Guidance for Topic 1 – Practical 1

Stages of mitosis in a root tip

Safety

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Guidance

The purpose of this practical is to enable students to observe chromosomes in dividing cells found in the meristem tissue of root tips of garlic or onion ($2n = 16$) or of the broad bean ($2n = 12$). The three-dimensional structure of the meristem is disrupted and a single layer of cells separated out. The middle lamella holding cells together is dissolved by hydrochloric acid, which does not damage the cellulose cell wall. The nuclei of the cells are stained with Feulgen stain, which does not stain the cytoplasm.

Apparatus and materials

Each student or pair will need:

- garlic bulb (or onion or beans)
- boiling tubes
- three test tubes with stoppers
- two Petri dishes
- fine forceps
- water bath at 60 °C
- 1 mol dm^{-3} hydrochloric acid (5 cm^3)
- Feulgen stain
- distilled water
- acetic alcohol (10 cm^3)
- mounted needles
- slides and coverslips
- tissue or blotting paper
- microscope

Setting up the practical

Roots need to be grown in preparation for this practical. For garlic, this is most easily done by cutting small holes in a polystyrene tile, pushing garlic cloves part way through these holes and then floating the tile in a tray of water. This is safer than pushing the clove into the top of a boiling tube as the combination of water uptake by the clove and water loss by evaporation can result in the developing roots drying up. Alternatively, a whole garlic bulb can be suspended over a boiling tube full of water for about 4 days, as described in the student's practical notes. Best results are obtained if disturbance is kept to a minimum.

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When the roots are about 2 cm long, remove 1 cm from the end. Fix the root material in a closed test tube of acetic alcohol overnight. Remove the root tips using fine forceps and wash in distilled water. Place the root tips in a test tube and cover with 1 mol dm^{-3} hydrochloric acid for about 5 minutes (slightly longer for bean roots) in a water bath at 60 °C. This treatment removes the middle lamellae and hydrolyses DNA. Transfer the root tips to a Petri dish and wash with distilled water. Transfer the root tips to a tube containing Feulgen stain. Seal the tube and keep cool for about 3 hours.

NB *Teachers may prefer technicians to carry out the preparatory stages of this practical so that students can concentrate on observing the stages of mitosis and drawing.*

Supporting the practical

It may help students if reference books or other visual materials are available so that they can check the various stages of mitosis that they observe through the microscope.

Answers to questions

1 Students make an appropriate drawing based on their observations.

2 20 mm = 20 000 μm

magnification = 20 000 ÷ 2 = ×10 000

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Guidance for Topic 1 – Practical 2

Demonstrating osmosis

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Guidance

In this practical, students use Visking tubing to represent a membrane. Visking tubing allows small molecules to pass easily through it while larger molecules are held back.

Apparatus and materials

This experiment can be carried out as a demonstration. The sugar solution can be coloured with food colouring to make the results very obvious.

Each student or pair will need:

- glass capillary tube 75 cm in length
- Visking tubing (approximately 25 cm)
- concentrated sugar or salt solution, prepared by dissolving table sugar or salt in water (approximately 30 cm^3)
- elastic band or wire
- 600 cm^3 glass beaker of water

Setting up the practical

The apparatus is set up as shown in the diagram in the student's practical notes. Visking tubing can be difficult to knot and seal effectively to prevent leakage so junctions should be checked.

Supporting the practical

Encourage students to make careful observations of the apparatus before the experiment, as well as afterwards, in order to note any changes. Some means of marking the level of the liquid surface in the capillary tube would be helpful.

Answers to questions

1 Students should be able to explain that only water molecules move and that the process by which they do this is osmosis. Osmosis results in the Visking tubing bag becoming turgid as water moves into it, and the excess liquid being pushed up the capillary tubing so that the surface level rises.

2 Students might suggest that since diffusion and osmosis are affected by the concentration gradient, different concentrations of sugar solution would result in different levels in the capillary tubing being achieved. They might also suggest that the rate of movement of water molecules

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would be different at different sugar concentrations and could design an extension to the practical to investigate this.

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Guidance for Topic 1 – Practical 3

Measuring cells and their nuclei

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Guidance

This practical is designed to familiarise students with using microscopes, eyepiece graticules and a stage micrometer. As these are expensive, students should be supervised as they use the equipment. Students prepare a temporary mount of onion cells to examine. Students should come to understand that cells vary in size and that for accuracy a number of cells must be measured to obtain an average. They should understand that nuclei are more or less constant in size unless cells are dividing and that the size of a cell is restricted by the diffusion distance from the cell nucleus to other parts of the cell.

Apparatus and materials

Each student or pair will need:

- microscope with magnification up to ×600
- eyepiece graticule
- stage micrometer
- scalpel
- board or tile for cutting
- hollow glass block
- fine forceps
- onion
- few drops 1 mol dm^{-3} hydrochloric acid
- toluidine blue stain
- microscope slide
- coverslip
- tissue or blotting paper
- 250 cm^3 beaker

Setting up the practical

Toluidine blue is an irritant and 1 mol dm^{-3} hydrochloric acid, although low hazard, can be harmful in the eyes or in a cut. Students should wear eye protection when using these substances. Only tiny quantities are needed so they are best supplied to the students in small dropping bottles.

Supporting the practical

Students should be supported in working out the calibration of the eyepiece graticule, which many find difficult.

Clearing up

Sharp instruments should be separated from glasswear for washing up.

Answers to questions

1 There is no correlation between the length and width of a cell and the diameter of the nucleus.

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2 Nuclei contain the same genetic material in every cell, and so will have a fairly constant diameter. Cell length and width will vary depending on the position of the cell in the tissue and its stage of growth.

3 Ten cells are measured so that an average value for each measurement can be obtained. This will improve the accuracy of the overall results used to answer question **1**.

4 Students may suggest taking more than one measurement of length for a cell, as onion cells are not perfect rectangles. Likewise, the diameter of each nucleus could be measured more than once, as nuclei are not perfect circles.

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Guidance for Topic 1 – Practical 4

Observing feeding in Paramecium

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Guidance

Students will be able to observe the process of feeding in *Paramecium*, or other available ciliates, including digestion and formation of food vacuoles. In addition, they will be able to observe other structures such as nuclei, contractile vacuoles and cilia under the light microscope.

Apparatus and materials

Each student or pair will need:

- yeast cells
- culture of *Paramecium* sp. or *Spirostomum teris*
- microscope
- depression slide
- coverslips
- petroleum jelly

Setting up the practical

Students will need a yeast suspension, which is prepared using about 2 g dried yeast in 100 cm^3 water. *Paramecium* sp. or *Spirostomum teris* can be obtained from biological suppliers and kept in culture for a few days. *S. teris* is a larger organism than *Paramecium* and may be easier for less-experienced students to see. If the organisms are fast moving they can be immobilised by adding a little cotton wool to the slide.

Supporting the practical

Students will need reference texts or diagrams to help them label the parts of their organisms. There is good visual material showing *Paramecium* digesting yeast cells stained with Congo red available on **www.youtube.com** and similar websites. This stain is no longer permitted for use in school laboratories but demonstrates the process of digestion well because it changes colour as pH changes in the vacuole.

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