

Experimental Protocols of Cell Divisions and Electrophoresis

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1. Aim of The Experiment- To study and demonstrate mitosis by acetocarmine staining of onion root tip cells.

Mitosis-In mitosis, the nucleus of the Eukaryotic cells divides into two, subsequently resulting in the splitting of the parent cells into two daughter cells. Hence, every cell division involves two chief stages:

Cytokinesis – Cytoplasm division, Karyokinesis – Nucleus division

Stages of Mitosis

The various stages of mitosis are:

1. Prophase

- The process of mitosis is initiated at this stage wherein coiling and thickening of the chromosomes occurs
- Shrinking and hence the disappearance of the nucleolus and nuclear membrane takes place
- The stage reaches its final state when a cluster of fibres organizes to form the spindle fibres.

2. Metaphase

- Chromosomes turn thick in this phase. The two chromatids from each of the chromosomes appear distinct
- Each of the chromosomes is fastened to the spindle fibres located on its controller
- Chromosomes align at the centreline of the cell

3. Anaphase

- Each of the chromatid pair detaches from the centromere and approaches the other end of the cell through the spindle fibre
- At this stage, compressing of the cell membrane at the centre takes place [1]

4. Telophase

- Chromatids have reached the other end of the cell
- The disappearance of the spindles

- Chromatin fibres are formed as a result of uncoiling of daughter chromosomes
- The appearance of two daughter nuclei at the opposing ends due to the reformation of the nucleolus and nuclear membrane
- At this phase, splitting of the cell or cytokinesis may also occur.

Post mitosis, the next stage is referred to as interphase which is part of the cell cycle that is non-dividing and between two consecutive cell divisions. A cell spends most of its life in the interphase. It comprises the G1, S and G2 stages.

Onion root tip used to demonstrate mitosis in this experiment because of the meristematic cells that are situated in the tip of the roots that render the most desirable and suitable raw material to study the different stages of mitosis. Onion is a monocot plant. Monocotyledonous plants possess large chromosomes that are clearly visible. Hence, their root tips are used. The period of time taken for mitosis varies as it is dependent on the cell type and type of species.[1]

Materials Required: Compound microscope, Acetocarmine stain, Water, Burner, N/10 Hydrochloric acid, Filter paper, Coverslip, Aceto alcohol (Glacial acetic acid and Ethanol in the ratio 1:3), Glass Slide, Onion root peel, Forceps, Blade, Watch glass, Dropper, Needle, Vial

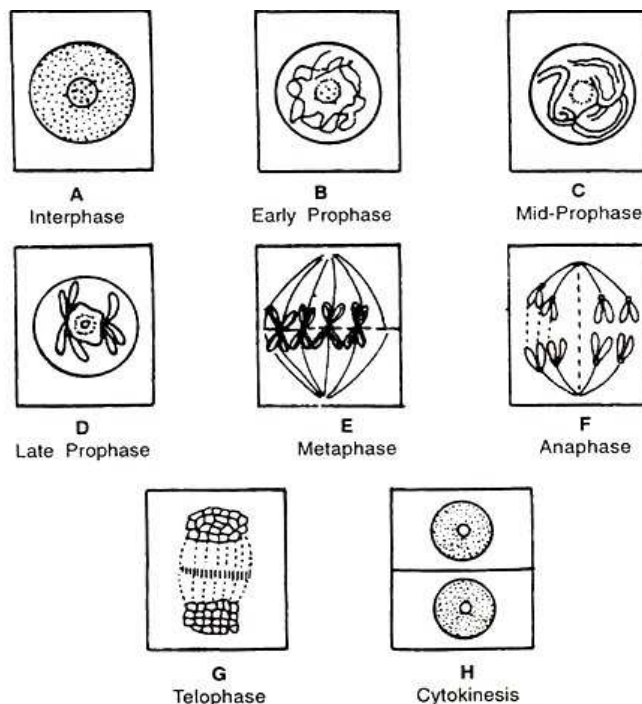
Procedure of The Experiment

- Place an onion on a tile
- With the help of a sharp blade, carefully snip the dry roots of the onion
- Place the bulbs in a beaker containing water to grow the root tips
- It may take around 4 to 6 days for the new roots to grow and appear

- Trim around 3 cm of the newly grown roots and place them in a watch glass
- With the help of forceps, shift it to a vial holding freshly prepared aceto-alcohol i.e., a mixture of glacial acetic acid and ethanol in the ratio 1:3.
- Allow the root tips to remain in the vial for one complete day
- With the help of forceps, pick one root and set it on a new glass slide
- With the help of a dropper, allow one drop of N/10 HCl to come in contact with the tip of the root. Additionally, add around 2 to 3 drops of the acetocarmine stain.
- Heat it lightly on the burner in such a way that the stain does not dry up.
- Excessive stain can be carefully treated using filter paper
- The more stained part of the root tip can be trimmed with the help of a blade.
- Discard the lesser stained part while retaining the more stained section.
- Add a droplet of water to it
- With the help of a needle, a coverslip can be mounted on it
- Gently tap the coverslip with an unsharpened end of a needle in order for the meristematic tissue of the root tip present under the coverslip to be squashed properly and to be straightened out as a fine cell layer
- The onion root tip cells' slide is now prepared and ready to be examined for different stages of mitosis
- Observe and study mitosis by placing the slide under the compound microscope. Focus as desired to obtain a distinct and clear image.

Observations and Conclusion

- The slide containing the stained root tip cells is placed on the stage of the compound microscope, changes taking place are noted and sketched.
- The different phases of mitosis, such as prophase, metaphase, anaphase and telophase can be observed.



2. To observe the stages of meiosis on onion bud cell or grasshopper testis through permanent slides.

Materials Required: Permanent slides of meiosis, Compound Microscope [2]

Procedure

1. Place the slide on the stage of the microscope.
2. Look for dividing cells with lower magnification.

Observations

The different stages of meiosis are observed along on the basis of the following features.

➤ Stages of Meiosis I

Prophase I

In this stage, the chromosomes condense and move towards the centre of the cell. It consists of five different sub-phases:

- Leptotene: The homologous chromosomes replicate.
- Zygotene: Synapsis between homologous chromosomes start.
- Pachytene: The sister chromatids separate but the homologous chromosomes remain attached.
- Diplotene: The two homologous chromosomes migrate apart and disintegrate between the chromosomal arms.
- Diakinesis: The condensation of chromosomes stops at this stage and the chiasmata is clearly visible under an electron microscope. The nucleolus and the nuclear envelope disappear at this stage and the centrosome moves to the equator.

Metaphase I

The homologous chromosomes that contain two different alleles for each gene, line up on the metaphase plate to be separated.

Anaphase I

The separated chromosomes are pulled towards the centrioles on either side of the cell.

Telophase I

The chromosomes are completely pulled apart and new nuclear envelope forms.

Stages of Meiosis II

Prophase II

In this stage, the nuclear envelope disintegrates and centrioles develop.

Metaphase II

The chromosomes line up on the metaphase plate and the chromatids are on either side of the metaphase plate.

Anaphase II

The sister chromatids separate and are known as sister chromosomes.

Telophase II

The cell divides into two and a new nuclear envelope surrounds the chromosomes.[2]

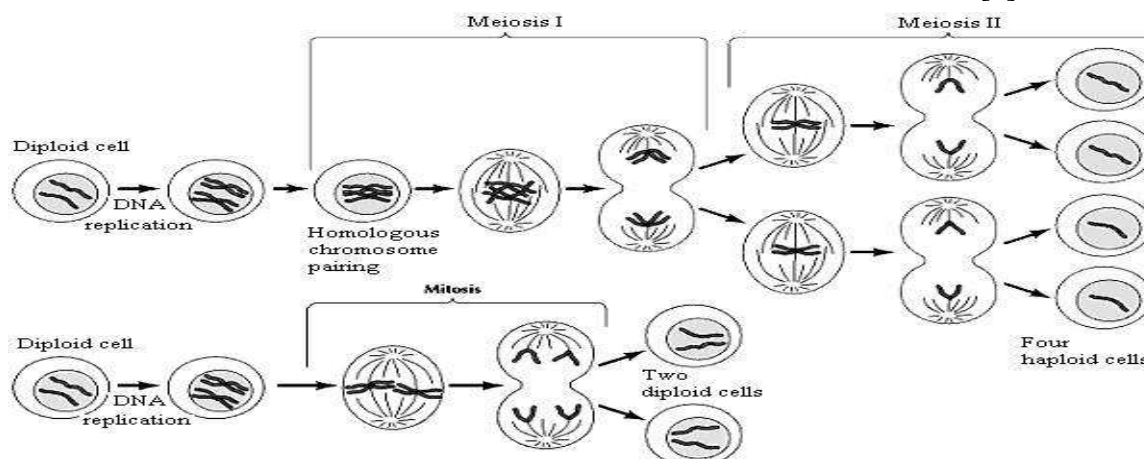


Figure showing meiosis

3. Agarose gel electrophoresis for the separation of DNA

1. Preparation of the Gel

- Weigh out the appropriate mass of agarose into an Erlenmeyer flask. Agarose gels are prepared using a w/v percentage solution. The concentration of agarose in a gel will depend on the sizes of the DNA fragments to be separated, with most gels ranging between 0.5%-2%. The volume of the buffer should not be greater than 1/3 of the capacity of the flask.
- Add running buffer to the agarose-containing flask. Swirl to mix. The most common gel running buffers are TAE (40 mM Tris-acetate, 1 mM EDTA) and TBE (45 mM Tris-borate, 1 mM EDTA).
- Melt the agarose/buffer mixture. This is most commonly done by heating in a microwave, but can also be done over a Bunsen flame. At 30 s intervals, remove the flask and swirl the contents to mix well. Repeat until the agarose has completely dissolved.
- Add ethidium bromide (EtBr) to a concentration of 0.5 µg/ml. Alternatively, the gel may also be stained after electrophoresis in running buffer containing 0.5 µg/ml EtBr for 15-30 min,

followed by destaining in running buffer for an equal length of time.[3]

Note: EtBr is a suspected carcinogen and must be properly disposed of per institution regulations. Gloves should always be worn when handling gels containing EtBr. Alternative dyes for the staining of DNA are available; however EtBr remains the most popular one due to its sensitivity and cost.

- A. Allow the agarose to cool either on the benchtop or by incubation in a 65 °C water bath. Failure to do so will warp the gel tray.
- B. Place the gel tray into the casting apparatus. Alternatively, one may also tape the open edges of a gel tray to create a mold. Place an appropriate comb into the gel mold to create the wells.
- C. Pour the molten agarose into the gel mold. Allow the agarose to set at room temperature. Remove the comb and place the gel in the gel box. Alternatively, the gel can also be wrapped in plastic wrap and stored at 4 °C until use (Fig.1)

2. Setting up of Gel Apparatus and Separation of DNA Fragments

- A. Add loading dye to the DNA samples to be separated (Fig. 2). Gel loading dye is typically made at 6X concentration (0.25% bromphenol blue, 0.25% xylene cyanol, 30% glycerol).

Loading dye helps to track how far your DNA sample has traveled, and also allows the sample to sink into the gel.

- B. Program the power supply to desired voltage (1-5V/cm between electrodes).
- C. Add enough running buffer to cover the surface of the gel. It is important to use the same running buffer as the one used to prepare the gel.
- D. Attach the leads of the gel box to the power supply. Turn on the power supply and verify that both gel box and power supply are working.
- E. Remove the lid. Slowly and carefully load the DNA sample(s) into the gel (Fig. 3). An appropriate DNA size marker should always be loaded along with experimental samples.
- F. Replace the lid to the gel box. The cathode (black leads) should be closer the wells than the anode (red leads). Double check that the electrodes are plugged into the correct slots in the power supply.
- G. Turn on the power. Run the gel until the dye has migrated to an appropriate distance.

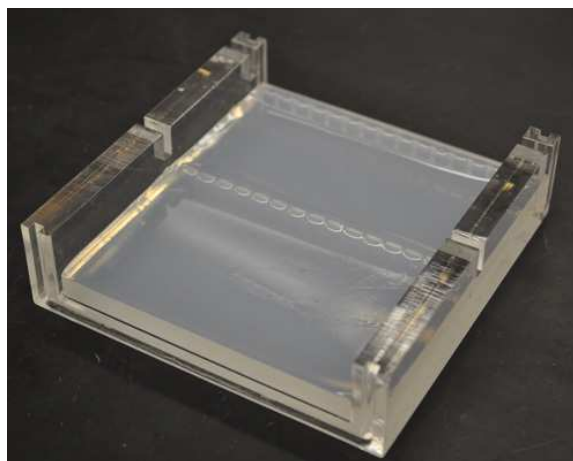


Figure 1. A solidified agarose gel after removal of the comb.



Figure 2. A student adding loading dye to her DNA samples.

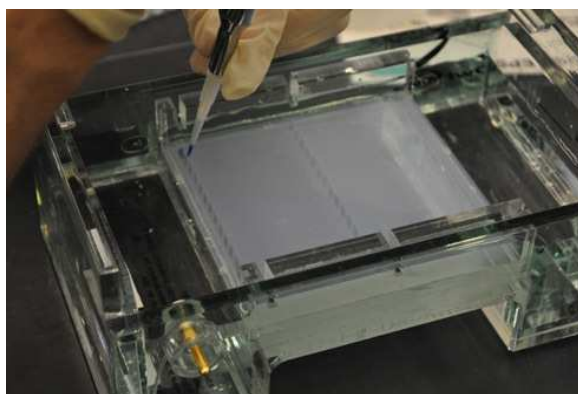


Figure 3. A student loading the DNA sample into a well in the gel.

3. Observing Separated DNA fragments

- A. When electrophoresis has completed, turn off the power supply and remove the lid of the gel box.
- B. Remove gel from the gel box. Drain off excess buffer from the surface of the gel. Place the gel tray on paper towels to absorb any extra running buffer.
- C. Remove the gel from the gel tray and expose the gel to uv light. This is most commonly done using a gel documentation system (Fig. 4). DNA bands should show up as orange fluorescent bands. Take a picture of the gel (Fig. 5).
- D. Properly dispose of the gel and running buffer per institution regulations.[4]

4. Representative Results

After separation, the resulting DNA fragments are visible as clearly defined bands. The DNA standard or ladder should be separated to a degree that allows for the useful determination of the sizes of sample bands. In the example shown, DNA fragments of 765 bp, 880 bp and 1022 bp are separated on a 1.5% agarose gel along with a 2-log DNA ladder.



Figure 4. An example of a gel documentation system.

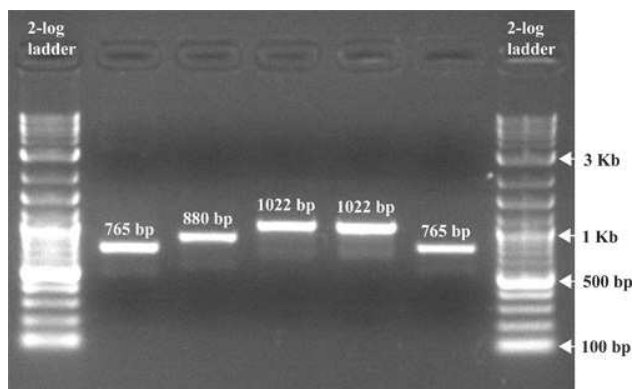


Figure 5. An image of a gel post electrophoresis.

EtBr was added to the gel before electrophoresis to a final concentration of 0.5 µg/ml, followed by separation at 100 V for 1 hour. The gel was exposed to uv light and the picture taken with a gel documentation system.

4. Aim: RNA gel electrophoresis

Reagents and Materials: for preparation: tank, tray, comb Diethylpyrocarbonate (from Sigma, cat. nr. D-5758); 0.1% DEPC (Diethylpyrocarbonate) H₂O: mix 1 ml DEPC in 1000 ml H₂O and autoclave. normal melting agarose powder, 10 Stainingx TBE buffer solution, gel stain (Eco Safe Nucleic Acid Solution) microwave, Erlenmeyer flask, measuring cylinder, scales; for loading: pipette, PCR tubes or tinfoil, power supply for documentation: gel documentation system[5]

Gel preparation:

1. Prepare sufficient 1 x TBE electrophoresis buffer (1:10 dilution of TBE:DEPC H₂O) - Clean all tools using DEPC H₂O. - Position the comb 0.5-1 mm above the plate so that a complete well is formed when the agarose is added.
2. Prepare agarose gel for a 1.2% agarose gel: 1.2 g agarose / 100 ml 1 x TBE buffer in Erlenmeyer flask - Cover the flask with kimwipes/ parafilm and heat with microwave until the agarose dissolves. Measure it again and complete the evaporated liquid with distilled water. - Leave it to cool down to about 60 °C on the bench for several minutes but do not leave it too long so the agarose should not start to solidify. - Stain the agarose solution: 5 µl ECO Safe Nucleic Acid Staining Solution / 100 ml gel - Mix the agarose solution well by swirling the flask. Pour the agarose into the mold. (3-5 mm thickness)
3. After 30 minutes at room temperature carefully remove the comb. - Position the gel into the gel electrophoresis tank. Avoid bubbles!

- Add enough TBE buffer to cover the gel to a depth of about 5 mm. Loading:
 1. Mix the samples of RNA with gel-loading buffer with pipettes: - 0,5 µg RNA - 2 µl loading dye to 10 µl RNase – free water [6]
 2. Load the mixtures slowly into the slots. Avoid making bubbles!
 3. Attach the electrical leads so that RNA can move toward the anode. (Red lead)
 4. Apply a voltage of 1-5 V/cm.
 5. Run the gel until the gel-loading buffer stain have migrated the appropriate distance (normally until the bromophenol blue dye front migrated ¾ of the way down the gel).

Documentation:

1. Turn off the current and remove leads.
2. Examine the gels: Carefully put it on an ultraviolet transilluminator and take a photo. Technical appendix: Type of Agarose: normal melting point, molecular grade
 - Agarose solution: The excess solution can be stored at 4 °C until later use.
 - 10 x TBE solution (per liter): from molecular grade reagents
 - + 108 g Tris + 55.65 g boric acid + 40 ml 0.5M EDTA (pH 8.0) stored at room temperature Gel stain: Eco Safe Nucleic Acid Staining Solution (Pacific Image Electronics)
 - Loading dye (6X concentration):
 - 0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol in water store at 4 °C[7]

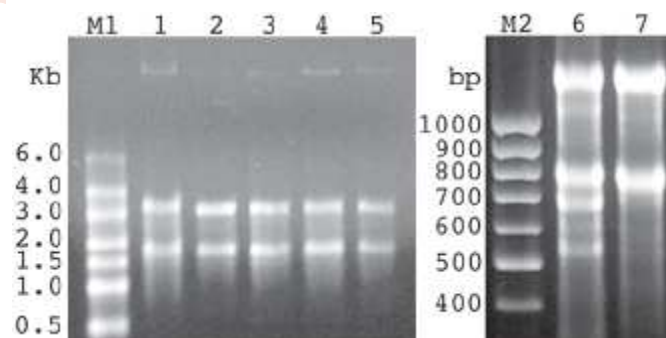


Figure showing RNA gel electrophoresis

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