Semester – I

19BTP112 CELL BIOLOGY AND MOLECULAR GENETICS - PRACTICAL II 4H-2C

Instruction Hours / week: L: 4 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**
End Semester Exam: 3 Hours

Course Objectives:

- To develop practical skills such as identification of cell types, cellular component and cell division, etc.
- Genetic laboratory course is to introduce the students to learn about prokaryotic and eukaryotic genetic system using modern techniques.

Course Outcomes (CO's):

1. It will provide an understanding of the unique features of plant cells and animal cell.
2. Gain understanding on the interaction between cells and the environment
3. This curse will provide the mechanics of experimentation methods of genetics.

CELL BIOLOGY

1. Identification of cell types- Microbe/plant /Human
2. Fractionation of cellular component – Nuclear Components, Mitochondria, Chloroplast.
3. Sucrose Fractionation of Castor Bean
4. Lipid Solubility of Membranes
5. Cell permeability – Plasmolysis - RBC/plant cells.
6. Cell division (Mitosis/Meiosis)

MOLECULAR GENETICS

1. Drosophila Giant Chromosome preparation.
2. Nuclear staining (Giemsa / acridine orange /feulgen)
3. Metaphase preparation and karyotyping (Human leucocytes/ onion root tip)
4. Conjugation
5. Transduction

SUGGESTED READINGS

1. Cappuccino, P., & Sherman, D. (2004). *Microbiology-A Lab Manual*. (7th ed.) Singapore: Pearson Education.
2. Dubey, R., & Maheswari, E. (2004). *Practical Microbiology*. New Delhi: S. Chand & Co.
3. Goldman, E., & Green, L.H. (2008). *Practical Handbook of Microbiology*. (2nd ed.). London: CRC press.
4. Kannan, P. (2002). *Laboratory Manual in General Microbiology*. (1st ed.) Tamilnadu: Palani Paramount Publishers.

Cell Biology and Molecular Genetics – Practical II

(Course code: 19BTP112)

Practical Manual

1. IDENTIFICATION OF CELL TYPES

IDENTIFICATION OF MICROBIAL CELL

The microbe cells were observed by different staining techniques like simple staining, gram‟s staining, fungal staining, endoscope staining.

IDENTIFICATION OF PLANT CELL TRANSVERSE SECTION OFF DICOT

From the study of the transverse section of the dicotyledons stem use will identified the following 3 regions of tissues epidermis vascular bundle or slab.

EPIDERMIS

It consist of a single layer of living cells which are closely packed. The walls are thickened and covered with a water proof layer called cuticles.

CORTEX

It is situated to the inside of the epidermis. This region compresses the collenchyma, parenchyma and endodermis.

COLLENCHYMA

These cells live under the epidermis constitute 3-4 layers of cells with the cell wall thickened at corners.

PARENCHYMA

Beneath the collenchyma cells are few layers of their cell walls, parenchyma cells with the intracellular space. The parenchyma cells make up the cortex.

ENDODERMIS (OR) STARCH SHEATH

It forms the innermost layer of the cortex. This is a single layer of lightly packed rectangular cells bordening the step of the cortex.

VASCULAR CYLINDER (OR) STEAL

The region comprises the pericycle, vascular bundle and pitch (medulla).

PERICYCLE

It is made of schlerenchyma cells which are lignified dead fibre cells. These cells have thick woody walls and tappening ends .it provides protection for the vascular bundle.

VASCULAR BUNDLES

They are situated in a ringed on the inside of the pericycle of the plant. This distinct tiny of vascular bundle is distinguished characteristics of dicotyledon stems.

A mature vascular bundles consists of 3 main tissues xylem, phloem and cambium. The phloem is located invert the outside of the bundle and the xylem towards the center of the cambium separate the xylem and phloem which belong about the 20 thickening.

PITCH (MEDULLA)

It occupy the large central part of the stem. It consist of thin walled parenchyma cells with intercellular air space.

TRANSVERSE SECTION OF MONOCOT ROOT EPIDERMIS

It covers the entire root except the root tip. It consist of concentric layer of tissue

HYPODERMIS

In many plant species especially these are from acid areas, the hypodermis is the outermost layer of cells of the cortex which have subrine enriched cell wall. These cells are only fully differentiated above the region of the root which is covered with root hairs. This layer is important in preventing the loss of water and nutrient which have been observed by the region of root hairs lower down the root.

STORAGE PARENCHYMA CELLS

The largest part of cortex consists of thin walled parenchyma cells which often have large intercellular space and contain starch.

ENDODERMIS

It is the layer of cells which surrounds steal. The endodermis cells are very highly packed with no intercellular air space.

STEAL

Monocotyledons roots have more groups of primary xylem and phloem arranged on a ring around a central part of parenchyma pitch core of nerve fibres the remaining are branched and known as dentrites.

The nerve cells (or) neuron has a cell body or cyton with a conspicuous nucleus. The cell body contains besides the usual mitochondria and the golgi complex.

Granular structure called lignid granules which supply energy for the activity of the cells.

2. FRACTIONATION OF CELLULAR COMPONENTS

AIM

To practice cell fractionation and isolate chloroplast from plant cells.

PRINCIPLE

The green colour of the leaves and sometimes the stem of plants is due to the presence of the green pigments chlorophyll a,b in the cytoplasm of the cell , unattached to other cellular components such as walls , membranes, nucleus and the mitochondria . when the cell wall is disrupted the cell membrane breaks and the sub cellular components are released as separate particles of various size and densities.

MATERIALS REQUIRED

- Freshly collected green leaves.
- Sterile & pre-chilled mortar and pestle.
- Ice cold 0.5m sucrose solution .
- Clean and sterile blades.
- Phosphate buffer
- Microscope

PREPARATION OF BUFFER

24g of sodium hydrogen phosphate was dissolved in 100ml of sterile distilled water to prepare 0.2m of monobasic salt.

Similarly 2.8g of sodium dihydrogen phosphate was dissolved in 100ml of sterile distilled water to prepare 0.2m dibasic salt. 89ml of monobasic salt was mixed with 60ml of dibasic salt and the volume was made to 200ml.

PROCEDURE

- Obtained 2g of deveined leaf tissue rinsed in ice water, blotted and cut into pieces about 1cm square.
- Place the leaf pieces in a pre-chilled blends cap containing 40ml of ice cold 0.5m sucrose blend for 15sec at top speed, pause about 10 sec, then blend again for 10 seconds.

- Remove the ice from the 100 ml beaker and then square the leaf homogenate through 4 layers of pre-chilled cheese cloth into the cold beaker by twisting the top corners of the cloth around the each other.
- Pour 15 ml of the homogenate into each of two centrifuge tubes and centrifuge at 2000rpm for 5mins.
- Using a Pasteur pipette transfer each supernatant containing the chloroplasts to a second centrifuge tube and centrifuge at 1000rpm for 7mins.
- Using the pipette, discard the supernatant but be careful not to disturb the pellet, pour 2ml of phosphate buffer onto the pellet and gently re-suspend it by moving it up and down in the pipette.
- Using a clean Pasteur pipette and buffer until you have a total volume of 8ml and mix the diluted suspension using the pipette.
- This is your chloroplast suspension, you should examine in the microscope.

3. SUCROSE FRACTIONATION OF CASTOR BBEAN

INTRODUCTION

Fractionation procedure is employed by cell biologists. Each organelle has characteristics (size, shape, density for examples) which make it different from other organelles within the same cells. If the cell is broke open in a gentle manner, each of the organelles can be subsequently isolated. The process of breaking open cells is homogenization and isolating the organelles requires those techniques can range from the use of simple sieves gravity sedimentation or differential precipitation to ultracentrifugation of fluorescent labeled organelles in computer generated density gradients.

MATERIALS REQUIRED

- Castor beans
- Gradient former sucrose solution in (0.01m, EDTA, Ph 7.5, 38%, 44%, 50%, 57%, 60% (w/v)).
- Grinding medium
    - 0.4m sucrose
    - 0.165m Tris-HCL buffer
    - 0.01m Kcl
    - 0.01mgcl2
    - 0.01m EDTA adjusted to Ph 7.5
    - 0.01m dithiotherital
- Chopping board & knife or razor blade.
- Motor & pestle
- Cheese cloth
- Refrigerated centrifuge (ultracentrifuge prepared) with swinging bucket rotor.
- Spectrophotometer & tubes
- Haemocytometer & phase contrast microscope.

PROCEDURE

- Germinate castor bean by soaking overnight, followed by germination in moist vermiculture or paper towels at 30˚C, castor beans are poisonous and fatal if taken internally. Wear gloves while handling.
- After approximately 5 days, remove embryos and cotyledons and discard. Wash the endosperm in distilled water and chill it.
- Prepare two centrifuge tubes for sucrose gradient, one for a linear 23-60% gradient and the other for a stepped gradient. The volume of each solution will depend on the centrifuge rotors to be used and its corresponding centrifuge tube capacity. The following are directions for the 35ml centrifuge tubes.

STEPPED GRADIENT

- Carefully layer, in order, the following solutions into a centrifuge tube.
- 3ml of 60% sucrose
- 6ml of 57% sucrose
- 9ml of 50% sucrose
- 9ml of 44% sucrose
- 3ml of 53% sucrose

LINEAR GRADIENT

- Add 5ml of 60% sucrose to the bottom of a centrifuge tube and form a linear 60-38% sucrose gradient on top of that.
- Combine 60g of endosperm tissue with 90ml of grinding medium and shake vigorously.
- Transfer the coarse material to a fire motor and pestle and continue to grind until a fine paste is formed.
- Filter through a layers of cheese cloth. Collect the fluid into a beaker, transfer it to a centrifuge tube and centrifuge the filtrate for 10mins at 27˚C to remove unbroken cells and large debris.
- Decant the supernatant into a clean, cold centrifuge tube and re-centrifuge for 30mins at 10,800rpm. Resuspend the pellet in 5ml of grinding medium and held on ice for further analysis.

- Carefully decant the supernatant and same for subsequent analysis. Gently resuspend the pellet in 6ml of grinding medium.
- Carefully layer 2ml of the pellet onto a stepped sucrose gradient and 2ml of the pellet onto a linear gradient.
- Team up with another lab section and carefully balance your corresponding tubes. That is be sure that the stepped gradients for both sections are exactly the same weight. Add a grinding media to balance where approximate have the instruction, check the balance before proceeding.
- Centrifuge tubes at 4˚C for 4 hours at 25,000rpm. Upon completion of the centrifugation, fractionate the samples to collect samples 15ml this yields a volume suitable for subsequent spectrophotometric analysis.
- This is accomplished most rapidly by gently inserting a long 10 gauge needle, with the tip, ground of, into the centrifuge tube moving the needle (so as not to stir the contents) attach 2.0ml syringe and pullout the bottom 1.5ml of the sample. Remove the syringe and place the 15ml fraction into a test tube marked it as 1. Re-attach the syringe without distributing the gradient and repeat as need to totally fractionate the gradient.
- Alternatively the gradient can be fractionated by prematuring the bottom of the tubes with a needle and collecting the fraction in 1.5ml portions by counting the approximate number of drops that from the premature tube.
- Read the O.D (absorbance) for each fraction on spectrophotometer at 540nm plot a double graph with fraction number on the x-axis (bottom to top) vs O.D 540nm on the y-axis and fraction number vs % sucrose on the y-axis.
- Carefully examine the fraction with a phase contrast microscope. Identify and count using a heamocytometer where appropriate all structures found in each fraction.

4. LIPID SOLUBILITY OF MEMBRANES

INTRODUCTION

Cell membranes are very important structure to cells because they function as a barrier between the components of the cell and the outside environment. The cell membrane is not only responsible for creating a wall between inside and outside the cell, it must also act as there should through which select molecules can enter and exit the cell when necessary. The cell membrane is what defines the cell and keeps its components separate from the outside cells or organism.

The cell membrane is composed of a double layer of that cells called lipid bilayer presents the free passage of must molecules into and out of the cell. We will begin our discussion of the structure of the cell membrane by discussing the structure and properties of the lipid bilayer, we will then go on to discuss the role of the membrane bound proteins and finally will discuss membrane transport structures.

MATERIALS REQUIRED

- Fresh beats
- Solutions of the following alcohol
- 22 ml methanol
- 0.5 ml ethanol
- 30 ml n-propanol
- 1.1 ml n-butanol
- 0.38 ml amyl alcohol (optional)
- Razor blade
- Depression slides
- Stop watch
- Microscope

PROCEDURE

- Beet cells contain a high concentration of the red pigment anthocyanin. When exposed to a compound which dissolves the cell membranes. The anthocyanin will leak out of the cells and causes a red colored occur in the surrounding media. Cut thin slices of a beet so

that they can be placed on a microscope depression slide and viewed with the lowest power (40x).

- While watching the edge of the sliced beet and n0.1ml of each of the above alcohols to the slides until the best section is submerged. Be careful not to allow the alcohol to flow off the slide. Isoamyl alcohol has a strong, obnoxious odor and as the fumes are somewhat irritating, adequate ventilation is required.
- Immediately begin to time the dissolution of the best cell membranes. Mark the time when a red colour is first observed in the surrounding alcohol solution.
- Repeat the entire series for the ½ and ¼ dilutions of each of the alcohol.
- For each dilution of each alcohol, calculate a penetration co-efficient by dividing the time of the pigment appearance by the molar concentration of the alcohol, plot this penetration co-efficient against the relative miscibility of the alcohol (known as the partition or distribution co-efficient).

5. CELL PERMEABILITY

AIM

To demonstrate the biological phenomenon observed in plant cells called "plasmolysis".

INTRODUCTION

Plasmolysis occurs when a plant cell membrane shrinks array from the cell wall. It happens when the cell losses water by osmosis from an area of high water potential in the cell to an area of low water potential outside the cell through the cell membrane. The cell loses target and the plant becomes wilter.

Cell to cell communication can occur through small pores in the cell wall called Plasmodesmata. The cytoplasm of one cell is continuous with the cytoplasm of a adjacent cell through these small pores. As the cytoplasm shrinks array from the cell walls during plasmolysis, the last point of the attachment is the plasmaderma for this reason different may be visible as each cell becomes plasmolysed.

MATERIALS REQUIRED

- 0.2, 0.4, 0.6, 0.8 and 1.0 ml solutions of sucrose.
- One piece of fleshy scale from onion submerged in distilled water.
- Dropper
- Scaled blade
- Cover slip
- Microscope

PROCEDURE

- Remove the piece of onion from the distilled water.
- Make small cuts on the inner concave surface on the onion scale to give at least 6 squares of about 5 x 5mm.
- Using the tip of the scalpel blade carefully peeled off the first 5mm x 5mm square of thin epidermal tissue and mounted it on a clean microscope slide in distilled water.
- Labeled the slide appropriately with a marker pen.
- Repeated the procedure with the other squares of epidermal tissue using a different concentration of sucrose (0.2, 0.4, 0.6, 0.8 and 1 ml) solution with each slide.

6. CELL DIVISION

Aim:

To prepare a slide of onion root tip and identify the different mitotic stages.

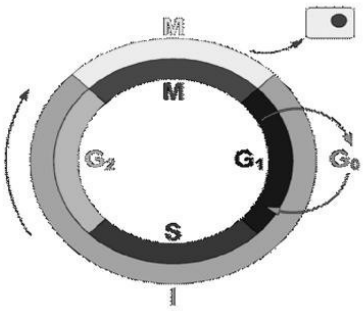
INTRODUCTION:

The cell cycle:

The cell cycle, or cell-division cycle, is the series of events that take place in a eukaryotic cell between its formation and the moment it replicates itself. These events can be divided in two main parts: interphase (*in between divisions* phase grouping G_1 phase, S phase, G_2 phase), during which the cell is forming and carries on with its normal metabolic functions; the mitotic phase (M mitosis), during which the cell is replicating itself (Figure 7). M phase is itself composed of two tightly coupled processes: mitosis, in which the cell's chromosomes are divided between the two daughter cells (karyokinesis), and cytokinesis, in which the cell's cytoplasm physically divides. Mitosis is also referred to as maintenance division. Cells that have temporarily or reversibly stopped dividing are said to have entered a state of quiescence called G_0 phase, while cells that have permanently stopped dividing due to age or accumulated DNA damage are said to be senescent. The molecular events that control the cell cycle are ordered and directional; that is, each process occurs in a sequential fashion and it is impossible to "reverse" the cycle. There are two key classes of regulatory molecules that determine a cell's progress through the cell cycle: cyclins and cyclin-dependent kinases.

Interphase

The mitotic phase is a relatively short period of the cell cycle. It alternates with the much longer *interphase*, where the cell prepares itself for cell division. Interphase is divided into three phases, G_1 (first gap), S (synthesis), and G_2 (second gap). During all three phases, the cell grows by producing proteins and cytoplasmic organelles. However, chromosomes are replicated only during the S phase.



Cell cycle

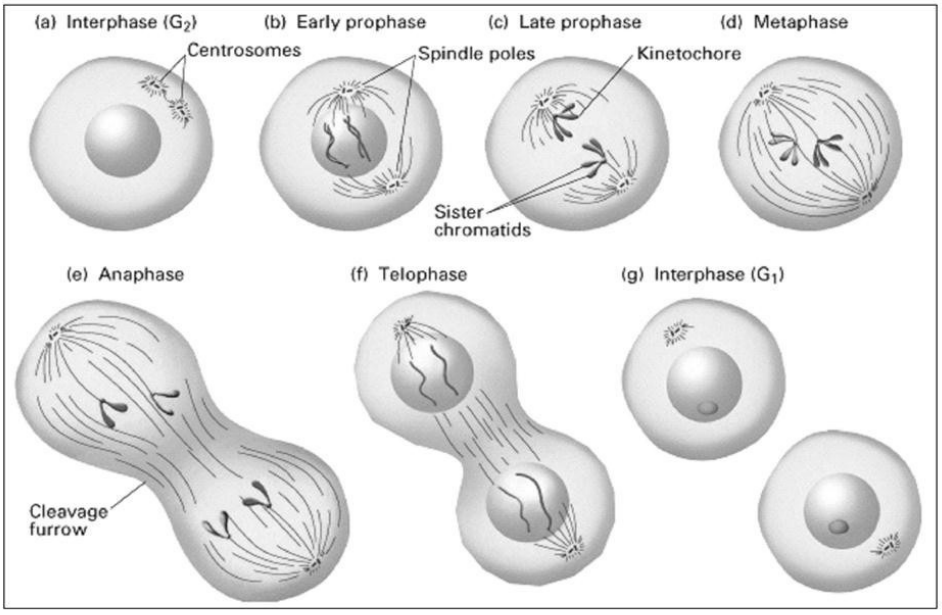


Figure 7. Different stages of mitotic cell division

Pre-prophase:

In plant cells only, prophase is preceded by a pre-prophase stage and followed by a post-prophase stage. In plant cells that are highly vacuolated and somewhat amorphoric, the nucleus has to migrate into the center of the cell before mitosis can begin. This is achieved through the formation of a phragmosome, a transverse sheet of cytoplasm that bisects the cell along the future plane of cell division. In addition to phragmosome formation, pre-prophase is characterized by the formation of a ring of microtubules and actin filaments (called pre-prophase band) underneath the plasmamembrane around the equatorial plane of the future mitotic spindle and predicting the position of cell plate fusion during telophase. The cells of higher plants (such as the flowering plants) lack centrioles. Instead, spindle microtubules aggregate on the surface of the nuclear envelope during prophase. The preprophase band disappears during nuclear envelope disassembly and spindle formation in prometaphase.

Prophase:

Normally, the genetic material in the nucleus is in a loosely bundled coil called chromatin. At the onset of prophase, chromatin condenses together into a highly ordered structure called a chromosome. Since the genetic material has already been duplicated earlier in S phase, the replicated chromosomes have two sister chromatids, bound together at the centromere by the cohesion complex. Chromosomes are visible at high magnification through a light microscope. Close to the nucleus are two centrosomes. Each centrosome, which was replicated earlier independent of mitosis, acts as a coordinating center for the cell's microtubules. The two centrosomes nucleate microtubules (or microfibrils) (which may be thought of as cellular ropes) by polymerizing soluble tubulin present in the cytoplasm. Molecular motor proteins create repulsive forces that will push the centrosomes to opposite side of the nucleus. The centrosomes are only present in animals. In plants the microtubules form independently. Some centrosomials contain a pair of centrioles that may help organize microtubule assembly, but they are not essential to formation of the mitotic spindle.

Prometaphase:

The nuclear envelope disassembles and microtubules invade the nuclear space. This is called open mitosis, and it occurs in most multicellular organisms. These microtubules can attach to kinetochores or they can interact with opposing microtubules. The kinetochore is the protein structure in eukaryotes which assembles on the centromere and links the chromosome to microtubule polymers

from the mitotic spindle during mitosis and meiosis.

Metaphase:

As microtubules (spindle fibres) find and attach to kinetochores in prometaphase, the centromeres of the chromosomes convene along the metaphase plate or equatorial plane, an imaginary line that is equidistant from the two centrosome poles. This even alignment is due to the counterbalance of the pulling powers generated by the opposing kinetochores.

Anaphase:

When every kinetochore is attached to a cluster of microtubules and the chromosomes have lined up along the metaphase plate, the cell proceeds to anaphase. The proteins that bind sister chromatids together are cleaved, allowing them to separate. These sister chromatids turned sister chromosomes are pulled apart by shortening kinetochore microtubules and toward the respective centrosomes to which they are attached. This is followed by the elongation of the nonkinetochore microtubules, which pushes the centrosomes (and the set of chromosomes to which they are attached) apart to opposite ends of the cell.

Telophase:

Telophase is a reversal of prophase and prometaphase events. At telophase, the nonkinetochore microtubules continue to lengthen, elongating the cell even more. Corresponding sister chromosomes attach at opposite ends of the cell. A new nuclear envelope, using fragments of the parent cell's nuclear membrane, forms around each set of separated sister chromosomes. Both sets of chromosomes, now surrounded by new nuclei, unfold back into chromatin. Mitosis is complete, but cell division is not yet complete.

Cytokinesis:

In animal cells, a cleavage furrow (pinch) containing a contractile ring develops where the metaphase plate used to be, pinching off the separated nuclei. In both animal and plant cells, cell division is also driven by vesicles derived from the Golgi apparatus, which move along microtubules to the middle of the cell. In plants this structure coalesces into a cell plate at the center of the phragmoplast and develops into a cell wall, separating the two nuclei. The phragmoplast is a microtubule structure typical for higher plants, whereas some green algae use a phycoplast microtubule array during cytokinesis. Each daughter cell has a complete copy of the genome of its parent cell. The end of

cytokinesis marks the end of the M-phase.

PRINCIPLE:

John Belling (1866-1933) born in Aldershot, England, was a cytogenetist who developed the iron-acetocarmine staining technique which is used in the study of chromosomes. Belling used plants such as lilies and hyacinths to demonstrate that segments between non-homogenous chromosomes can interchange. He was able to make accurate estimates of chromosome numbers and proposed that chromomeres, the small condensations along the chromosome, were individual genes. Acetocarmine is a basic dye used in staining chromosomes and is utilized in viewing the different stages of mitosis in fixed cells. Carmine is a basic dye that is prepared from the insect *Coccus cacti.*

MATERIALS REQUIRED:

Onion root tips, Carnoy's fixative, forceps, needles, slide, acetocarmine stain, 1N HCl, cover slip, microscope, *etc*.

Carnoy's fixative: Absolute alcohol 60ml, chloroform 30ml and glacial acetic acid 10 ml.

PROCEDURE:

1. The 1cm long grown root tips of the onion plant was washed properly with excess amount of distilled water to remove soil attached to the roots.
2. The root tips were fixed in carnoys fixative for 24 hrs (can be stored in 95% alcohol for future use).
3. The root tips were placed in a small beaker containing 1N HCl which is necessary to hydrolyze the tissue.
4. The beaker was warmed to 60^{o}C in a water bath for 10 – 15 minutes.
5. The HCl was rinsed out using fresh water.
6. The meristematic region (1mm) of the root tips were cut and placed on a slide.
7. A drop of acetocarmine was added to the root tip and the tips were squashed well using a clean glass rod and covered with a cover slip.
8. The squashed, stained preparation was then viewed under the microscope.
9. The meristematic region was located with the 10X objective, and then the 40X objective to study individual cells.

Note: Make sure the specimen is a root tip. Avoid excess staining in order to have clear visibility of

nuclear materials.

OBSERVATION

RESULT

AIM:

To observe the meiotic stages in the anthers of *Rheo discolor* and Datura buds.

INTRODUCTION:

Meiosis is the process that allows one diploid cell to divide in a special way to generate haploid cells. It is essential for sexual reproduction. It therefore occurs in most eukaryotes, including single-celled organisms. A few eukaryotes, notably the *Bdelloid rotifers*, have lost the ability to carry out meiosis and acquired the ability to reproduce by parthenogenesis. Meiosis does not occur in prokaryotes, which reproduce by asexual cell division processes.

During meiosis, the genome of a diploid germ cell, undergoes DNA replication followed by two rounds of division, resulting in haploid cells called gametes. Each gamete contains one complete set of chromosomes, or half of the genetic content of the original cell. These resultant haploid cells can fuse with other haploid cells of the opposite gender or mating type during fertilization to create a new diploid cell, or zygote. Because the chromosomes of each parent undergo genetic recombination during meiosis, each gamete, and thus each zygote, will have a unique genetic *blueprint* encoded in its DNA. In other words, meiosis is the process that produces genetic variation. The different stages in meiosis include:

First meiotic division – Meiosis I

Prophase I

In the *prophase* stage, the cell's genetic material, which is normally in a loosely arranged pile known as chromatin, condenses into visible threadlike structures called chromosomes.

Leptotene: The first stage of Prophase I (Figure 10) is the leptotene stage, during which individual chromosomes begin to condense into long strands within the nucleus.

Zygotene: The *zygotene* stage then occurs as the homologous chromosomes pair up with each other. The combined homologous chromosomes are said to be *bivalent*. They may also be referred to as a *tetrad*, a reference to the four sister chromatids.

Pachytene: The *pachytene* stage heralds crossing over. Non-sister chromatids of homologous chromosomes randomly exchange segments of genetic information. Because the chromosomes cannot be distinguished in the synaptonemal complex, the actual act of crossing over is not perceivable through the microscope.

Diplotene: During the *diplotene* stage, the synaptonemal complex degrades. Homologous chromosomes fall apart and begin to repel each other. The chromosomes themselves uncoil a bit, allowing some transcription of DNA. They are held together by the chiasmata.

Diakinesis: Chromosomes recondense during the *diakinesis* stage. Sites of crossing over entangle together, effectively overlapping, making chiasmata clearly visible. The nucleolus disappears and the nuclear membrane disintegrates into vesicles.

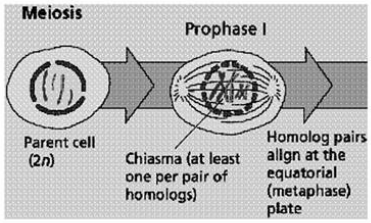
During these stages, the centrioles, duplicated during interphase, migrate to the two poles of the cell. Centrioles sprout microtubules, which invade the nuclear membrane after it disintegrates, attaching to the chromosomes at the kinetochore. The kinetochore functions as a motor, pulling the chromosome along the attached microtubule toward the originating centriole. There are two kinetochores on each tetrad, one for each centrosome. Prophase I is the longest phase in meiosis.

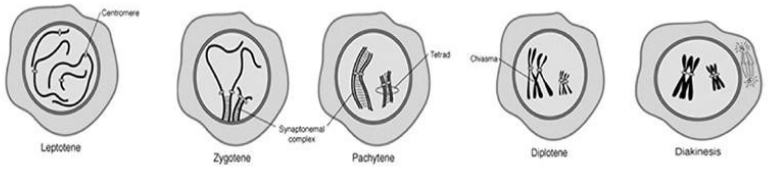
Metaphase I

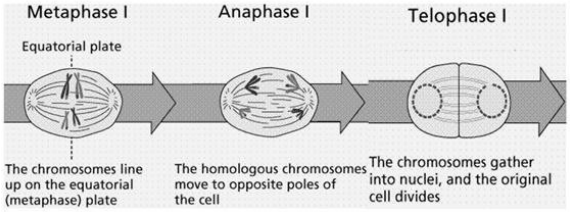
As kinetochore microtubules from both centrioles attach to their respective kinetochores, the homologous chromosomes align equidistant above and below the equatorial plane, due to counterbalancing forces exerted by the two kinetochores of the bivalent.

Anaphase I

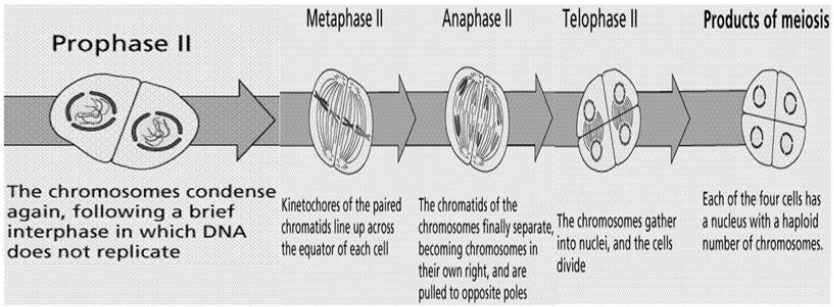
Kinetochore microtubules shorten, pulling homologous chromosomes apart. Since each chromosome has only one kinetochore, whole chromosomes are pulled toward opposing poles, forming two haploid sets. Each chromosome still contains a pair of sister chromatids.







Meiosis I



Meiosis II

Figure 10. Different stages of meiosis

Telophase I

The first meiotic division ends when the centromeres arrive at the poles. Each daughter cell now has half the number of chromosomes but each chromosome consists of a pair of chromatids. The microtubules disappear, and a new nuclear membrane is formed. The chromosomes uncoil back into chromatin. Cytokinesis occurs, completing the creation of two daughter cells.

Second meiotic division - Meiosis II

Prophase II

The nucleoli and the nuclear envelope disappear once again and the chromatids thicken. Centrioles move to the poles and the spindle fibres appear. The new equatorial plane is rotated by 90 degrees when compared to meiosis I, perpendicular to the previous plane.

Metaphase II

The centromeres contain two kinetochores, organizing fibers from the centrosomes on each side.

Anaphase II

Metaphase II is followed by anaphase II, where the centromeres are cleaved, allowing the kinetochores to pull the sister chromatids apart. The sister chromatids are now called daughter chromosomes, and they are pulled toward opposing poles.

Telophase II

The process ends with *telophase II*, which is similar to telophase I, marked by uncoiling, lengthening, and disappearance of the chromosomes occur as the disappearance of the microtubules. Nuclear envelopes reform; cell wall formation eventually produces a total of four daughter cells, each with an haploid set of chromosomes.

MATERIALS REQUIRED:

Immature anthers, forceps, needles, glass slide, cover slip, 1N HCl, acetocarmine, carnoy's fixative, coverslip, microscope, *etc*.,

PROCEDURE:

1. Immature flower buds were collected and the anthers were removed.
2. The anthers were fixed using carnoy's fixative for 24 hrs.

3. The fixed anther was hydrolysed using 1N HCl for few minutes.
4. The anther was mounted on a slide. A drop of acetocarmine was added and a squash was made.
5. A cover slip was placed.
6. Excess stain was removed with the help of soft tissue paper.
7. The slide was viewed first under 10X and later under 40X to see the different meiotic stages.

Note: Locate the pollen grains and do the acidification. Add minimum stain to have clear visibility of nuclear materials.

OBSERVATION

RESULT

7. DROSOPHILA GIANT CHROMOSOME PREPARATION

INTRODUCTION

The polytene chromosome found in the salivary glands of Drosophila larvae [add other diptera] provide a model valuable system in which microscopical techniqes can be used to study the functioning of the interphase genome chromosomes banding patterns revalued markers by which specific genes (or) genomic regions (or) associated with specific chromatin functions (eg: transcription) however procedures that give the best polytene chromosomes preparation (squashes) with the most clearly resolved bands involved treatment with concentration acetic acid is unmodified, such produces results in extration of air (or) nearly all the histone and most non-histone proteins.

AIM

To prepare giant polytene chromosome squashes from Drosophila.

MATERIALS REQUIRED

KCM BUFFER:

120 mm kcl, 20mm Nacl, 10mm tris HCL (PH8) 0.5MM EDTA, 0.1% v/v triton x100.

PBS:

14M Nacl, 0.02M Kcl, 0.05M Na2Po4, 0.02M NAH2PO4.

SOLUTION 1:

0.1% triton x 100 in phosphate buffered saline [PBS] Ph – 7.5.

SOLUTION 2:

3.7% Paraformaldehyde, 1% triton x 100 in PBS Ph 7.5, this should be made fresh every 2-3 hrs.

SOLUTION 3:

% Praformaldehyde, 50% acetic acid, this should be made squash (or) fresh every 2-3hrs.

PROCEDURE

PREPARATION OF THIRED INSTAR LARVAE:

- Drosophila Melanogeseter strawn was maintained in polystyrene vials on yeast. Dextrose agar culture medium, supplemented with additional active yeast at room temperature.

- Adult flies were allowed to lag eggs for 2-3 days, to the point where the larvae would hatch under uncrowded conditions (to allow optimum growth) and were then transferred to fresh vials.
- "Wandering" third instar larvae were used for the preparation of polytene chromosome squasher ie , larvae that have migrated out of the media up the walls of the tubes but have not yet formed paper.

POLYTENE CHROMOSOME SQUASHES:

- Third instar larvae were dissolved in solution one using two sets of fine forceps (no: 3 forceps). One pair of forceps is used to grip the body of the larvae while the other pair. The forceps are slowly down apart to separate the mouth hooks with little other tissue attached to the salivary glands and as much as possible should removed before squashing.
- As 500m as the gland has been extracted it was placed into a well of a 96 well microlitre plate [flexible-round-bottomed] containing 250ml of solution.
- After 30-60 sec, solution 2 was removed by pipetted and replaced by 250ml of solution.
- The glands were left in solutions 3 for 2mins and placed a cover slip.
- Following this, hard downward thumb pressure was applied to the pair of glands in the same directions as the moment of the zigzag line and of solution three.
- This slide was labelled so as to mark the position of the coverslip and hence of the gland.
- The good slide were frozen in liquid nitrogen and the coverslip flicked off with a razor blade the slide were then immersed in 80% ethanol and stored at 20oc until use.
- Never store slides for more than few days before labelling.

LABELLING POLYTENE CHROMOSOME SQUASHES:

- This slides were taken out of 20% ethanol and placed in 40% ethanol for 10mins.
- The slides were placed in a humid chamber for 1hr at 4^{o}c.
- The slides were immersed in PBS for 10mins.
- The slides were washed in kcm for 15mins slides were labelled with 50ml of secondary antibody for 1hr.
- Slides were washed in kcm for 15mins slides were removed and mounted in rock shield containing DA PIC(1 mg/ml) as a DNA counter stain.

8. NUCLEAR STAINING [GIEMSA STAIN]

AIM

To perform nuclear staining by the given sample.

PRINCIPLE

Giemsa stain is used to differentiate nuclear, cytoplasmic morpology of platelets, RBC"S,WBC and paracites the stain must be diluted for use with water buffered to pH 6.8 /7.0-7.2, depending on the specific technique used the aqueous working dilution of stain is good only for one day.

SPECIMENS

The specimens usually consists of fresh whole blood collected by finger puncture (or) of whole blood containing EDTA that was collected by vein puncture and is less than 1 hold. if slide have been prepared, the specimen may be a thin blood film that has been fixed in absolute methanol and allowed to dry the combination thick, thin blood is also acceptable.

MATERIALS REQUIRED

REAGENTS:

Giemsa stain,

Giemsa buffer.

EQUIPMENTS:

Microscope, binocular with mechanical stage,10w (10x),high dry(40 x) and 0.1 immersion (100 x) objectives, 10x occulars, calibrated occular. micrometer, light sources equipment to 20 w halogen (or) 10 w tungsten bulb and while grown glasss diffuses filters.

Timer (1hr or) more in 1 min in increments.

PROCEDURE

A. wear gloves when performing this procedure.

B. Thin blood films(only)

EAR GLOVES WHEN PERFORMING THIS PROCEDURE

- Fix air dried film in absolute methanol by dipping the film briefly in a coplin for containing absolute methanol.
- Remove and let air dry

- Stain with diluted giemsa stain (1:20 v/v) for 20 mins for a 1:20 dilution. Add 2ml of stock giemsa to 40ml buffered water in a coplinjar.
- Wash by briefly dipping the slide in and out of acoplin jar of buffered water (1/2 dips)
- Let air dry in a vertical position.

HIN BLOOD FILM

- Allow film to air dry thoroughly for several hrs/overnight. Don't dry films in an incubator/by heat because this will fix the blood and interfere with lysing of the RBC‟S.
- Do not fix
- 1ml of stock giemsa to 50ml of buffered water in a coplin jar wash by water for 3-5mins.
- Let air dry in a vertical position.

9. METAPHASE PREPARATION AND KARYOTYPING [ONION ROOT TIP]

AIM

To study the different stages of mitosis in onion root tip cells.

PRINCIPLE

Onion root tip has meristematic tissue just behind the root cap. hence this serves as a good material for studing various stages of mitosis. the root can be easily grown if an onion is placed for a water filler conical flask is such a way that onion disk touches water or else making the onion to sit on wet sand in such a way that the bulb is baried partially in sand.in two day time nearly 1cm long root develop which can then be cut, fixed and stored. longer grown roots are not good as it will have more of non-dividing tissue.

MATERIALS REQUIRED

Onion root tips

1 NHCL

1:3 acetomehanol fixation

70% and 90% ethanol

2% acetocarmine stain

[2 gm of carmine mixed with 100ml of 45% of acetic acid and boiled using a condenser for 1 hrs to disslove carmine].

45% of acetic acid

Slide

Cover glass

Sealing wax or nail polish

PROCEDURE

FIXATION OF ROOT TIPS

- Fix the freshly cut 1 cm long root tips in acetomethanol fixative for overnight in a specimen tube.
- Remove fixative and add 90% ethanol, leave for 2hrs.
- Decant 90% ethanol and add 70% ethanol the root tip can be stored in 70% ethanol for a long period of time if the tube is tightly closed storing at 4oc is even better.
- Staining and making squash preparation.

- Treat the root tips with 1NHCL for 1min, this will soften the cell wall.
- Rinse the tips once in water, transfer to acetocarmine stain and stain for 30mins.
- Take a drop of 45% acetic acid on slide place a root tip on the drop, leave for 1-2mins, if acetic acid drop becomes closed, it can be decanted and a fresh 45% acetic acid drop can be added.
- Place a cover glass on the root tip and squash ot using a rubber end pencil under the folds of a blottting paper.
- Seal the edges of the cover glass with malten wax or with nail polish immediately to prevent drying of acetic acid film and entry of air bubbles.
- The slide is ready for observation under the microscope.

10. CONJUGATION

INRODUCTION

Bacterial conjugation is often regarded as the bacterial equivalent of sexual reproduction mainly. It is actually sexual as it doesn't involve the fusing of gametes and contain of a zygote. It is infact the simple exchange of genetic material and information from one bacterial to another. The bacterial has to carry the F- plasmid F- factor. the plasmid is a specialized plasmid known as an episome that has 100 kbp in length, the F- plasmid has its own origin of replication is originated within a single bacterium.

AIM

To pass the drug resistance of one bacterial to another by the process of bacteria conjugation, make agar plates the day before the experiment .

PREPARE IB AGAR PLATES

- Empty the entire contents of one LB agar plates into an autoclavable container and add 150ml distilled H2O, autoclave for 15mins qt 121oc.
- Once the LB agar has cooled to hand hot temperature, pour ~0.5cm layer of agar into a 6 petriplates. That is 20-25 ml each plates.
- Let the plates set for 20-30mins to solidify.
- Distribute two LB agar plates tube shared by 2 groups.

PREPARE LB AGAR PLATES WITH ANTIBIOTICS:

- Prepare the antibiotics add 200ml twtracycline buffer to the vial of ampicillin and tetracycline mix.
- Label the bottom of petriplates,(or) petriplates with the antibiotics and then pour a N 20-25ml each plate.
- Let the plates set for 20-30mins to solidity.

PREPARE OVERNIGHT CULTURE OF BACTERIA:

- Label one culture tube with ".BB4" and another "SCBI".
- Using aseptic techniques transfer 7ml of LB broth provided in the Rit to each of the labelled culture.
- Pipette 0.8ml LB broth into the C-C "SCSI" agar slub incubated at 37oc for 30mins.
- Culture the bacteria in a 37oc shaker overnight.

- The following day, label six centrifuge tube 2ml with "SCSI" dispense 1ml of each overnight culture into the respective labelled six centrifuge tubes.

MATERIAL REQUIRED

- Supply each group with the following components the LB agar plate are shared between two group of four students.

  1 vial 1ml E.Coli BB4 (F+) overnight culture.

  2 vial 1ml E.coli BCSI (F-) overnight culture.

  2 ampicillin discs

  2 tetracycline discs

  2 LB agar plates

  1 ampicillin tetracycline LB agar plates

  1 pair of forceps

  1 centrifuge tube (2ml)

  1 inoculation loop

PROCEDURE

- Add 0.5ml of E.coli BB4 to he labelled 2ml tube and 0.5ml of E.coli SCSI.
- Incubate the mixture in a 37oc incubator for 60mins any shaking.
- Allow the LB agar to dry for 15-20mins upside down .so that the agar is at the top and place in a 37oc.
- At the end of incubation (step 4) vortex.
- Vigorously shake the tube for 1min to stop the conjugation process, allow the plate to dry for 15-20 mins.
- Once the plates are dry , turn the plates upside down ,so that the agar is at the top and place in a 37oc incubator for 18-24hrs.the following day examine the plates and record your results in the followig section.

11. TRANSDUCTION

AIM

To learn the process of transduction which is a genetic transfer of a particular gene from one E.coli host to another through a bacteriophage.

PRINCIPLE

Bacteria can exchange (or) transfer DNA between bacteria in 3 different ways ¡)transformation , ¡¡)conjugation,¡¡¡) transduction.

In call those cases the sources of cells the “DNA are called” “doners” “receptor” .

The ability of virus to carry bacterial DNA between bacteria was discovered in 1952 and named as transduction.during the lysogenic cycle. The phage chromosome is intergrated into the bacteria chromosome and can remain dorment for several generation.

MATERIALS REQUIRED

Donor strain

Recepient strain

Strain c

Chloramphenical

Bacteriophage lysate

LB broth

CaCl2.

GLASSWARES:

Conical flask

Measuring cylinder

Sterile tubes[15 and 50ml]

Petriplates

REAGENTS:

Distilled water

OTHER REQIUREMENTS:

Centrifuge

Incubator

Shaker

Water bath

Micropipette

Tips

Loops and 0.45µm filter.

PROCEDURE

DAY 1:

Streak a loopful of culture from stab of donor LB 20 plate susceptible host on LB plate.

Along with streaking and incubate loopful of culture from given stabs in 5 ml LB broth with respective antibodies.

DAY 2:

Store the 5ml culture tubes at 4oc for inoculation on day 3.

Inocubate 10-15 colonies from received donor plant into 5ml LB (20 and label as donor)

Inocubate at 30o c in shaker for 2hrs.

Keep a 5ml aliquat of sterile LB broth in water bath ,set at 60-65oc.

Add 100µl of given phage lysate to above labelled donor tube, continue incubation for 30mins at 30oc.

Add 2ml of pre heated sterile LB broth to donor tubevmix well and incubate this tube at 42oc another 20mins.

Transfer this tube to 37oc and incubate for 3hrs.

DAY 3:

Incubate 100µl of overnight grown recepient culture in 5ml fresh LB broth with ampicillin(100ms/ml).incubate on shaked at 37oc for 2hrs.

After incubation take 50µl of this culture in 2ml collection tube,and 50ml of this culture in 2ml collection tube,add 50µl 0.1 m cacl2 along with 250µl of phage lysate 2 obtained and stored at 4oc on day 2.

Mix well and incubate further at the 30oc for 2hrs.

After 2hrs take each 50µl of culture overnight incubation on LB plate.

Along with it, take 50µl of overnight grown culture of receipient stain and donor strain.

On next day, store these plates at 4oc for observation and results.

DAY 4:

Incubate 20,25 colonies from received plate of susceptive host in 15ml of fresh LB broth.incubate this culture on shaker at 37oc for 2hrs.

After this incubation spin down 1.5ml of this culture at 800rpm for 10mins before starting the titration protocol, keep 5ml of LB broth at 60oc.

To conform presence of phages in lysogenized colonies of infected receipient culture inoculated 10-15 colonies from LB 120 and 110 plate in 5ml LB broth, Lbel this as lysogenized culture.

Incubate this tube at 30oc for 3hrs.

After incubation add 2ml of hot LB broth to this lysogenized culture.

Further incubation this tube at 42oc for 20mins, again transfer this tube to 37oc and incubate for 2hrs.

PHAGE TITRATION

Take 7 collection tubes label them as concentrated lysate 10-1,10-2,10-3,10-4,10-5,10-6,.

Take 900µl of sterile LB broth from tube from 10-1 to 10-6 and add 100µl of concentrated lysate to 10-1 tube.

In 100 l of plating cell tubes add 20µl of concentarated and 20ml phage lysate dilutions respectively and incubate all tubes at 37oc for 15mins.

Mean while,melt the soft agar , dispence 5ml of melted soft agar in 15ml pipetted out the mixture of plating cells and concentrated lysate in soft agar tube mix and immediately pour on labelled LB hard agar plate.

Repeat step 13 for phage dilutions from 10-1 to 10-6 close the lids of petriplates and incubate these plates at 37oc .

Note down the results and observation next day.