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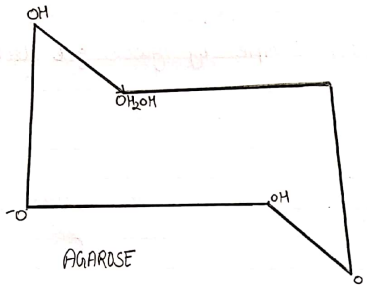

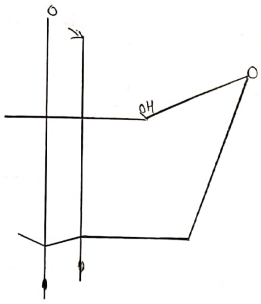

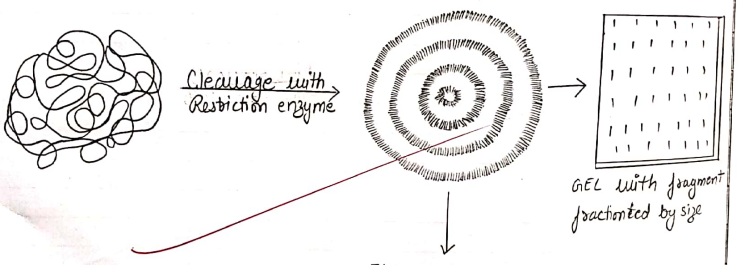

GEL - ELETROPHORESIS

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Aim ⇒ To analyse the legated sample by Agarose gel electrophoresis.

Introduction ⇒ Agarose gel electrophoresis is a procedure used to separate fragments based their weight and an intrinsic part of almost all carried out in molecular biology.

(A) Preparation of Agarose gel.
(B) Gel electrophoresis of the DNA fragments.
(C) Visualisation of the DNA fragments.

A. Preparation of Agarose gel

Agarose is a linear primer extracted from it seaweed purified Agarose is a powder insoluble in H_2O or buffer at room temperature but dissolve on boiling. molten solution is then poured into a mould and allowed to solidify. As it cools agarose undergoes polymerisation ie, sugar polymerase cross link with each other and cause the solution to gel. The density or porisize of ⊂ is determined by concentration of Agarose.

(B). Electrophoresis of DNA fragments

Electrophoresis is a technique used to separate charged molecules. DNA is -ve charged at neutral PH and when electric field be applied across the gel. DNA migrates the gel dependent upon →

(i). Molecular size of DNA.
(ii). Agarose concentration

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(iii) Conformation of DNA.
(iv) Applied current.

Matrix of gel (Agarose) acts as a molecular sieve. Through c DNA fragments move on application electric current. Higher concentration of agarose gives firmer gel that is space b/w cross-linked molecules is less and hence smaller DNA fragments easily cross-linked these spaces as the length of DNA increases it becomes harder for the DNA to pass through the space. while lower concentration of agarose helps in movement of larger DNA fragments as the space b/w the cross-linked molecules is more. the process of gel electrophoresis is monitered.

The migration of a visible dye (trading dye) through the gel. Two commonly used dye are Xylene & Bromophenol blue that migration at the same speed as DNA of size 5000 bp & 3000 bp. These trading dye as negatively charged. low molecular weight compounds. these are loaded along c each sample at the start of run. when the trading dye reaches towards the Anode run terminates.

As the voltage applied to gel increased layer fragments migrate proportionally faster that of small fragments, But resolution of fragments is attained by applied net more than 5 volt / cm to the gel.

Different buffer have been recommended for electrophoresis of DNA. The most common uses are.

TAE - [Tris Acetate - EDTA]
TBE - [Tris Borate - EDTA]

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DNA fragments migrates at some what different rates in these two buffer due to different in ionic strength. Buffer not only establish PH but provides ions to support conductivity.

[C] Visualisation of DNA fragments.

Since DNA is not naturally coloured it will not be visible on the gel. Hence the gel after electrophoresis is strained ~ a dye. DNA as well as RNA are normally visualized by staining with ethidium bromide, which intercalates into the grooves of the DNA and fluoresces under UV light.

When stained with ethidium bromide, the gel is viewed with an ultraviolet transilluminator. The UV light excites the electrons within the aromatic ring of ethidium bromide, and once they return to the ground state, light is released, making the DNA and ethidium bromide complex fluoresce.

Procedure - Prepration of 1% Agarose gel.

(1). Prepare 1X TAE by diluting appropriate amount of 50 x TAE buffer [for one expt Approximate 200 ml of 1X TAE is required] makeup 4 ml of 50 ml 1X TAE - 200 ml distilled water.

(2). Weight of 0.5 gm of agarose and to 50 ml of 1X TAE this gives 1% agarose gel.

(3). Boil till agarose dissolve completely and a clear solution result.

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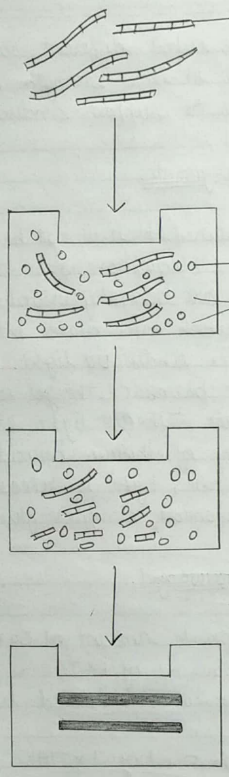

SEPRATION OF DNA FRAGMENTS OF DIFFERENT LENGTH BY GEL ELECTROPHORESIS

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(4) Meanwhile place the combs of electrophoresis set so that it is approximately 2 cm away from the cathode.

(5). Add ether to the matrix agarose to a final conc^n of 0.5 ml (from a stock of 10 mg/ml in water) and mix gently.

(6). Pour the agarose solution in the central part of tank when the temperature reaches approximately 50°c Don't generate air bubbles. The thickness of the gel should be around 0.5 to 0.9 cm. Keep the gel undisturbed at room temperature for the agarose too.

Electrophoresis :-

(7) Pour 1X TAE buffer into the gel tank till the buffer level stands at 0.5 to 0.8 cm above the gel surface.

(8). Gently lift the combs ensuring that will remain connect the power cord to the electrophoresis power cord to the electrophoresis power supply a/c to the connection red anode and cathode.

(9). Load 10 ml of the 3 different DNA samples in the well desired order and record the order.

(10) Set the voltage to 50V and switch on the power supply.

(11). Switch off the power supply when the tracking dye. (Bromo phenol blue) from the well reaches 3/4 of the gel. This takes approximately 1-2 hours.

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12. After electrophoresis DNA samples can be visualized under UV transilluminator (They appears fluroscent no cleasting is required in the case).

Precaution :- Ethidium bromide must be handeled carefully as it is a mutagen and while handling Ether solution and gel stained with it.

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Aim → To analysis protein by SDS Page.

Principle → SDS page is the most widely used method for qualitatively analysis any protein mixture. monitering protein purity and to determine their molecular weight. It is based on the separation of Protein acc to their size and locating them by binding to a dye.

SDS or sodium - di - sulphate is an ionic detergent that binds strongly to protein causing their denaturation. In the presence of excess SDS about 1-4 gm of detergent binds to each gram of protein giving.

$$RF = \frac{\text{Distance migrated By Protein}}{\text{Distance migrated by tracking dye.}}$$

- RF values of protein makes of known size is used to generate a standard curve by plating the molecular weight ~~against~~ the RF values on semi log graph, The molecular weight of the unknown protein can then be extrapolated from its RF values.

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The technique consists of three Basic steps →

I. Preparation of Polyacramide gel → Cross linked polyacramide gel are formed by co-polymerization of acrylamide monomer and a crosslinked agent N, N methylene, Bis acrylamide, This reaction is catalysed by N, N, N, N - tetramethyl (TMED) and initiation by ammonium per sulphate (APS) of acrylamide and bisacrylamide mix used lower % gel there larger pore sized, there by offering less resistance to passage to larger molecules while higher % gel favours. Separation of smaller molecules.

II Electrophoresis → The polyacralamide gel slab is prepared and fixed to a verticle electrophoresis apparatus protein sample are usually denatured by boiling in sample buffer containing ions of PH 6.8, SDS β-microcepto ethanol.

Differences in PH and loaded there 2 gels causing the sample to be concentrated into narrow bonds by iso-technophoresis, The sample migrates through the separing gel. Protein get resolved depending on their molecular weight electrophoresis is stopped when the dye front reaches the bottom of the gel.

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III. Visualization of Protein :→

Generally proteins are colourless and hence can't be visualized directly suitable dye are used to stain the sample.

Example → Brilliant Blue R - 150.

Material Required →
Equipment ⇒ Rocker (Rules) apporinal.
Glass ware – Measuring cylinder
Reagents – Alcohol, Distilled water
other equipment – Lamps, gloves, micropipette, lids, water bath.

Procedure → The assembly of Plates, ensure the assembly is leak proof by filling water b/w plates Sigrease can be applied to spacesses 1% agarose can be use for sealing to make it leak proof.

2. Add 50% of ammonium disulphate to 5ml of separting gel mixture and mix through
3. Add 200 – 250 ml of water to make the surface even.
4. Add 20 ul of APS sol^{n} to 2ml of stacking gel mixture

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pore directly on the polymerized sepatiy gel.

- Pipette out 25 µl of Protein sample (A, B, C) and 10 µl of Protein makes into indivisible the approximately to each of these vials add 15 µl of sample loading buffer.
- Place the vials in boiling water bath for 5 min.
- Fill the bottom reservion ē x 1 x reservoir buffer.
- Carefully fr the plate to poye apparatus without bapiy any air bubbles bw the buffer and the bottom of the gel ē the notched plate facing the top reservions.
- The relative mobilitie of the proten (markers and Sample C) is calculated as follows)

$$RF = \frac{\text{Distance migrated by Protein}}{\text{Distance migrated by tracking dye.}}$$

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Aim ⇒ To perform Restrication Digestion of DNA of E coli and HIND III enzymes. To analyse the Restriction pattern by Agarose gel electrophoresis.

PRINCIPLE ⇒ Bacteria are under constant attack by bacteriophages to perfect protect themselves, many types of bacteria have overlapped defance mechanisms in the form of Enzyme Ca Endonuclease that any foreign DNA. Since these enzymes restrict the infaction of Bacteriophage they are termed. "RESTRICTION ENDONUCLEASE" Thes molecular restrict found in the bacterial cytoplasm can prove dangerous to cell do bacterial protect their own DNA by methylating the adenine & cytosine bases. The methyl groups block the binding of restriction enzymes but not the normal reading and replication of genetic information.

DNA from an attacking bacteriophage will not have these protective methy group and will be destroyed.

Together R·E and its modification methyl trans-ferase form a "RESTRICTION MODIFICATION (RM) System" four kinds of RM System are known. These are distinguished based on subunit composition kinds of reduence recognised and cofactors needed for activity most characterized Enzyme to belong to type II Class. They comprise the commercialy available restriction used for DNA analysis and other manipulation Each R·E has a unique name derived from genus spp. and strains of bacteria that produce them.

Eg :- E·CoRI is from E.coli.

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Note :- R.E are powerful tools of molecular genetics used to →

- Map DNA molecules.
- Analyse population polymorphism.
- Rearrange DNA molecule.
- Prepare molecular probes.
- Create mutants.

TYPE II - R.E recognism specific DNA reference and the DNA at fixed location near the recognition sites. They not acts as dimers each subunit recognising the same 5' - 3' not sequence in complimentary DNA strands and are said to recognise palindrome sequence before the RE unit and its specific recognition site on a long DNA. It bind to a site on a long DNA it binds to a site on a very long no. of sites.

At recognisation site in presence of sliding of Mg^{2+} enzymes undergoes a conformational change & the helix end cleavage the DNA producing blunt or sticky ends.

5' — GAACC — 3' ⟶ 5' — GAACC — 3'
3' — CCGG — 5' ⟶ 3' — CC GG — 5'

However many R.E cut in an affect fashion to give sticky ends & have pasturing ends (5' or 3' ends) & unpaired bases depending upon the R.E for eg - Ecoli recognise the following sequence and each backbone b/w G & A base residue given 5 pasturing ends.

5' — GAATTC — 3' ⟶
3' — CTTAA G — 5'

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The resulting sticky ends can base pair ~ any DNA molecules that has the complementry sticky ends to give a recombinant DNA molecule. Any R.E will cut only a specific base seq. no. matter what DNA molecule its acting on However a given recognition seq. can be recognised by multiple enzyme c/a isoschizones.

Example → SMA I & XMA I

These can called the DNA at same or different Position ~ in the recognition sequence.

Frequency of cleavage depends on the probability this enzyme ~ longer recognition seq. cut less frequently and consequently produces larger fragments that so enzymes ~ shorter recognition sequences.

FACTORS AFFECTING RESTRICTION ENZYME ACTIVITY ⇒

(a) **TEMPERATURE** ⇒ most digestion are carried out at 31°c however these are few exception.

Example :- Digestion ~ SMA-I is carried out a lower temp. (~20°c).

(b). **Buffer System** ⇒ HCl is the most commonly used buffering agent incubation mix enzyme are active in 7-8 PH.

(c). **Ionic conditions** ⇒ Mg^{2+} is an absolute requirement for all R.E but the requirement of other ions varies with different enzymes.

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(d) **Methylation of DNA :-** Methylation of specific adenine or cytidine residues within the recognition sequence of the R.E effects the digestion of DNA.

MATERIAL REQUIRED ⇒

- Equipment ⇒ Dry bath.
- Glass-ware ⇒ Beaker, flask, measuring cylinder.
- Reagent ⇒ water, crushed ice, micropippete.

PROCEDURE ⇒ Place the vials containing RE (E.coli & HINDTT,) on ice.

- Then the vails containing substance (λ-DNA and assay buffers.
- add 20 ml of λ DNA to each of enzymes.
- add 25 ml of assay buffer to the enzyme and DNA mixture mix by tapping the tube.
- Incubate the vial at 37°c for 1 Hours.
- Mean while prepare 1% agarose gel for electrophoresis.
- After 1 hour add 5ml of gel loading buffer to each of enzyme.
- Table a new 1.5 ml vial as add 10 ml of sub strate and 1 ml of gel loading buffer to each of enzyme.
- Take another 1.5 ml vial as add 10 ml of substrate and 1 ml of gel loading buffer.
- Load the digestive sample, substrates and markers note down the order of loading.
- Electrophoresis the sample at 50 - 100°c for 1-2 hours.

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- Stain the agarose gel ē staining dye.
- Destain to visualize DNA bands.
- observe the band b/w bottom obtained on digestion ē E.CORI & HIND-III compare these ē marker (λ/HLVI).

INTER·PREPRATION OF RESULT ⇒ Restriction pattern obtained on digestion ē E·CORI & HIND III are markedly different demonstrating the fact. each R·E recognise and cut only a particular base seq. unique to it.
By compairing the migration distance ē that of marker we can also determine approximatly size fragments.

RESULT ⇒ The DNA was cut with R·E dyed and loaded on agarose gel and electrophoresis.

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GENERAL CYTOLOGICAL TECHNIQUE

CYTOLOGY =>

The term cytology means "The study of cell" it is a biological science which deals ē the study of cell from morphological, biochemical, Physiological, developmental, genetical, Pathological and evolutionary point of views.

CHROMOSOMES => These are the thread like filamentous structure containing DNA. They are capable of self reproduction & maintaining morphological and physiological properties through successive generation. They are capable of transmitting heridity materials of the next generation they are also called heredity vehicles. They are 2 type of chromosomes.

(A). Somatic
(B). Reproductive.

Study material => mostly root tips cells are used for cells flower bud is used to for female meiosis, we use pollen mother cell and for female meiosis, egg mother cell is used. Chromosome appears only during cell division for meiotic study we use stem, root or leaf part should have meristematic gene. for studying the chromosome the basic requirement is that the cytoplasm should be colourless.

No oil pigment should be there as pigment decrease the visiblity of chromosome root tips are colourless where as stem and leaves coloured so they are suitable (root tip are suitable) for study than stem & leaves.

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S.no.	Chromosomes of MONOCOTS	Chromosomes of Dicots.
1.	Generally larger	smaller in size.
2.	less in number	more in number.
3.	more polyploids	less polyploids.

Example :→ • Triticum aestivum is hexaploid → 7 x 6 = 42.
• Ophioglossum reticulatum 2n ⇒ 1025 + 25 fragments.

TIME PERIOD ⇒ Cells have a particular time period for division s vary from plant to plant. generally it occurs in morning day time. In winter and rain cell divide throughout the day as the optimum conditions are provided. Basically it is a temperature dependent process.

TECHNIQUES :- (A) FIXATION
(B) MORDATING
(C) STANING
(D) PERMANENT SLIDE PREPRATION.

(A). FIXATION ⇒ To fix cell is very important as the material is not available every time, so we have to use started material. fixation is done to maintain stages undistribute and undamaged.

FIXATIVE ⇒ An agent that brings fixation is c/a fixative.

(a) Coenoj's fluid I ⇒ Glacial acetic acid + absolute alcohol (1:3 ratio), material are kept for 12-24 hours at room temp. at fixed at proper conditions.

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(B) Coenoj's fluid II ⇒ Glacial acetic acid + absolute alcohol + Chloroform in core when Coenoj's fluid II is unable to stain Conjons fluid II is used.

STORAGE ⇒ change the fixed material in 70% alcohol (Permanent Staining chemical), Sprit (90%) absolute alcohol, FAA should Store in 70% alcohol after 15 days, we should add it to FAA to prevent bacterial infection.

(B) MORDATING ⇒ mordating is a process of making storage permanent chromosome. we use $FeCl_3$ + 2-3 crystals of $(NH_4)_2$ $FeSO_4$ in fixative, stain should stick on surface. stain gradually disappears mordant increase long activity of stain.

(C) COMMON STAIN ⇒ carmine, aceto carmine, Basic fuschin.

Aceto carmine ⇒ most commonly used stain it is basically prepared or extracted from ground dried bodies of insects carmine is organic other chemical are synthetic.

Acetocarmine solution ⇒ Glacial acetic acid ⇒ 45cc.
Distilled water ⇒ 55cc
100 cc

In 1gm Carmine powder mix all these and boil till dissolving.

(II) PERMANENT SLIDE PREPRATION =>

- Take set of petridishes having glass rod.
- Keep slide on these rods and add Acetic acid in 1:1 ratio.
- Normal butyl alcohol + acetic acid are prepared in bottle.
- 4 days old slide is where coverslip is present as solution is pressed, coverslip attaches.
- Coverslip is inverted now both slide and coverslip are sunk.
- Take blotting paper keep slide on it. DPX is mounting material.

→ In case of root tips 1 drop of 1N-HCL + 2% aceto carmine (drops).

PRE-FIXATION TECHNIQUE => The work of making permanent slide is done round the year except rainy season. as during rainy season cell don't dry properly.

TECHNIQUE FOR SMEAR PREPRATION FOR MEIOTIC STUDIES:-

1. Fix the flowers bud of different size (smallest to largest) in Coenjs fluid -I for 12 hours at room temp. Stamens have pollen grains.
- In bud, grains are present while in flowers they are dehised Salt in 70% alcohol in seprate bottle.
- Take the stamens from a bud of suitable size mostly medium sized buds used, stain it in 1% acetocarmine. Disect the material, Heat it over sprit lamp for 1-2 minutes for good staining.
- Slightly press the coverslip & Blotting paper stain again from

a side and heat it.

- Seal the coverslip & Nail polish.

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Object :→ To study MITOTIC Index (M.I) in given root tip cells of onion.

Requirement ⇒ Root tip cells of onion 1N HCL 2% aceto-carmine blotting paper, sprite lamp, slide coverslip, microscope etc.

Procedure ⇒

1. Collect some root tips (of about 5mm 1cm) long in a test tube containing 2% acetocarmines and 2% in a ratio 9:1 and boil them for 15-20 min.
2. Take a root tip on slide, cut and remove the pointed part of tip (containing root apical meristem).
3. Stain it in a drop of 2% aceto carmine.
4. Put coverslip over it.
5. Press uniformly after putting a piece of blocating paper.
6. Stain it, heat it and remove air bubble and pressing till cells get separated, stained and show some stage chromosome.
7. When stages are visible the fined meiotic index by using formula.

Observation table :→ To study frequency of different stage by mitotic for mitotic index.

Calculation ⇒ M.I $\frac{\text{Total no. of Dividing cell}}{\text{Total no. of cell observed}} \times 100$

M.I ⇒ $\frac{B}{A} \times 100$

⇒ $\frac{17.3}{51.3} \times 100$

M.I = 34%.

Means - A ⇒ $\frac{513}{10} = 51.3$

Means - B ⇒ $\frac{173}{10} = 17.3$

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S.no.	Total no. of cells observed	Frequency of mitotic stages				Total no. of Division cells.
		Prophase	metaphase	Anaphase	Telophase	
1.	55	12	2	–	2	16
2.	48	15	–	1	1	16
3.	52	13	–	1	2	16
4.	61	11	2	–	2	15
5.	51	13	2	1	2	18
6.	60	14	1	2	5	22
7.	45	11	2	–	2	15
8.	58	16	–	–	1	17
9.	40	12	2	1	2	17
10.	43	14	2	2	4	22

SUCROSE SOLUTION TEST ⇒ Its a specific test for pollen c contain starch (example - maize). The visible pollen tube the blue stain of KI & the non-starch remain unstained.

DETERMINE THE % FREQUENCY OF ⇒

% viable pollen ⇒ $\frac{\text{No. of Pollen}}{\text{Total no. of Pollen}} \times 100$

Result :⇒ Acetocarmine stain test in TEPM → out of 20, only 12 are.

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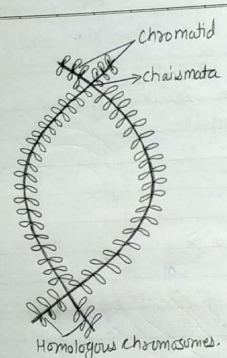
% viability in tephrasiapupia ⇒ $\frac{12}{20} \times 100$ ⇒ 60%.

In Casia spp. ⇒ out of 12 only, 9 are v.

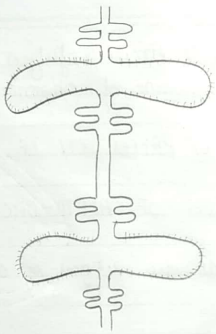
$\frac{9}{12} \times 100 = 75\%$.

In tabermontana spp. ⇒ out of 10, cently 2 are viable

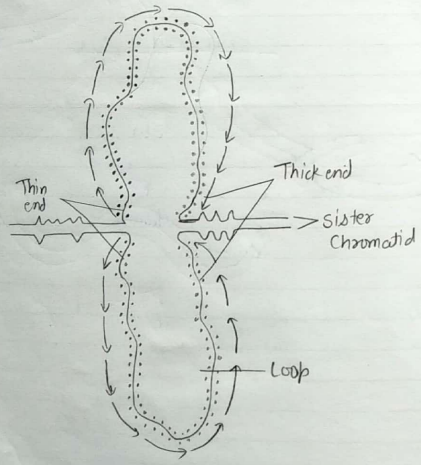
% viability = $\frac{2}{10} \times 100$ ⇒ 20%.



(A) LAMP BRUSH CHROMOSOME



(B) DOUBLE LOOP



(C) SINGLE LOOP

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OBJECT ⇒ Aim is flugen staining of the salivary gland chromosome of chironomous and Drosophilla.

METHOD OF PREPRATION OF SLIDE ⇒

I. PREPRATION OF ACETO-ORCEIN STAINING ⇒

① firstly dissolve 2.2 gms of orcein in 100 ml of glacial acetic acid by heating in gently.

② Now cool and dilute and filter it (the best stain is obtained when 2% of orcin is dissolved in 50% acetic acid).

II. DISSECTION OF LARVA ⇒

- It's done for getting salivary gland and after getting, these glands are fixed in carnoys fluid.

III. Now a slide is prepared ē mayer's albumin & salivary glands are placed on it. After it these glands are stained ē 10 to drops of aceto-orcin stain (2%) and allow them to remain as such for 3-5 min. After this the previous stain is drained off and fresh drops of the stain are utilized.

IV. For prepration of slide, this is gently heated cover spirt lamp for some time, Now this material ē cover slip is sealed ē wax.

THE IMPORTANT FEATURE OF SUCH CHROMOSOME ARE GIVEN BELOW ⇒

(a) LAMP BRUSH CHROMOSOME ⇒ These are present in the oocytes of vertibrates and there can be best studied in diploid stage.

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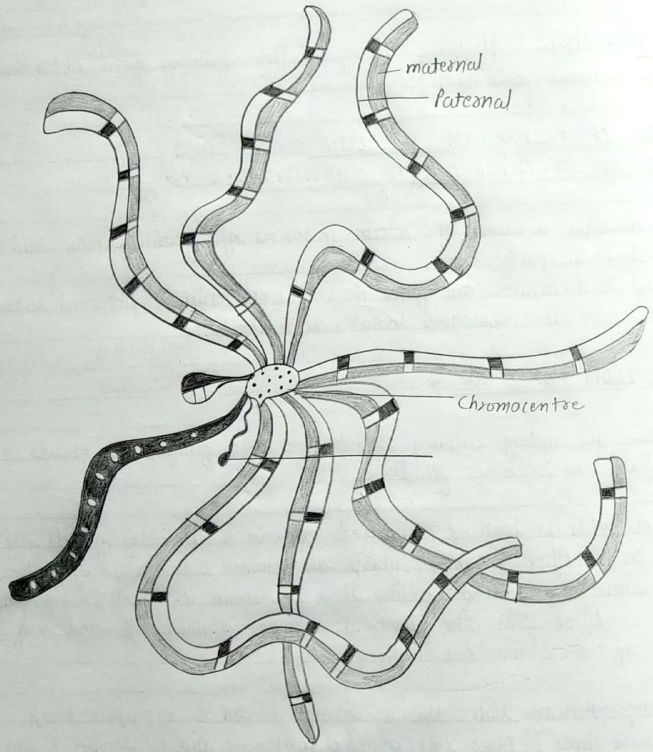

Salivary Gland Chromosome
or
Polytene Chromosome

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of meiosis-I.

- These chromosomes are very larger in size in comparision to normal chromosomes in the central axis of each chromosome, numerous lateral loops Emerge out in pairs due to presence of paired loops emerge out in pairs. These give an appearence a lampbrush Therefore these are known as lampbrush chromosome.
- These axis of the loops is made up of DNA fibres and produced from this axis & are covered by RNA and protein matrix.
- The number of loops is max^m in diploid stage.
- As the process of cell division proceedes further their no decreases gradually and ultimately these loops disappear totally.

(B) SALIVARY GLAND CHROMOSOME
OR
POLYTENE CHROMOSOME ⇒ These chromosomes are found in the salivary gland of some insects belonging to order diptera.

Example ⇒ DROSOPHILA.

- In each of chromosome the no. of chromonemata is up to 2000, hence these are known as polythene chromosome.
- These chromosomes are always found in paired conditions, their size become larger by the duplication may be reported 9-10 times and after this process the duplicated chromosome don't separate.
- The process involved in this phenomenon is known as endomitosis during a abnormal somatic synapsis is observed.
- These polythene chromosomes are having permanent light and dark bands.

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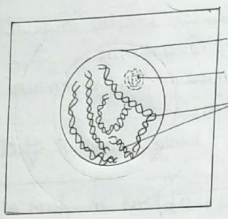
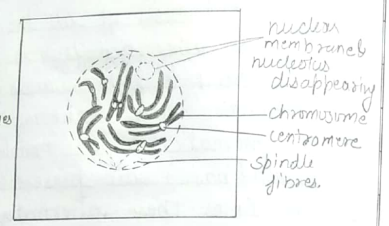
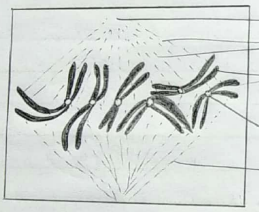
- The centromeres of all the chromosomes present in a cell unit c each other and form a common chromocentre.
- The dark & light bands of chromosome are known as chromatic and achromatic centre.
- In chromatic region mainly DNA c bance amount of RNA and protein (basic) are present.
- Some-times these alternating bands, appear in form of chromosomal swelling and known are chromosome puffs which are known as "BALBIANI RINGS".



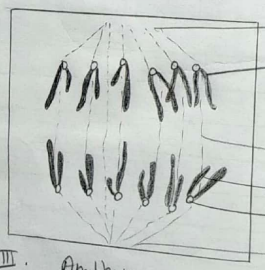
I. Early Prophase



I. Late Prophase



II. Metaphase



III. Anaphase

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OBJECT ⇒ To Study the various stages of mitosis.

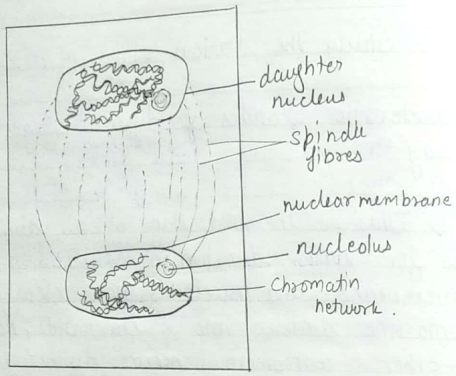
The characterstic features of different stages of mitosis are as following →

I. PROPHASE ⇒ This is the first stage of cell division or mitosis and continuous for longer duration in comparision to other stages.
- Nuclear membrane and nucleolus are clearly observed.
- The chromosome divides into 2 chromatids, both are attached to each other & centromere become distinct late prophase.
- In later prophase, nuclear membrane and nucleous gradually disappear.
- Spindle fibres appears on both the ends.
- The zone of equitorial plate gradually become distined.

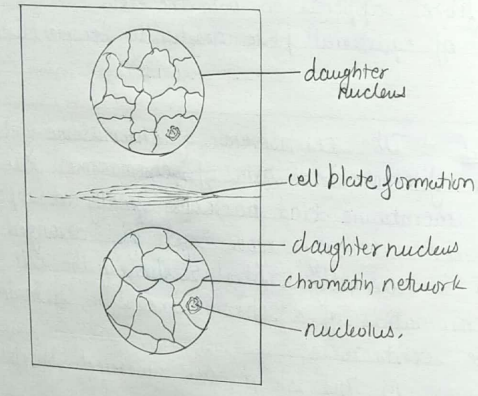
II. METAPHASE ⇒ The centromere of chromosome get attaches to spindle fibres and arms of chromosomes are free.
- nuclear membrane and nucleolus finally disappears.
- Chromosomes actively move and get arranged on equitorial plate & is usually central region of the cell.
- Both chromatids of a chromosome still remain undivided at the centromere.
- Chromosomes in this stage are observed shortest and thickest.

III. ANAPHASE ⇒ The centromere of each chromosome also get divided into 2 parts and due to this both the chromatid get separated.

Teacher's Signature :



IV. Telophase



V. Cytokinesis

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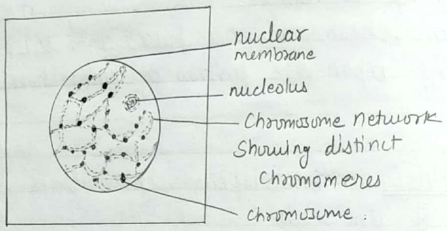
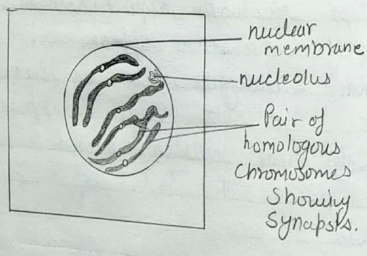
- These chromatids are know known as chromosome.
- These chromosomes or daughter chromosomes move at opposite poles due to contraction of spindle fibres.
- Movement of chromosomes to the opposite poles takes place due to repulsion c centromere.
- ultimately chromosomes are pulled into V, L, T or I shapes. depending upon the position of kinetochore.

IV TELOPHASE ⇒ In telophase chromosomes shift to the opposite poles of the cells.

- Chromosomes gradually uncoil and become long and cylinder finally they become long and cylinder.
- Gradually chromosomes form chromatin network.
- Nuclear envelope gradually appear around each group of chromosomes. Nucleolus also appear.
- In this manner 2 daughter nuclii are formed.
- Spindle fibres and astral rays disappear.
- ultimately 2 daughter cells are formed.



(a). Leptotene



(b). Zygotene

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Object :→ To Study various stages of meiosis.

(A). **MEIOSIS** ⇒ This is also known as reduction division in c prophase-I, metaphase-II, Anaphase-I and Telophase I are included.

This process continues for max^m duration it can be differentiated in 5 substages. These are →

(a) Leptotene
(b). Zygotene
(c). Pachytene
(d). Diplotene
(e). Diakinesis

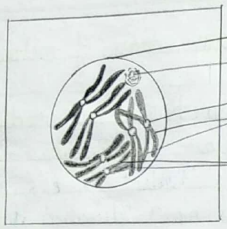
(a). **Leptotene** ⇒ In this stage chromosomes appear long thread like structure c are interweave.

- Chromosomes are present on chromosome.
- Chromosome doubling takes place but chromatids are very closely present, therefor each chromosomes appears like a thread.
- Nuclear membrane and nucleolus are distinct.

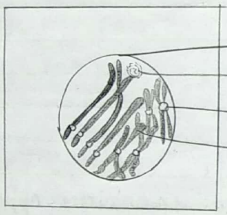
(b). **Zygotene** ⇒ The homologous chromosome come to lie side by side in pairs. This pairing of chromosomes is known as synapsis.

- The structure is formed during the process of Pairing or Synapsis is known as synaptonemal complex.
- The process of synapsis is like and may start at one or more points of chromosomes.
- Synapsis takes place at particular point segments of 2 chromosomes.

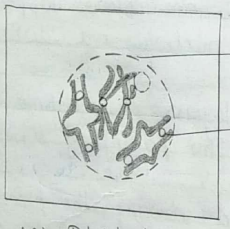
Teacher's Signature :



(c) Pachytene



(d) Diplotene



(e) Diakinesis.

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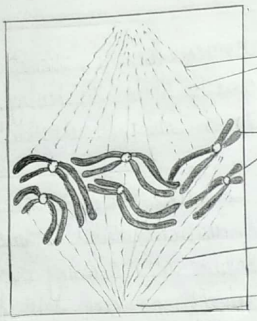
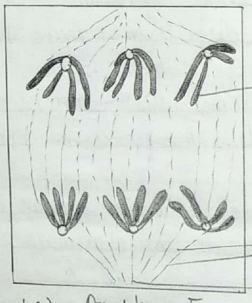
(c) Pachytene ⇒ The chromosomes become short and looked become more distinct due to their thickening.
- The homologous chromosomes closely adhesion together they are known as bivalents.
- In each chromosome of a bivalent there 2 chromatids i.
- Recombination or exchange in chromatid segments takes place during this stage. It's known as crossing over.
- Nucleolus and nuclear membrane are still present.

(d) Diplotene ⇒ The homologous chromosomes start seperating from each other due to some repulsion.
- This process is known as disjunction. it makes the chromatids more distinct towards very clear.
- The separating of chromosomes starts on centromere and proceds towards the ends. This process is known as terminalization.
- The homologous chromosomes are a seperate c each other at certain points ē are known as chaismata.

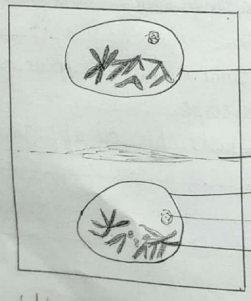
(e) Diakinesis ⇒ The chromosomes (Bivalent) are once again condensed to aquire their characteristic size and form.
- Nucleus is disappeared.
- The chaismata become terminal or disappear by sliding towards periphary near nuclear envelope.
- Nuclear membrane now starts to disappear gradually.



(B). Metaphase I



(C). Anaphase I



(d) Telophase I

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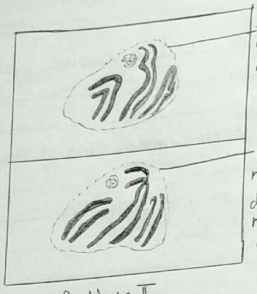
(B). METAPHASE-I ⇒ Nuclear membrane and nucleolus get disappeared completely.
- Bivalents are arranged on the equitorial plate.
- Spindle fibers get developed and each chromosome of the bivalent is attached to spindle by centromere.
- Chromosome become most condensed in this stage as a result of which this appear short and thick.
- Centromere remain undivided.

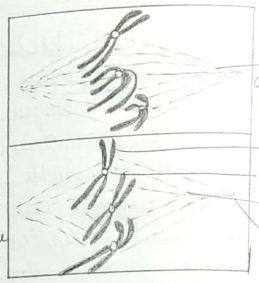
(C). ANAPHASE-I ⇒ The chromosome of a bivalent separate from each other and move towards the opposite pole.
- In this manner, on both sides of equatorial plate a group of chromosome of a bivalent is present in each group.
- each chromosome is having 2 chromatids.
- Chromosome appear V, L or I shaped in this stage.

(D). TELOPHASE-I ⇒ Single group of chromosomes (one each) is located on both the poles.
- The chromosomes become uncoiled and appear like distinct cluster or bunch of threads, on the pole this bunch like structure is known as chromatin.
- Formation of nucleolus and nuclear membrane around the chromatin take place at each other poles.
- Spindle fibre disappear.
- In this manner single daughter nucleus is formed at each pole.
- In each daughter nucleus the no. of chromosome become half in comparision to parent cell. Therefore meiosis-I is also known as reduction Division.

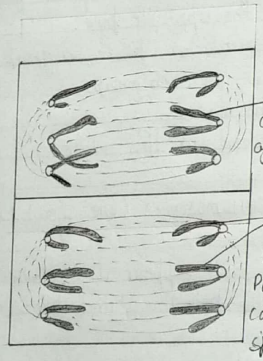
Teacher's Signature : _____



* Prophase II.



* Metaphase II



* Anaphase II

* Telophase II

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MEIOSIS - II

Meiosis II starts after the completion of meiosis-I this stage can be differentiated into following substages.

PROPHASE - II
METAPHASE - II
ANAPHASE - II
TELOPHASE - II

All these sub-stages are similar to the substages of mitosis. Therefore its also known as meiotic mitosis.

Its brief account is as follows →

PROPHASE - II → Disappear of nucleolus and nuclear mem. dehydration and coiling of chromosome.

METAPHASE - II → Chromosomes arranged on equatorial plate formation of spindle fibres.

ANAPHASE - II → Concentration of spindle fibres sepration of chromatids and movement towards opposite site.

TELOPHASE - II → Formation of two daughter nuclei.

Teacher's Signature : .....