104 / ml = percentage viability = 97.8% viability

PRACTICAL NO 9

Counting of leukocytes through improved neubaur haemocytometer

Material:

Microscope

Improved nubaur haemocytometer

Fresh blood

Syringe

Vacutainer

Diluent (WBCs)

Bulb pipette (with red bead)

Cover slip

Ethanol 70%

Spirit

Procedure:

Take improved naubaur haemocytometer and wash it with water.

Disinfect the haemocytometer and coverslip with 70% ethanol.

Put cover slip on haemocytometer.

Clean your arms vein with spirit and get blood with syringe. (If have to save for later work put it in vacutainer otherwise use it from syringe).

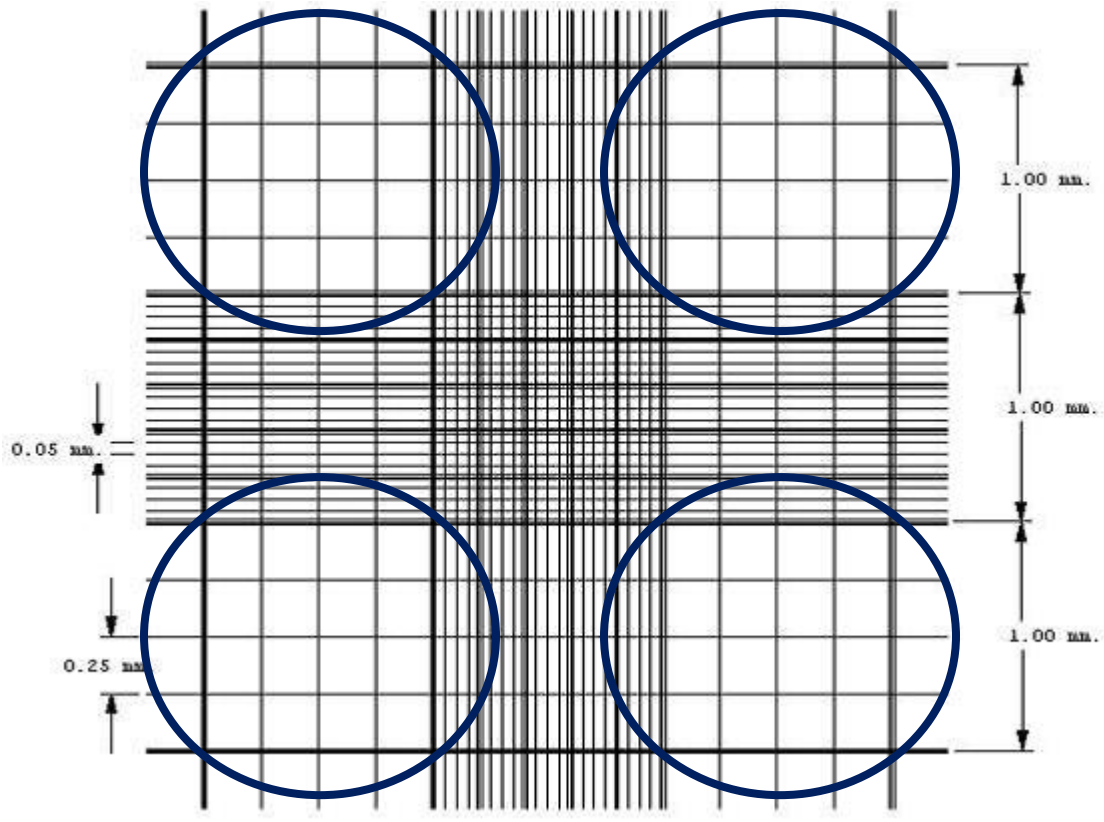
Take blood in bulb pipette upto 0.5 mark.

Suck diluent in bulb pipette and make the volume upto 11 mark.

Then rotate gently to mix the blood and diluent for 1-2 mins.

Then discard 1^{st} two drops and put 3^{rd} drop on the haemocyometer by slightly moving the coverslip from haemocyometer and after that properly cover the haemocyometer.

Put it on microscope, observe and count the WBCs from the central box of haemocyometer in this pattern.



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Live cell count (not including trypan blue cells)

Total cell count including those stained with trypan blue.

Live cell count

Total cell count = percentage viability

Example:

45 x 104 / ml

46 x 104 / ml = percentage viability = 97.8% viability

PRACTICAL NO 9

EXTRACTION AND QUANTITATIVE ESTIMATION OF CHROMOSOMAL DNA

Material:

Blood Sample

EDTA

Labeled container

Falcon tube

Freezer (-20 ^{0}C)

Lysis Buffer

Centrifugation machine

70% ethanol

NaCl

SDS 10%

Protenase K

HCl

Isopropanol

Incubator

Digital pH meter

SAMPLING:

Took a labeled container already had EDTA.

Took 3-5ml blood sample and poured it into the falcon tube adds EDTA. (3-5ml blood; 25ml EDTA)

Mix EDTA and blood gently so blood didn't clot.

After mixing put the falcon tube in freezer at -20 ^{0}C for 1-2 hours

Then added lysis buffer in falcon tube

Lysis buffer: tris HCL + EDTA

14ml 10ml + 4ml

CENTRIFUGATION:

Centrifuged the falcon tube at 3500 RPM at 4 ^{0}C for 15 mins

If RBCs found after centrifugation then repeated the centrifugation again and again by adding buffer

DIGESTION:

Digested the cell membrance

For lipids used 10% SDS

For proteins used protenase K.

The buffer A_1 is used to maintain the pH of the enzyme.

Buffer A_1 ⟶ tris HCL + NACL + EDTA

SDS 10% ⟶ 10µl

Protenase K ⟶ 45-50µl

Incubate it at 45 ^{0}C for overnight**.**

PRECIPITATION:

Added 1ml NaCl in it and shake the tube and freeze it at -20 ^{0}C for 10 mins.

CENTRIFUGATION:

Centrifuged the sample again at 4000 RPM at 4 ^{0}C for 15 mins

After this step a pellet is appeared in the form of a thread like structure.

DNA PRECIPATATION:

Now add the equal amount of isopropanol to supernatant that caused DNA-precipitation.

Again centrifuged it at 4000 RPM at 4 ^{0}C for 15 mins

The thread like structure of DNA then adhered with the wall of falcon tube.

WASHING OF DNA:

3ml of 70% ethanol is used in washing. Shaked it well for 1-2 hours.

Centrifuged at 4000 RPM at 4 ^{0}C for 5 mins and air dried it.

Till the smell of ethanol was gone

PRACTICAL NO 6

AGAROSE-GEL-ELECTROPHORESIS

Agarose os a component of agar which is a mixture of polysaccharides obtained from certain sea weeds.

SIGNIFICANCE:

Agarose gel electrophoresis is a technique to separate DNA fragments by their size. It is a common diagnostic procedure used in molecular biology labs.

COMPOSITION:

Mostly agarose gel is made in the range of 0.7 % - 02%.

0.7% gel: show good separation of large DNA fragments.

02% gel: show good separation of small DNA fragments**.**

FORMATION:

Gel is formed by suspending dry agaraose in aq.buffer.

The mixture is loaded then; until a fine solution is formed.

This solution is then poured into a glass tube/ tray. Plastic combs are placed into the gel as to form wells for loading the samples.

The gel is then cooled at room temperature. After it is kept in electrophoresis tank which is filled with buffer so that the gel submerged

BUFFER OF CHOICE:

For carrying out gel electrophoresis of DNA numbers of tris buffers are available.

The most commolnly buffered used are;

Tris acetate and EDTA (TAE)

Tris borate and EDTA (TBE)

MIXING OF BUFFER:

The samples of DNA that has to be separated did not directly load into the wells of agraose gel.

The samples firstly mixed with gel loading dye prior to loading.

DYES:

Bromophenol blue

Glycerol

It increased the density of the sample making the DNA to sink evenly into the wells of agarose gel.

Added the colour to the samples by simplifying the loading process

Also help to visualize the movement of DNA fragements during electrophoresis.

PRACTICAL NO 10

EXTRACTION AND QUANTITATIVE ESTIMATION OF CHROMOSOMAL RNA

Material:

Blood Sample

EDTA

Labeled container

Falcon tube

Freezer (-20 ^{0}C)

Lysis Buffer

Centrifugation machine

70% ethanol

NaCl

SDS 10%

Protenase K

HCl

Isopropanol

Incubator

Digital pH meter

SAMPLING:

Took a labeled container already had EDTA.

Took 3-5ml blood sample and poured it into the falcon tube adds EDTA. (3-5ml blood; 25ml EDTA)

Mix EDTA and blood gently so blood didn't clot.

After mixing put the falcon tube in freezer at -20 ^{0}C for 1-2 hours

Then added lysis buffer in falcon tube

Lysis buffer: tris HCL + EDTA

14ml 10ml + 4ml

CENTRIFUGATION:

Centrifuged the falcon tube at 3500 RPM at 4 ^{0}C for 15 mins

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DIGESTION:

Digested the cell membrance

For lipids used 10% SDS

For proteins used protenase K.

The buffer A_1 is used to maintain the pH of the enzyme.

Buffer A_1 $\longrightarrow$ tris HCL + NACL + EDTA

SDS 10% $\longrightarrow$ 10µl

Protenase K $\longrightarrow$ 45-50µl

Incubate it at 45 ^{0}C for overnight**.**

PRECIPITATION:

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CENTRIFUGATION:

Centrifuged the sample again at 4000 RPM at 4 ^{0}C for 15 mins

After this step a pellet is appeared in the form of a thread like structure.

RNA PRECIPATATION:

Now add the equal amount of isopropanol to supernatant that caused RNA-precipitation.

Again centrifuged it at 4000 RPM at 4 ^{0}C for 15 mins

The thread like structure of RNA then adhered with the wall of falcon tube.

WASHING OF RNA:

3ml of 70% ethanol is used in washing. Shaked it well for 1-2 hours.

Centrifuged at 4000 RPM at 4 ^{0}C for 5 mins and air dried it.

Till the smell of ethanol was gone

PRACTICAL NO 11

PCR (POLYMERASED CHAIN REACTION)

What is PCR?

The **Polymerase Chain Reaction (PCR)** is a technique that is used widely in molecular biology.

The process is used to amplify a single sequence of RNA into many more identical copies, PCR can produce millions of copies from one **RNA template** strand in a couple of hours.

The name PCR is derived from a key component used in the process, **RNA polymerase**, which can be used as it can work at high temperatures which would denature many other enzymes.

The first process in PCR involves heating to a high temperature resulting in the RNA becoming **denatured**; this causes the doubled stranded molecule to separate giving 2 single strands which act as templates from which copies can be made.

The second stage requires the use of oligonucleotides called **RNA primers,** which are simply short sections of single stranded RNA. The primers will find the specific nucleotide complimentary sequence on both template strands and **anneal** to them giving a starting point from which copies can be made.

The final stage of PCR is where the short primers have RNA building blocks called nucleotides added to them resulting in the **extension** of the RNA strand. This is where the RNA polymerase does it job by adding the nucleotides according to the sequence of the complimentary strand, it starts at the point on the strand where the primers have annealed in the previous step and continues to work its way along the template. This process occurs to both single template strands simultaneously so from one section of original RNA 2 identical copies will be made.

The 3 stages are referred to as a **cycle.** Each cycle will double the number of RNA template strands. These results in 2 copies of RNA being produced after the 1st cycle, 4 copies produced in the 2nd cycle, 8 copies in the 3rd cycle, etc. so by the time the 30th cycle has completed there will be 1,073,741,824 identical copies of RNA! PCR can be applied to many forensic and medical techniques used widely today.

PRINCIPLE:

Similar to DNA replication

CHEMICALS:

Taq.polyemerase

Buffer

DNA

$MgCl_2$

Nucleotides (dNTPs)

H_2O

Primer forward

Primer reverse

AMOUNT AND SEQUENCE OF INGERDIANTS:

H_2O	16µl
Buffer	2 µl
$MgCl_2$	2.5 µl
dNTPs	2.5 µl
DNA	2 µl
Primer reverse	1 µl
Primer forward	1 µl

Taq.polymerased for proof reading

CYCLE NO. 1- 4:

CYCLE NO. 1:

Lid heat will be 94 ^{0}C for 4 mins.

1st cycle is called as initial denaturation.

CYCLE NO. 2:

94 ^{0}C for 30 sec

52 ^{0}C for 45 sec

72 ^{0}C for 45 sec

The cycle is called as cyclic denaturation. 52 ^{0}C is known as the annealing temperature.

Cycle no. 2 is repeated for 35 times to produce multiple copies of desired DNA.

CYCLE NO. 3:

72 ^{0}C for 10 mins

This step is known as extension. Cycle no. 3 filled the gaps between nucleotides.

CYCLE NO. 4:

4 ^{0}C for ∞ time

Every single cycle make two copies each time. Then polymerase added for proof reading.

PRACTICAL NO 13

CELLULAR REPRODUCTION

STUDY OF MITOSIS IN ONION ROOT TIP

All new cells come from previously existing cells. New cells are formed by the process of cell division which involves both replication of the cell's nucleus (karyokinesis) and division of the cytoplasm (cytokinesis) to form two genetically identical daughter cells. There are two types of nuclear division: mitosis and meiosis. Mitosis typically results in new somatic (body) cells. Formation of an adult organism from a fertilized egg, asexual reproduction, regeneration, and maintenance or repair of body parts is accomplished through mitotic cell division. Meiosis, on the other hand, results in the formation of either gametes (in animals) or spores (in plants). These cells have half the chromosome number of the parent cell.

Materials

- Microscope
- Blade
- Slides with cover sip
- Onion Root Tips
- 1M HCL
- Forceps
- Aceto Orcein Stain

Procedure:

Preparing an onion root tip squash

It is possible for you to make your own stained preparations of onion root tips and observe mitotic figures. Onion bulbs have been rooted in water. Growth of new roots is due to the production and elongation of new cells. Mitotic divisions are usually confined to the cells near the tip of the root. Follow the procedure outlined below to make your own root tip preparation.

- Obtain an onion bulb that has been rooted in water. Cut 2 or 3 roots off near the base of the bulb.
- Then, cut off the bottom 1 or 2 mm of the root tip and place it in a petri plate. With a Pasteur pipette, add a small puddle of 1M HCl and let root tip stand for 4 min.
- Remove the root tip from the HCl with forceps and place on a slide.

- Wash the tips with tap water to remove acid and place it on clean slide
- Add 2-3 drops of aceto orcein stain
- Give longitudinal cut with sharp razor blade
- Place the slide, cover slip down on a paper towel. Using a pencil eraser, carefully apply pressure to the cover slip area in order to squash and spread the root tip tissue.
- Mount the slide on your microscope.
- Use the low power objective on your microscope to look for thin layers of cells and then use the 40X power objective to observe mitotic stages in individual cells.
- Identify chromosomes at the various stages of mitosis.
- Compare your slide to the prepared slides that contain stained sections of root tips.
- Make sketches of the mitotic stages observed.

Observation

The phases of plant mitosis are:

Interphase: The non-dividing cell is in a stage called interphase. The nucleus may have one or more dark-stained nucleoli and is filled with a fine network of threads, the chromatin. Interphase is essential to cell division because the genetic material (DNA) is duplicated (replicated) during this stage.

Prophase: The first sign of a division is prophase, in which a thickening of the chromatin threads occurs. Thickening continues until it is evident that the chromatin has condensed into chromosomes. With somewhat higher magnification you may be able to see that each chromosome is composed of two chromatids. As prophase continues, the chromatids continue to shorten and thicken. In late prophase the nuclear envelope and nucleoli are no longer visible, and the chromosomes are free in the cytoplasm. Just before this time the first sign of a spindle appears in the cytoplasm; the spindle apparatus is made up of microtubules, and it is thought that these microtubule may pull the chromosomes toward the poles of the cell where the two daughter nuclei will eventually form. It appears that centrioles are basal bodies that give rise to flagella and cilia in animals and lower plants such as mosses and ferns. Centrioles are not found in non-flagellated “higher” plants such as angiosperms.

Metaphase: At metaphase, the chromosomes have moved to the center of the spindle. One particular portion of each chromosome, the centromere, attached to the spindle. The centromeres of all the chromosomes lie at about the same level of the spindle, on an imaginary plane called

the metaphase plate. At metaphase you should be able to observe the two chromatids of some chromosomes.

Anaphase: At the beginning of anaphase, the centromere regions of each pair of chromatids separate and are moved by the spindle fibers toward opposite poles of the spindle, dragging the rest of the chromatid behind them. Once the two chromatids separate, each is called a chromosome. The daughter chromosomes continue pole ward movement until they form two compact clumps one at each spindle pole.

Telophase: Telophase, the last stage of division, is marked by a pronounced condensation of the chromosomes, followed by the formation of a new nuclear envelope around each group of chromosomes. The chromosomes gradually uncoil to form the fine chromatin network of interphase, and the nucleoli and nuclear envelope reappear. The cell develops into two new cells. In plants, a new cell wall is laid down between the daughter cells. In animal cells, the old cell will pinch off in the middle to form two new daughter cells. This division of the cytoplasm, in contrast to nuclear division (mitosis), is called cytokinesis.