

Teaching Pack

Investigating the effect of temperature on the permeability of plant cell membranes

Cambridge International AS & A Level
Biology 9700

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Icons used in this pack:



Briefing lesson



Planning lesson



Lab lesson



Debriefing lesson

Introduction

This pack will help you to develop your learners' experimental skills as defined by assessment objective 3 (AO3 Experimental skills and investigations) in the course syllabus.

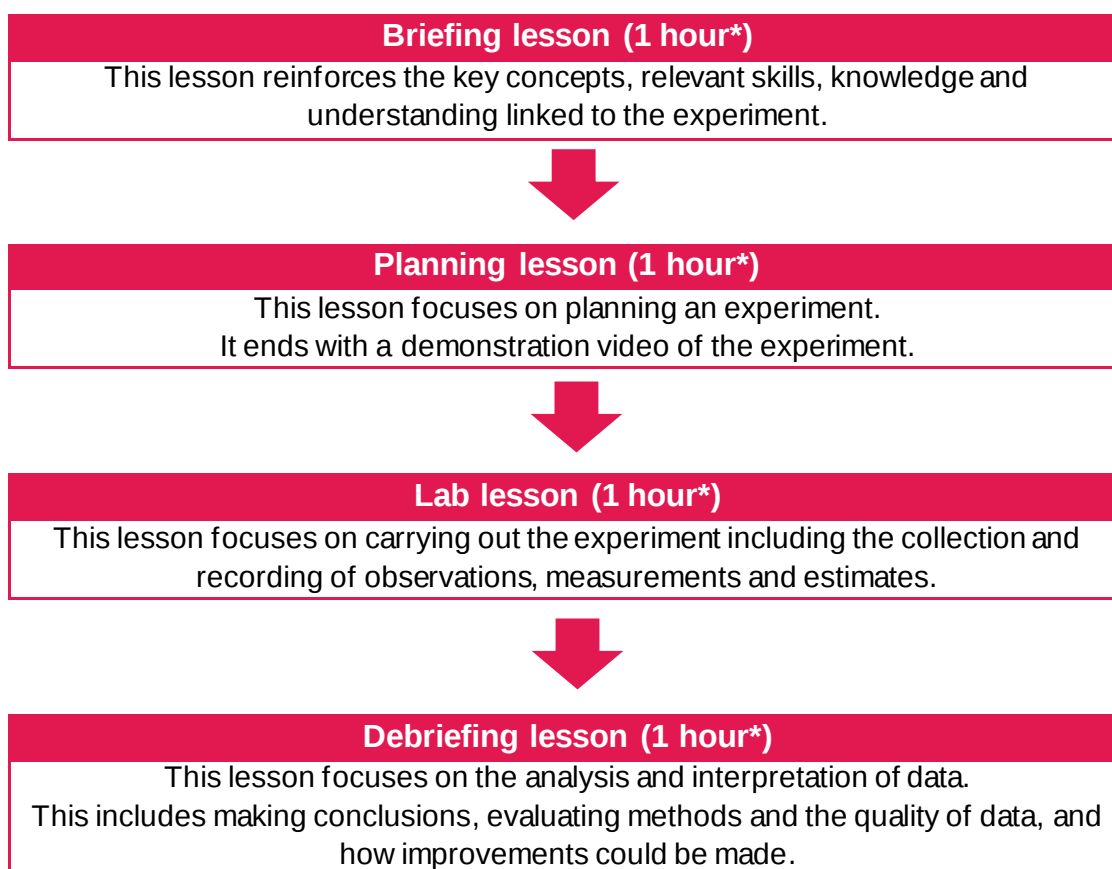
Important note

Our *Teaching Packs* have been written by **classroom teachers** to help you deliver topics and skills that can be challenging. Use these materials to supplement your teaching and engage your learners. You can also use them to help you create lesson plans for other experiments.

This content is designed to give you and your learners the chance to explore practical skills. It is not intended as specific practice for Paper 3 (Advanced Practical Skills) or Paper 5 (Planning, Analysis and Evaluation).

This is one of a range of *Teaching Packs* and each pack is based on one experiment. The packs can be used in any order to suit your teaching sequence.

The structure is as follows:



** the timings are a guide only; you may need to adapt the lessons to suit your circumstances.*

In this pack, you will find lesson plans, worksheets and teacher resource sheets.

Experiment: Investigating the effect of temperature on the permeability of plant cell membranes

This *Teaching Pack* focuses on an investigation into the effect of temperature on the permeability of plant cell membranes in the beetroot.

The cell membrane forms an effective barrier that separates the contents of a cell from its external environment. It is described as fluid, in that the molecules can move relative to each other, and mosaic in that different types of molecules – including phospholipids, proteins and cholesterol – are interspersed. The fluidity of the membrane, and its protein components, explains why at high temperatures the structure loses its integrity and allows cell contents to escape into its surroundings, including the red pigment in the beetroot.

This experiment has links to the following syllabus content (see syllabus for detail):

- 4.1. Fluid mosaic membranes
- 4.2. Movement into and out of cells

The experiment covers the following experimental skills, as listed in **AO3: Experimental skills and investigations**:

- plan experiments and investigations
- collect, record and present observations, measurements and estimates
- analyse and interpret data to reach conclusions
- evaluate methods and quality of data, and suggest improvements.

Prior knowledge

Knowledge from the following syllabus topics is useful for this experiment.

- 2.2. Carbohydrates and lipids
- 4.2. Movement of substances into and out of cells

Briefing lesson: Seeing the invisible



Resources

- Worksheets A and B
- Teacher instructions 1, 2 and 3
- Items to represent components of a fluid mosaic membrane, for examples see Teacher Instructions 1
- 250 cm³ beaker containing 150 cm³ tap water
- Bunsen burner, tripod, gauze, 2 × heat-proof mats
- Vegetable that releases chlorophyll (green pigment) upon boiling, e.g. broccoli or spinach
- Whole beetroot (or suitable alternative)
- Cork borer/sharp knife
- White tile
- 6 large ice cubes containing red food colouring
- 2 × very small plastic, 'zip-lock' 'sandwich' bags, with 15–20 holes punched in each
- 2 × 500 cm³ beakers, one containing 300 cm³ water at 60–70°C and the other 300 cm³ water at 5–10°C
- 2 × thermometers

Learning objectives

By the end of the lesson:

- **all** learners should be able to describe the structure and key properties of the cell membrane
- **most** learners should be able to explain how an immeasurable factor can be investigated indirectly by measuring another factor with which it is strongly correlated
- **some** learners will be able to evaluate models to explain how they represent a scientific concept.

Timings

Activity



Starter/Introduction

Give pairs of learners [Worksheet A](#). Challenge them to model the arrangement of molecules in a fluid mosaic membrane using the items provided (see [Teacher Instructions 1](#)). Explain that this task gives them the opportunity to construct a visual representation of a mechanism that cannot be seen with the naked eye. Take a photo of some of the models so you can use them in the *Planning lesson*.

If time allows, challenge learners to describe to each other how their models illustrate key properties of the cell membrane such as:

- how it self assembles due to the amphipathic nature of the phospholipids
- is fluid (items can move in relation to each other)
- is a mosaic (items are scattered and interspersed).

Circulate the class to give support and to stretch learners' thinking by asking further questions (extension questions and items are given on Teacher Instructions 1).

Timings	Activity
	Main lesson Teacher demo: Gather learners around a demonstration table. Show how a piece of broccoli (or spinach leaves) cause the colour of the water to gradually turn light, then dark, green during heating. For instructions see, Teacher Instructions 2. During the demo, pose the following questions: <i>‘What is the green pigment that is colouring the water?’; Why does chlorophyll not enter the water in cold conditions?; Why does the chlorophyll gradually begin to leave the broccoli cells as the water heats up?; Why does the water become suddenly much darker after about 60°C?</i> Ask learners to discuss with a partner. If necessary, refer them back to the model they produced at the start of the lesson and provide clues to scaffold thinking, such as <i>‘chlorophyll is only found in chloroplasts, which are only found inside cells; heating the water increases the kinetic energy of molecules within the cell and the cell membrane’</i>. Pairs of learners should then form a group of four with another pair to continue the discussion (in a ‘snowballing’ technique). Ask learners to think about how they might improve their answers now that they’ve discussed them with others. Elicit that the chlorophyll cannot leave the plant cells until the membranes have either been made more fluid, or have been damaged. Explain that as the permeability of the membrane increases (due to the kinetic energy of the molecules in the membrane creating gaps between them), more and more chlorophyll is able to diffuse out of the cell (chlorophyll molecules will also have increased kinetic energy and therefore are more likely to diffuse out of the cell through the gaps in the membrane). Beyond a certain temperature (around 60–70°C), the surrounding water suddenly becomes a much darker green as the membrane proteins denature, collapsing the membrane structure so that chlorophyll leaves the cells in great quantities. Ask learners how this behaviour might be useful when investigating the effect of environmental factors on membranes in a laboratory. Develop the idea that this allows us to indirectly determine the integrity of cell membranes, which we cannot observe directly. Prompt discussion between learners to get to this point and ask them how and why different environmental factors affect the integrity of the structure. An extension question would be to ask what they would predict to see if the vegetable had been placed into ice-cold water, and to explain their predictions.  Show learners a beetroot (or suitable alternative if not available) and give them Worksheet B. Inform them that the cells of beetroot plants contain the water-soluble, dark-red pigment betalain in their vacuole. Prompt discussion to elicit the idea that the intensity of the red colour in the water can be used as an indicator of the integrity of the cell membrane, and hence can be used to assess the effect of a factor on this structure. Cut a slice from the beetroot, or use a cork borer to extract a cylinder, and elicit from learners a simple protocol that could be used to investigate membrane permeability. Learners then spend 10 minutes completing the summary activity on Worksheet B based on the demonstration.

Timings	Activity
 A circular timer icon with 15 dots around the perimeter. The number '15' is in the center, with 'min' below it.	Plenary Teacher demo: Gather learners around a demonstration table. Show them a model of betalain leakage from a beetroot cell as per the guidelines on Teacher Instructions 3. Ask them what each part of the model represents: • the plastic bag is the cell membrane • the water inside the bag is the cytoplasm • the ice-cube is the vacuole containing betalain • the water outside of the bag is the external environment of the cell. Challenge learners to discuss what they have seen. As a class, they should decide how the model does and does not accurately illustrate betalain leakage at a molecular level. Write down suggestions on the board for the whole class to see.

Planning lesson: Accuracy, reliability and validity



Resources	• 'Investigating the effect of temperature on the ...' video • Worksheets C, D and E • Tray containing all equipment as per Teacher notes (ideally one tray per pair of learners)
Learning objectives	By the end of the lesson: • all learners should be able to describe what is meant by the terms <i>accuracy</i>, <i>reliability</i> and <i>validity</i>, as applied to data collected in investigations • most learners should be able to explain how and why the choice of procedure in an investigation can affect the accuracy, reliability and/or validity of data • some learners will be able to plan and evaluate a procedure to enhance the accuracy, reliability and/or validity of data collected in an investigation.
Timings	Activity
 15 min	Starter/Introduction Give pairs of learners Worksheet C, which shows a set of scores from a competition between five archers. Give learners a couple of minutes to consider the five scores, then ask 'Who was the best archer and why?' Have a class discussion and make sure you bring in the following five key terms that are important when considering the quality of data collected in a scientific investigation: validity, reliability, accuracy, random errors and systematic errors; make sure learners understand what each term means. To emphasise how this ties in with scientific experiments, explain that 'hitting the centre target' is equivalent to obtaining the true value through an investigation that involves repeats, precise use of calibrated equipment and controlling other factors that would otherwise invalidate the results. Give each learner a copy of Worksheet D and ask them to consider the answers to each question on their own, before sharing their answers in a whole-class discussion. Resolve any misconceptions.
 30 min	Main lesson Tell learners that in their next lesson they are going to investigate the effect of temperature on the permeability of beetroot cell membranes. Explain that they need to use what they have learned so far to help them devise a method to follow. Show learners the equipment required for the practical. You might need to discuss and explain how a colorimeter works and what the measure (absorbance) represents in the context of their investigation, i.e. the greater the absorbance the more concentrated the red betalain pigment is in the solution. Give each learner Worksheet E and explain that they need to choose one option from each pair of alternatives given, stating if the choice would enhance the validity (V), reliability (R) or accuracy (A) of the data they collect. They must justify their choices. They also need to consider how they will carry out the experiment to minimise the possibility of random and systematic errors. It is important that the learners explore the equipment provided to act as a stimulus to help them make their method choices. Emphasise that the knife/scalpel should be handled with extreme care.

Timings	Activity
	Plenary Play the '<i>Investigating the effect of temperature on the permeability of plant cell membranes</i>' video and explain that this shows someone carrying out the experiment. Challenge learners to correct and modify their method / answers on their copy of Worksheet E. For step 20, discuss the advantages and disadvantages of each option. Explain that there isn't time for them to repeat the whole experiment, so they will have to pool the class data in order to calculate the mean. The completed Worksheet E should be read and reviewed for homework in advance of conducting the practical task next lesson; alternatively, it could be collected by you for formative assessment and to ensure that the class has a common understanding of the task ahead. Inform learners that they will begin the practical work immediately at the beginning of the next lesson. Make sure that all learners have a clear idea of the method and the underlying purpose of each step prior to the <i>Lab lesson</i>.

Lab lesson: Getting practical



Resources	- Completed Worksheet E - Graph paper (1 sheet per learner) - Equipment as per <i>Teacher notes</i> - Access to common spreadsheet or whiteboard markers
Learning objectives	By the end of the lesson: all learners should be able to undertake an investigation into the effect of temperature on cell membrane permeability most learners should be able to obtain accurate, reliable and valid data in an investigation into the effect of temperature on cell membrane permeability some learners will be able to suggest how aspects of the method used to investigate the effect of temperature on cell membrane permeability could be improved.

Timings	Activity
 45 min	Main lesson Make sure you are happy with learners' Worksheet E, as they will use this to carry out the experiment. Make sure they understand why each option was chosen. Ask learners to identify any aspects of the method they find problematic and to make a note of these as they go. They should write down possible solutions that they could employ to these problems, if they were to perform the practical again. This will develop their evaluative skills and prepare them for the task in the <i>Debriefing lesson</i>. Ask learners to record the mean absorbance for each sample on a common class spreadsheet or on the whiteboard. (The mean from the repeated readings of the same sample in step 18.) Safety Circulate the classroom at all times during the experiment so that you can make sure that your learners are safe and that the data they are collecting is accurate.
 15 min	Plenary Discuss how the class data can improve reliability (by effectively taking repeats of each temperature), illustrate anomalous data points, and show significant differences between data collected at different temperatures. However, discuss the possible impact of different people carrying out the experiment. Ask learners to calculate the mean value of light absorbed and the standard error for each temperature using the class data (step 20). Worksheet F contains the required formulae and support for these calculations. Ask what kind of graph they should plot of their data. Elicit that as the data is continuous, they should draw a line graph. Ask if the points should be joined by a series of straight lines, a smooth curve, or a line/curve of best fit and why. Ask them to draw their graph and calculate the 95% confidence interval values in order to add the error bars for each temperature; Worksheet F provides support for this. Learners could undertake this section of the task for homework (they would need to take a photograph, or save a copy, of the class data).



Teacher notes

Watch the *Teacher walkthrough* video and read these notes.

Each group will require:

- 6 × water baths (5°C, 25°C (or room temperature), 40°C, 60°C, 75°C and 90°C)
- colorimeter set to transmit blue-green light (520–570 nm)
- 1 half of a raw beetroot
- 6 × thermometers (0–100°C), one for each water bath
- scalpel
- sharp knife
- white ceramic tile
- piece of white card or paper
- small sieve
- tweezers
- ruler (length 30 cm, with mm graduations)
- distilled water in wash bottle
- measuring cylinder (volume 10 cm³)
- 6 × boiling tubes
- boiling tube rack (with 6 spaces)
- 6 × cuvettes
- paper towels
- marker pen
- petri dish/lid
- cork borer (size 4–6)
- dropping pipette

Safety

The information in the table below is a summary of the key points you should consider before undertaking this experiment with your learners. The information is **not** exhaustive and does not include storage or handling instructions.

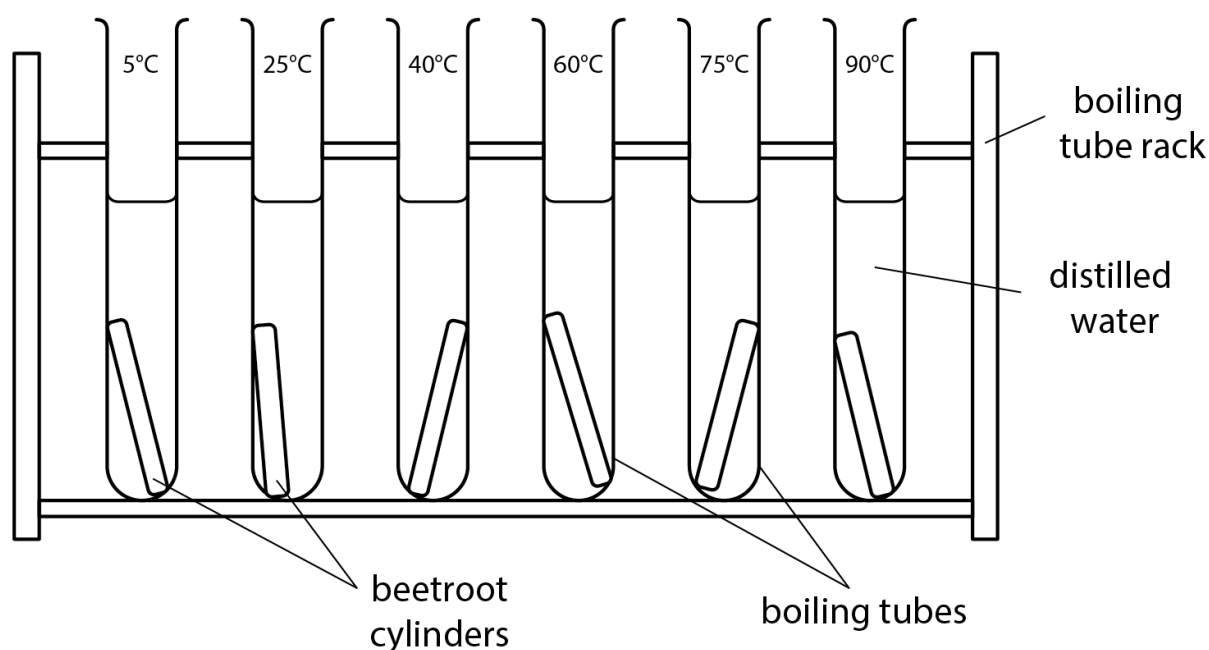
Learners should always wear gloves, eye protection and lab coats. There should not be any eating or drinking in the lab. Hands should be washed thoroughly at the end of the experiment.

It is your responsibility to carry out an appropriate risk assessment for this experiment.

Hazard	First aid
Allergies – food	Do not consume any foodstuffs in the labs. If discomfort persists see a doctor.
Allergies – latex gloves	Remove the gloves and wash hands under water. Look out for severe allergic reactions such as difficulty breathing and/or swelling of the face, body or tongue. Seek emergency medical attention immediately.

Hazard	First aid
Burns	Flood burnt area with water for at least 10 minutes. For serious injuries see a doctor.
Cuts or puncture wounds due to sharps (scalpels, knives, dissection scissors, cork borers, mounted needles, broken glassware). Wounds can lead to infection, especially if the blade or point is contaminated.	Minor cuts: Rinse the wound with water. Get the casualty to apply a small, sterile dressing. Severe cuts: Lower the casualty to the floor. Raise the wound as high as possible. If feasible, ask the casualty to apply pressure on or as close to the cut as possible, using fingers, a pad of cloth or, better, a sterile dressing (adding further layers as necessary). If the casualty is unable to do so, apply pressure yourself, protecting your skin and clothes from contamination by blood if possible. Leave any embedded large bodies and press around them. Send for a first aider.

Experiment set-up





Teacher method

This is the version of the method that accompanies the *Teacher walkthrough* video.

Before you begin

Plan how you will group your learners during the experiment session.

Think about:

- the number of groups you will need (group size 2–3 learners)
- the amount of equipment/chemicals required
- the questions you could pose to learners during less active periods in the practical task, for example during incubation periods or during the clean-up (some examples are provided on Teacher Instructions 4).

Experiment

Walk around the learners during the experiment in case they encounter any difficulties.

Steps	Notes
1. Learners should collect the equipment they require from the front of the class.	<i>Asking different learners to collect different items can improve the smooth running of the practical task.</i>
2. They should find a space in the classroom where the equipment can be assembled safely.	<i>Learners should remain standing for the duration of the investigation, due to the use of hot water, which could scald if spilt.</i>
3. Make sure your learners are briefed with regard to aspects of safety, including the hazard warning symbols associated with the sharp knife, and the water baths.	<i>You could provide learners with beetroots already cut into halves in order to minimise the risk of using sharp knives. Ensure clear notices are placed on each water bath to show its temperature.</i>
4. Learners should measure 20 cm ³ of distilled water into each of six boiling tubes.	<i>This should be done using a small measuring cylinder or a syringe.</i>
5. Learners should then place each boiling tube into a different water bath for 10 minutes.	<i>The purpose of this step is to ensure the water reaches the required temperature.</i>
6. Learners should carefully cut a beetroot in half using a sharp knife onto a white tile.	<i>5–6 beetroots should be sufficient for a class of standard size, with each group having one half. Although using beetroot gives the most reliable results, red cabbage is a possible alternative.</i>

Steps

7. A cork borer is used to cut cylinders from the beetroot. The cylinders of tissue are pushed out of the borer with a plastic straw or a pencil and the ends are trimmed with a scalpel to remove the peel. Finally, the cylinders are cut into pieces 2 cm in length using a scalpel or sharp knife.

8. Learners should wash the cylinders thoroughly under running water, for at least a minute, to remove any excess pigment found on their surface. Using tweezers, the beetroot pieces are then carefully removed from the distilled water and gently blotted dry on a paper towel.

9. A beetroot sample is placed into each boiling tube at the different water temperatures and the timer is immediately started. The tubes are then incubated in the water baths for 20 minutes.

10. The boiling tubes are removed from the water bath and gently agitated to ensure that any pigment is fully dissolved in the water.

11. For each sample, a dropping pipette should be used to transfer the solution to fill a colorimeter cuvette. The pipette should be rinsed in distilled water between uses.

12. Care is taken to make sure that the correct sample is associated with the correct water bath temperature. Temperatures are labelled on a piece of paper and the samples are placed accordingly.

13. The colorimeter is set to transmit green light and to measure absorbance. It is then calibrated by using distilled water or as per the manufacturer's instructions using a standardisation solution.

Notes

Preferably use a size 4 cork borer, which will provide cylinders with a diameter appropriate to the size of the boiling tubes used in this investigation. This can be done overnight prior to the experiment if time in the lab on the day of the practical is short. If this is done, beetroot cylinders should be wrapped in cling film and placed in the refrigerator for up to 24 hours.

The purpose of this step is to ensure that no pigment remains on the surface of the cylinders. Cutting the beetroot will damage some of the cells, causing pigment to be released.

A pair of learners may wish to recruit help from other groups in order to ensure that the beetroot cylinders are placed in the water baths at the same time; alternatively, they should stagger the incubations 1–2 minutes apart.

Inverting the tube once is a better way to ensure that the pigment is completely mixed with the water, although a bung will need to be used with the hot solutions to avoid scalding the thumb.

It is important to keep the beetroot sample at the bottom of the boiling tube because pieces of beetroot tissue may otherwise be transferred to the cuvette, which would interfere with the colorimeter readings.

Cuvettes should not be labelled directly as this will interfere with the operation of the colorimeter.

Green light passes most effectively through dark red solution. Remind learners to make sure the colorimeter is set to absorbance, and that the colorimeter reading is calibrated to zero using distilled water at the start and between separate readings.

Steps

14. This process is repeated for each sample, with recalibration repeated between measurements. The results are entered into a table. The observation of the colour is also recorded. A mean is calculated from the three repeats for each sample.

Notes

Ask learners to collate their data to create a class mean for all temperatures, as there is not time to repeat the experiment two more times each.

Clean-up

After the experiment learners should:

- place the pieces of beetroot in a bin
- pour any water and solutions down the sink and wash with plenty of water
- clean all glassware
- tidy up their work space
- ensure any spillages have been mopped up.

Debriefing lesson: Modifying a method



Resources

- Worksheets G and H
- A3 plain paper (3–4 sheets per group)
- Sticky tape (1 dispenser per group)
- String (at least 10–15 m in length)
- Paper clips (1 per group)

Learning objectives

By the end of the lesson:

- **all** learners should be able to recognise that more than one factor can have an effect on a dependent variable
- **most** learners should be able to describe how a procedure used to investigate the effect of one factor on a dependent variable can be modified in order to investigate the effect of a different factor
- **some** learners will be able to evaluate the modifications made to a procedure used to investigate the effect of a different factor on a dependent variable.

Timings	Activity
 10 min	Starter/introduction Learners will need their graphs from the <i>Lab lesson</i> as they are going to interpret and evaluate the results of their investigation. Arrange learners in pairs and ask them to show each other their graphs. They should spend 2–3 minutes comparing their graphs (note that all learners should have plotted graphs that look roughly the same, as they were based on the class' collective data). Challenge learners to answer Worksheet G on their own. This will prompt deeper independent thought about what they did during the practical, and why, and encourage further analysis and evaluation of their plotted data.
 10 min	Ask learners to discuss their answers with a partner. Invite each pair to offer their refined answers to the class and generate a class-wide discussion. Ask different pairs of learners whether they agree or disagree with the responses offered, and encourage them to develop on their own answers.
 30 min	Main lesson Arrange learners into groups of 3–4 and explain that you want them to modify the method they employed in the <i>Lab lesson</i> in order to investigate the effect of ethanol concentration on membrane permeability. Tell them that phospholipids dissolve in ethanol, and that high concentrations of ethanol will denature proteins. The investigation is given the following context: 'A chef wants to know the concentration of ethanol that will keep beetroot fresh, but will not cause damage to the tissue and the leakage of pigment.' Their method needs to include plotting a graph to find the point at which the plotted line intersects the appropriate axis (interpolation) to estimate an appropriate concentration.

Timings	Activity
	Provide each group with 3–4 sheets of A3 plain paper, and each learner with a copy of Worksheet H. Inform them that they will need to collaboratively evaluate the method they employed in the previous lesson and decide which steps should be modified, how and why. Their work should take the form of a poster (which can be made larger by taping sheets of A3 paper together). This will be used as the basis of a presentation at the end of the lesson. You can withhold the checklist on Worksheet H for the first 5 minutes in order to keep the discussion regarding evaluation and planning more open, and then provide an opportunity for learners to reflect on what they had missed in their initial plan.
	Plenary Tie a piece of string so that it goes across the room. Learners are requested to hang their poster onto a 'washing line' and then circulate around the room in a 'marketplace activity' to view their peers' work. Ideally, one member of each group should remain with their poster to answer any questions that are posed by other learners as they circulate. Collaboration and collective reflection will enable learners to reflect on how other groups have approached the problem, which will lead to deeper consideration of the reasons and rationale that underpin modifications to the steps. Conclude with a whole-class discussion to give learners the opportunity to reflect on their own investigation and state how they felt another group's work had offered something that theirs did not. The expected outcomes of this activity are given in the answers to Worksheet H; use this to make sure all learners have met the expected outcomes and to extend their thinking. Based on their experiences during this activity, learners should be asked to individually write a formal plan for this investigation for homework.

Worksheets and answers

	Worksheet	Answers
For use in <i>Briefing lesson</i>:		
A: Building a fluid mosaic membrane	20	–
B: Investigating the invisible	21	34
Teacher Instructions 1: Fluid mosaic membrane model	31	–
Teacher Instructions 2: Boiling broccoli demo	32	–
Teacher Instructions 3: Betalain leakage	33	–
For use in <i>Planning lesson</i>:		
C: Aiming for accurate data 1	22	–
D: Aiming for accurate data 2	23	35
E: Planning an ideal investigation	24–26	36–37
For use in <i>Lab lesson</i>:		
F: Calculating mean and standard error	27–28	–
For use in <i>Debriefing lesson</i>:		
G: Analysing data	29	38–39
H: Modifying our investigation	30	40–41



Worksheet A: Building a fluid mosaic membrane

The cell membrane, shown in Figure 1 below, is a vital component of all cells and separates the contents of the cell from its external environment. Membrane proteins allow for the transfer of some molecules between the two sides of the structure.

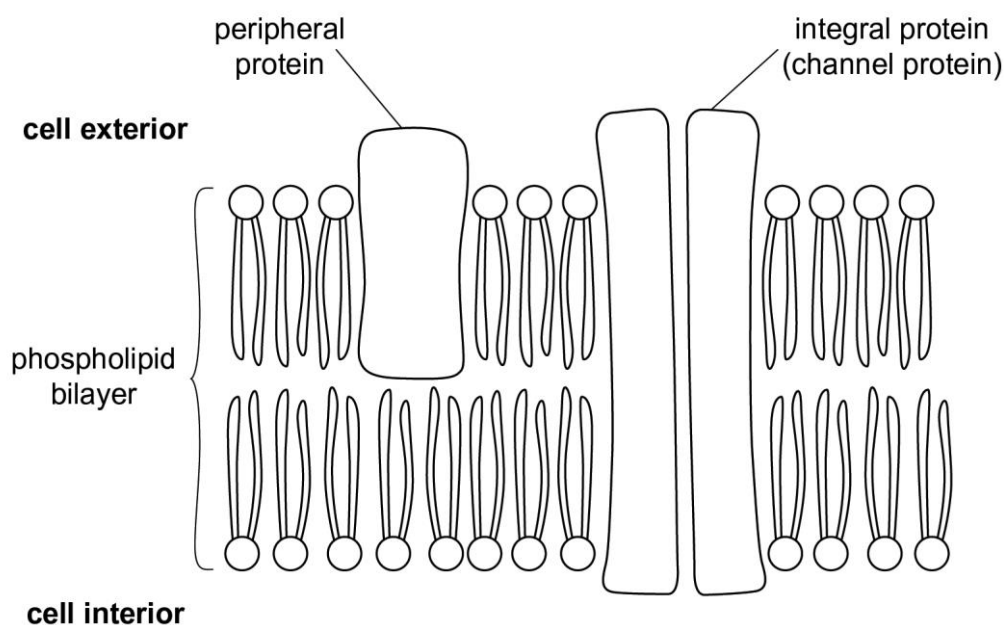


Figure 1 Phospholipid bilayer of cell membranes.

Your task is to produce a three-dimensional visual representation of the molecular structure of a cell membrane. You should ensure that it conforms to the fluid mosaic model.

You will be given some items to represent the components of the membrane. You must decide for yourself:

- which items represent which molecules
- how to arrange the items in the membrane to accurately represent its structure
- what to do in order to show that your membrane is both fluid and a mosaic.

When you have completed your model, put your hand up and ask for it to be checked by your teacher. They will provide you with further materials and ask you to demonstrate how your model can be used to illustrate other key properties of a cell membrane.

Worksheet B: Investigating the invisible



The effect of environmental factors on the integrity of the cell membrane cannot be measured directly in the school laboratory. However, it is possible to measure membrane integrity *indirectly* by measuring the release of detectable substances from the cell. The release of these substances correlates with the integrity of the cell membrane.

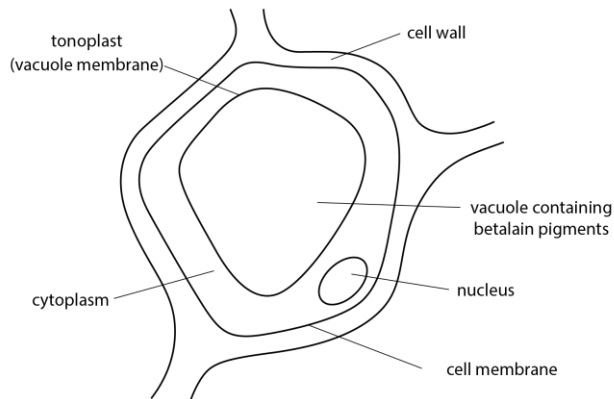


Figure 1 A beetroot cell.

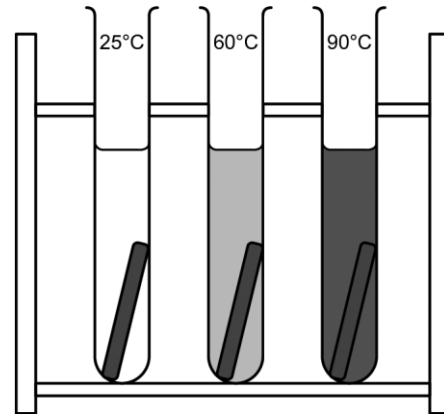


Figure 2 The contents of boiling tubes containing beetroot samples that have been heated in water of different temperatures.

Using the information in the two figures to help you, outline how you could investigate the effect of temperature on the integrity of the cell membrane in beetroot cells.

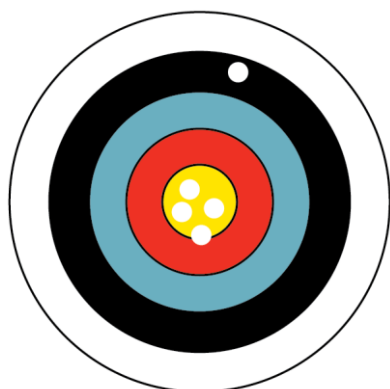
You should use as many of the following 10 key terms as you can in your summary.

vacuole
cell membrane
tonoplast
denature
diffusion
kinetic energy
beetroot cylinder
colour intensity
membrane proteins
betalain

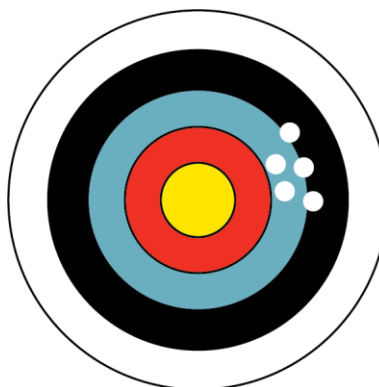
Worksheet C: Aiming for accurate data 1



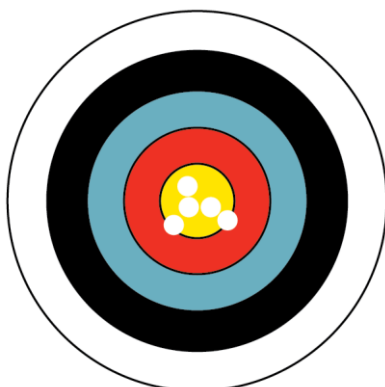
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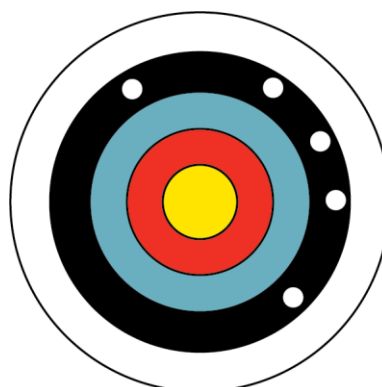
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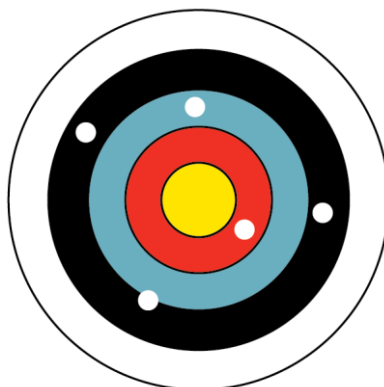
C



D



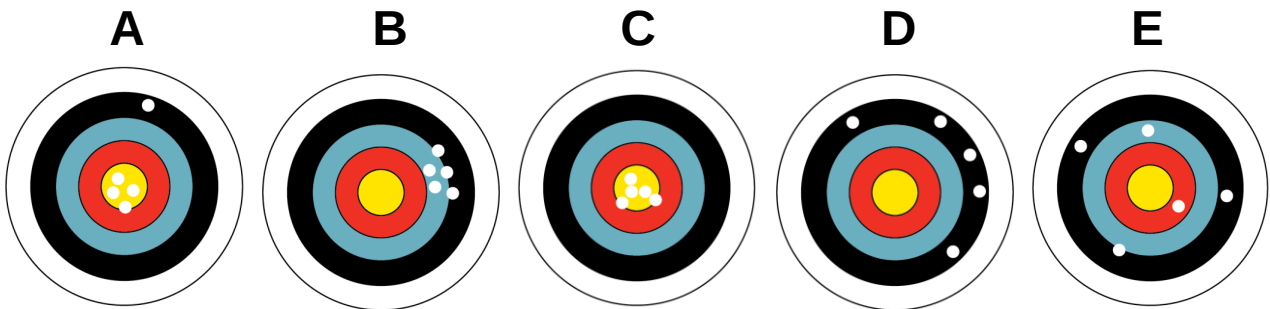
E





Worksheet D: Aiming for accurate data 2

In a competition, five archers stand 20 metres from a target. Their aim is to hit the central 'bullseye' with five arrows. The results of the competition are shown below.



To answer the following questions, assume that hitting the centre of the target is equivalent to obtaining the **actual** (true) value for a piece of data in a scientific investigation.

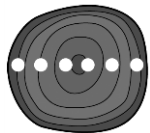
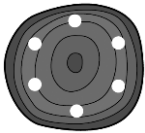
1. Which archer had the most **precise** technique for shooting their arrows? Explain your choice.
2. Identify which **two** archers obtained the most **reliable** results with their technique. Explain your choice.
3. Archer A stood 1 metre closer to the target than the other four archers. Explain why this would invalidate any comparisons of the scores.
4. Which score shows a random error? Explain your choice and suggest a reason for this.
5. Identify which **two** archers have scores that show a systematic error. Explain your choice **and** suggest why the error might have occurred.

Worksheet E: Planning an ideal investigation



Select the most appropriate option for each step in the method. Indicate if your choice enhances the validity (V), reliability (R) or accuracy (A) of the data. Justify your choice. If your choice is (A), identify how your choice helps to minimize **systematic error** and/or **random error**.

You will use this sheet during the *Lab lesson*, so cross out the option you decided against. The first step has been done for you.

	Option 1	Option 2	V	R	A	Justify your choice
1	Use 3 boiling tubes to carry out incubations at 5 °C, 40 °C and 75 °C	Use 6 boiling tubes to carry out incubations at 5 °C, 25 °C, 40 °C, 60 °C, 75 °C and 90 °C ✓			✓	Using a greater range and more intervals of the independent variable will provide data that allows for a more accurate trend to be determined.
2	Measure 20 cm ³ distilled water into each of the boiling tubes using a 10 cm ³ measuring cylinder	Measure 20 cm ³ distilled water into each of the boiling tubes using a 50 cm ³ measuring cylinder				
3	Place the boiling tubes containing water into the separate water baths immediately	Wait until the beetroot pieces have been prepared before placing the boiling tubes containing water into the water baths				
4	Use cooked beetroot for use in the investigation	Use a fresh, uncooked beetroot for use in the investigation				
5	Use a cork borer to obtain 6 cylinders of beetroot, and then trim them to the same length with a sharp knife	Use a sharp knife to cut 6 cubes of beetroot				
6	Take the pieces for step 5, from left to right (longitudinally) 	Take the pieces for step 5, from the same inner circle (in a circle) 				

	Option 1	Option 2	V	R	A	Justify your choice
7	Soak the beetroot pieces in a beaker of warm water to remove pigment released during cutting	Wash the beetroot pieces under cold running water to remove pigment released during cutting				
8	Dry the beetroot pieces using a paper towel	Leave the beetroot pieces exposed to the air to dry for 10–20 minutes				
9	Place the beetroot into the boiling tubes, containing water, wait 5 minutes, and then start a stop clock	Place the beetroot into the boiling tubes, containing water, and start a stop clock immediately				
10	Leave the boiling tubes within the water baths for the entire duration of the incubation period	Remove the boiling tubes from the water baths and place them into a rack for the duration of the incubation period				
11	Leave the boiling tubes to incubate for 5 minutes	Leave the boiling tubes to incubate for 20 minutes				
12	Gently shake the boiling tubes upon removing them from the water baths	Be careful not to agitate the boiling tubes upon removing them from the water baths				
13	Qualitatively judge the intensity of the colour of the first solution by comparing it to a colour chart	Use a colorimeter to obtain a quantitative reading of the intensity of the colour of the first solution				
14	Pick up the beetroot piece with tweezers or a needle and then pour the solutions into separate cuvettes	Use a syringe or dropping pipette to collect the solution from the boiling tube and place it into separate cuvettes, and avoid disturbing the beetroot pieces				
15	Set the colorimeter to transmit blue-green light and measure the absorbance of distilled water to get a baseline (in a cuvette)	Set the colorimeter to transmit red light and measure the absorbance of distilled water to get a baseline (in a cuvette)				

	Option 1	Option 2	V	R	A	Justify your choice
16	Write the temperature of the first (coldest) solution on the cuvette and measure the absorbance of the first solution	Write the temperature of the first (coldest) solution on a piece of paper placed under the cuvette and measure the absorbance of the first solution				
17	Measure the absorbance of the baseline solution in a cuvette again to recalibrate the colorimeter	Do not measure the absorbance of the baseline solution in a cuvette again				
18	Repeat the measurement of the solution's absorbance value two more times	Repeat the collection of the solution's absorbance value one more time				
19	Measure the absorbance of only the hottest solution in the series	Measure the absorbance of all other solutions in the series				
20	Carry out the entire experiment again two more times to identify anomalous results and to calculate a mean	Pool class data for each temperature reading to identify anomalous results and to calculate a mean				

Safety precautions

To conclude your plan, list three safety precautions you will follow to minimise risk in this investigation.

1.

2.

3.



Worksheet F: Calculating mean and standard error

1. How to calculate the mean of a dataset

The sample mean is the most commonly used average value in most scientific investigations. It represents the mean of a number of data points from one sample of a population, and can be calculated by dividing the sum of all the data points by the number of data points:

$$\bar{x} = \frac{\sum x}{n}$$

Where:

$\bar{x}$ = mean

$\sum$ = sum of

n = sample size

2. How to calculate the standard error of a mean of a dataset

When it is impractical to record data for all members of a population, the data is collected for just a sample of that population. In these cases, it is important to determine how accurately the sample mean reflects the population mean; this measure is called the standard error of a mean. The standard error provides an indication of the degree of similarity of the mean of the sample population to the mean of the whole population.

The standard error of the mean (S_M) is found by first calculating the standard deviation of the sample (s) using the following formula.

$$s = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

Where:

s = sample standard deviation

$\bar{x}$ = mean

$\sum$ = sum of

n = sample size

The value of the standard deviation is then divided by the square root of the sample size. This provides the value for the standard error:

$$S_M = \frac{s}{\sqrt{n}}$$

Where:

S_M = standard error

s = sample standard deviation

n = sample size

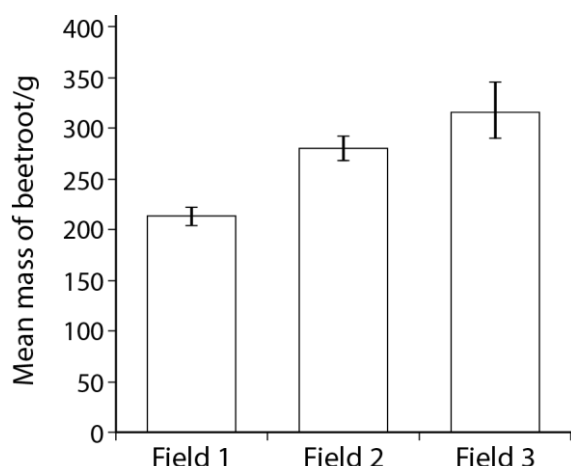
3. How to calculate 95% confidence interval for the sample mean of a dataset and plot these as error bars on a graph

The standard error can be used to calculate the 95% confidence interval (CI) for a sample mean.

$$95\% \text{ CI} = \pm 2 \times \text{SE}$$

The 95% CI represents the range of the sample data in which the true value of the population mean lies, with 95% probability. This can be indicated using an error bar on a graph or chart. For a given sample mean, the error bar extends to the value of 95% CI either side of the sample mean.

In the bar chart below, the values of the three sample means have been plotted, with error bars included for each mean.

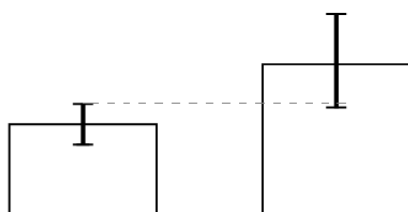


If the error bar is small, then the calculated mean is close to the true mean and the data is reliable. This is the case for Field 1.

Smaller error bars are less likely to overlap. The greater the overlap between any two bars, the greater the probability that there is not a significant difference between the two datasets. This suggests that any perceived difference between the sample means may be due to chance (although a statistical test should be conducted to confirm this).

In the chart above:

- There is likely to be a significant difference between the population mean of the mass of beetroots grown with and without fertiliser as the error bars for the sample means of Fields 1 and 2, and Fields 1 and 3 do not overlap.
- Although the mean values for Field 2 and Field 3 are different, the significance of this difference is questionable, because the error bars for these samples slightly overlap. The error bar for Field 3 is particularly wide, suggesting that its sample mean may not be a true representation of the population mean for beetroot mass in this field.





Worksheet G: Analysing data

You should use your graph from the *Lab lesson* to help you answer the following questions.

Write your answers in your notebook or on paper.

1. Describe in detail the relationship between temperature and the absorbance of the surrounding solution.

Hint: summarise the overall trend, then talk about the shape of the plotted line in more detail. It can help to break up the graph into distinct sections and describe the shape of each section with reference to temperature and light intensity. Key figures, for example temperature in this case, should be cited.

2. Explain the relationship between temperature, the colour intensity and the absorbance of the surrounding solution.

Hint: explain what is happening to cause the observed trend. What does the colour intensity and absorbance of the surrounding water represent?

3. Use your graph to predict the absorbance that would be obtained if a temperature of 65 °C had been used in this investigation.

4. Describe whether the error bars indicate the absorbance at each temperature are significantly different. Explain your answer.

5. Use your answer to Question 4 to comment on the accuracy of the prediction you made in Question 3.

6. List any sources of error that you were unable to fully avoid in your investigation.

7. A student claimed that the results would be more accurate if the beetroot and water had been mixed regularly during the 20-minute incubation period. Explain why this would improve the accuracy of the data collected.

Hint: use what you saw during your investigation to help you answer this question – did you notice anything different immediately surrounding the beetroot cylinders that were placed in the boiling tubes at higher temperatures?



Worksheet H: Modifying our investigation

Ethanol is an organic solvent that dissolves phospholipids and denatures proteins. The permeability of cell membranes is **increased** when the membranes are exposed to ethanol. A chef wants to know the concentration of ethanol that will keep beetroot fresh, but will not cause damage to the tissue.

Design an investigation to find the minimum concentration of ethanol that can be used to preserve beetroot, without causing a detectable loss in pigment from cells.

You are provided with:

- 100 cm³ of 100% ethanol
- 100 cm³ of distilled water
- 10 cm³ pipette
- 6 × boiling tubes with the capacity to hold 10 cm³ liquid each
- 1 fresh beetroot
- cork borer
- sieve
- white tile
- sharp knife
- colorimeter
- cuvettes

In this investigation, you are **not** allowed to combine class results. Therefore, you will need to consider how you could check for anomalous readings in your data and calculate a mean in another way to ensure that your results are reliable.

You will present your method as a poster to the rest of the class.

To help structure your poster, use the following checklist. Has your group:

1. Identified an appropriate range of concentrations of ethanol?
2. Identified an appropriate number of different concentrations of ethanol, separated by appropriate intervals?
3. Described how different concentrations of ethanol will be prepared from the stock solutions?
4. Described how other variables that could affect membrane integrity will be controlled?
5. Described a control experiment for the investigation and explained why this is important?
6. Described how the intensity of the red colour in the solution will be objectively and precisely measured to minimise the effect of random and systematic errors on the accuracy of the data?
7. Identified a hazard in the experiment and how a safety precaution will be followed to minimise risk?
8. Shown how you will record your data in a table and how you will improve the reliability of your data?
9. Made a prediction, shown in the form of a sketch of a graph, of the relationship you would expect to see between ethanol concentration and membrane permeability?
10. Identified, on the graph, how you will estimate the maximum concentration of ethanol that has no detectable effect on membrane permeability?

Teacher Instructions 1: Fluid mosaic membrane model



This activity requires learners to create a visual representation of a cell membrane, and in so doing demonstrate what is meant by the **fluid mosaic model**. The models should demonstrate how the cell membrane is both fluid (items can move in relation to each other) and is a mosaic (items are scattered and interspersed).

Give each pair of learners the resources to represent each part of the membrane – you can put these on one side of the lab and ask learners to collect them on a disposable plate.

The learners must decide for themselves:

- which items represent which molecules
- how to arrange the items in the membrane to accurately represent its structure
- what to do in order to show that the membrane is both fluid and a mosaic.

Example resources	Represents
Dried pasta spirals (3–4 pieces per pair of learners)	integral/peripheral proteins
Plastic drinking straws (2–3 per pair of learners)	channel proteins
Cotton buds with one end removed (20–30 per pair of learners)	phospholipids
Elastic bands (2 per pair of learners)	a means to hold the items together

Extension questions

As you circulate, be prepared to provide the following supplementary items to learners as they finish their model. As you do so, ask the question as per the table below.

Supplementary item to provide	Extension question to ask	Expected response
Putty-tack (e.g. Blue Tack) ball of 2–3 cm diameter	Show the effect of cholesterol on the fluidity of the cell membrane.	The role of cholesterol is to restrict the fluidity of the membrane by interacting with the phospholipid tails, so learners should show how several lumps of the putty-tack sticks some of the cotton bud tails together.
White and brown rice grains (× 2 of each)	Show how the cell membrane is selectively permeable: how molecules that are water-soluble (white grain) and lipid-soluble (brown grain) can pass across the cell membrane.	White grains can only pass through the protein channels (plastic straw pathways), while brown grains can pass directly between the phospholipid tails (cotton buds).
Ruler (30 cm)	Calculate the magnification of your fluid mosaic membrane (width of cell membrane = 10 nm (1×10^{-5} mm))	The exact answer will depend on the width of the learners' model, but can be found by dividing this measurement (in mm) by 1×10^{-5} .
N/A	Show what would happen if the phospholipid were spread as a monolayer.	They should show the tails in air and the heads in water.

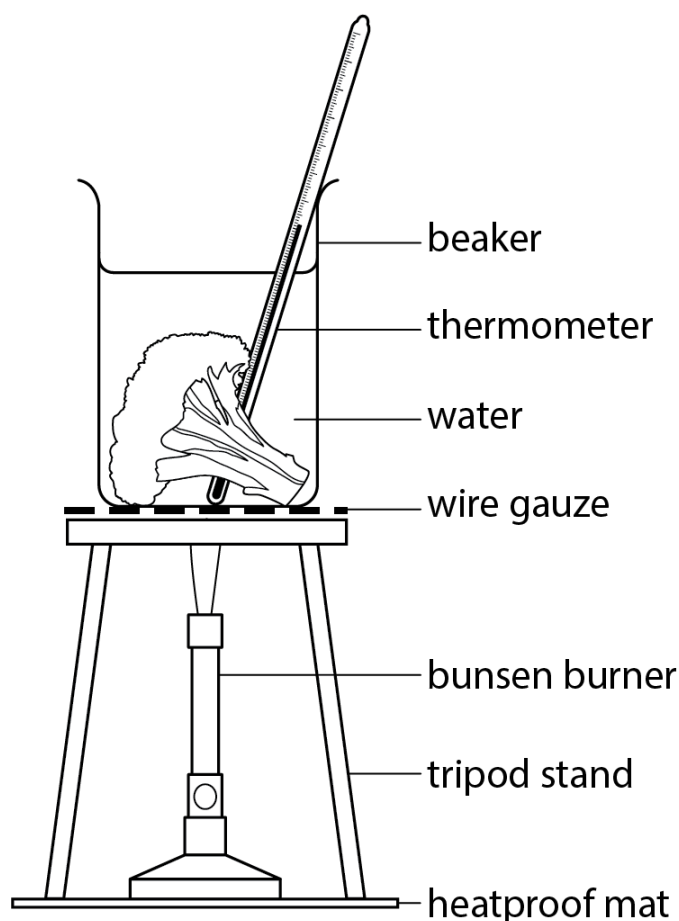
Teacher Instructions 2: Boiling broccoli demonstration



This demonstration shows what happens to the colour of water that holds a piece of broccoli as it is heated. The duration of the demonstration is approximately 10–15 minutes, during which time an active class discussion progresses in parallel with this ‘working model.’ Note you can use any vegetable/food that changes the colour of the water as it boils.

Instructions

1. Place a small sprig of broccoli into a 250 cm³ beaker containing 150 cm³ tap water. Pass the beaker around the class and confirm with learners that the surrounding water remains clear, even after a few minutes.
2. Arrange the equipment as shown below.
3. Heat the beaker strongly and call out the temperature at each 60–90 second interval. Ask the learners to watch the surrounding water closely for any signs of the green pigment leaving the vegetable.
4. When the temperature reaches 60–65 °C, inform the learners to watch the surrounding water with extra attention (there should be a relatively sudden release of green pigment).
5. Once the water reaches boiling point, stop the investigation and carefully place the beaker onto another heatproof mat. Ask learners to describe and explain their observations.





Teacher Instructions 3: Betalain leakage

This demonstration produces a partially flawed model of a beetroot cell and its exchange of substances with its environment. Through discussion of the results, learners will gain a deeper understanding of the molecular events that occur during the investigation they will carry out in the *Lab lesson*.

Three ice cubes containing red food colouring are placed into two 'cells' (small plastic 'zip-lock' 'sandwich' bags) that have holes in them. These two 'cells' are placed into two 500 cm³ beakers containing 300 cm³ tap water (one cold, one hot) to show the clear difference in pigment leakage over time.

It is important that the 'cells' are set up in view of the learners. They must see that there are holes in the plastic bags, that the ice cubes contain red pigment and, using a thermometer, that the temperature of the water in the two beakers is significantly different.

Instructions

1. Ask two learners to make 15–20 holes in two small plastic 'zip-lock' bags using a hole punch; ask them what these represent.
2. Use two thermometers to show that the temperature of the water in beaker A is 5–10 °C and the temperature of water in beaker B is 60–70 °C.
3. Add three ice cubes containing red food dye, of equal size, to each plastic bag and seal the bag tightly.
4. Ask two learners to gently lower the plastic bags (containing the ice cubes) into each beaker at the same time. Before they do so, ask the learners to predict what they will see.
5. Upon viewing the results, challenge learners to consider how this model shares similarities with actual beetroot cells exposed to similar temperatures, and how it does not model this process accurately.

Expected contributions – The lists below are **not** exhaustive

Ways the model accurately represents the molecular theory:

- The pigment is contained inside two barriers (the frozen state of the ice cube, and the plastic bag).
- The pigment is released if the barrier is disrupted (melting of the ice cube and punching holes in the bag).
- There is more leakage at a higher temperature (ice cube melts faster in higher temperatures) as shown by a higher intensity of colour in the surrounding water.

Ways the model does **not** accurately represent the molecular theory:

- The starting state of each cell membrane is fully permeable (holes that are not selectively permeable); you would not expect any leakage in cold temperatures.
- The permeability of the cell membrane in the model (size of the holes) does not change at higher temperatures.
- The cell membrane (plastic bag) is not fluid nor mosaic in nature.
- The pigment in the ice cubes does not need to move through an internal membrane (vacuolar tonoplast).



Worksheet B: Answers

An example response is provided below. The key terms provided in the worksheet to help scaffold the learner's response are underlined.

The integrity of the cell membrane of beetroot cells cannot be measured directly. Instead, a variable that strongly correlates with it must be measured. We can use the colour intensity of a solution into which a beetroot cylinder has been immersed for this purpose.

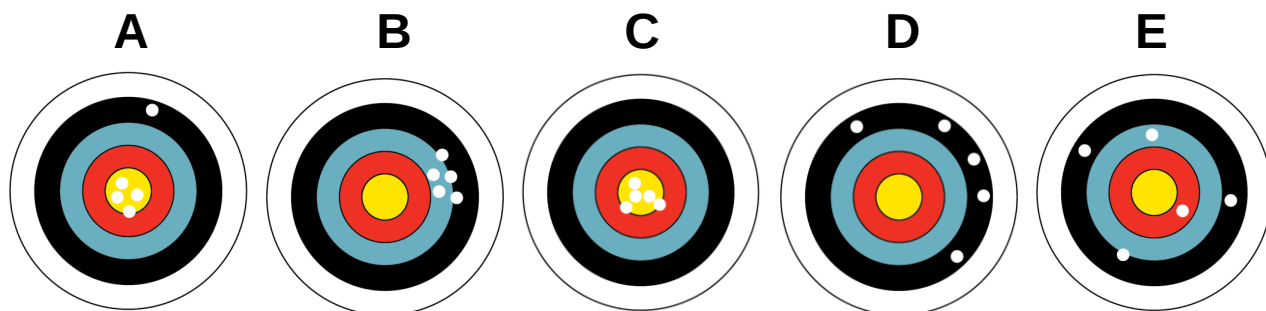
At cold temperatures, the betalain pigment found in the vacuole of beetroot cells cannot leave through the tonoplast and cell membrane. Therefore, little or no pigment will enter the surrounding solution and the colour intensity will be low.

At warmer temperatures, the kinetic energy of both the betalain molecules and molecules in the tonoplast and cell membrane increases. This increases the likelihood that more betalain molecules will be able to move by diffusion from the vacuole and into the cytoplasm, and then through the cell membrane to enter the surrounding solution. Therefore, the colour intensity of the surrounding solution will be higher.

At high temperatures, membrane proteins in both the tonoplast and the cell membrane will denature. This will result in the formation of 'holes' in the structure through which large amounts of betalain can diffuse out. This means that the colour intensity of the surrounding solution at these temperatures will be the highest.



Worksheet D: Answers



1. Archer C. The results were the most accurate as all five arrows hit the target (recorded a reading close to the true value), suggesting that their technique was the most precise.
2. Archers B and C. For both, their arrows hit their target in roughly the same area each time (they got the same result each time) suggesting their technique was the most reliable.
3. Archer A. The closer an archer is to the target, the more precise their aim can be as they can see the target more clearly and the arrow has less far to travel. As Archer A was closer to the target, they had an unfair advantage over the other archers; their technique *and* the distance from the target affect the shot here. Therefore, comparison of the scores obtained using their technique against those using the technique of the other archers would not be valid. (The distance from the target is a variable that needs to be standardised so that only the dependent variable (archer technique) is being investigated.)
4. Archer A. One of their five shots is very inaccurate compared to the others. All the other shots are clustered together towards the centre and one shot is towards the outer edge of the target. (There is an anomalous result.) This may have been because the archer was distracted when she released this arrow, or perhaps she released the arrow too early and before she had enough time to take aim.
5. The results of Archers B and D are the most likely to show a systematic error because both results are consistent, or reliable, but in all cases the arrows hit a section of the target away from the centre. This error could be due to aiming at the wrong place on the target, or because of a fault in their bow or arrows. (The equipment used must be sufficiently precise and calibrated correctly in order to ensure that the data it yields is accurate.)



Worksheet E: Answers

Step	Option	V/R/A	Justification
1	2	A	Using a greater range and more intervals of the independent variable will provide data that allows for a more accurate trend to be determined
2	1	A	The precision of a 10 cm ³ measuring cylinder is greater than a 50 cm ³ cylinder, allowing for a more precise measurement of the water
3	2	V	This allows for the water in the boiling tubes to reach the stated temperature before the beetroot is added
4	2	V	Cooked beetroot would contain cells that have lost much of their cell membrane integrity and lost a large quantity of the betalain pigment
5	1	V	This increases the likelihood that the diameter of the different beetroot pieces is uniform, which is a factor that needs to be controlled
6	2	V	The concentration of pigment in cells and/or the density of cells may be different at different depths of the vegetable, both of which are factors that need to be controlled
7	2	A	This removes any pigment that remains on the surface of the beetroot pieces without releasing any further pigment from undamaged cells
8	1	A	This ensures that the beetroot pieces do not dehydrate and form an impermeable layer that could interfere with the experiment
9	2	A	Pigment may be released immediately, so this ensures that all pigment released is accounted for in the results
10	1	V	This would keep the temperature constant during the incubation period
11	2	V	This allows for the beetroot pieces to reach the temperature of the water
12	1	A	This ensures that the released pigment is spread consistently throughout the water
13	2	A	This removes subjectivity and bias from recording the results
14	2	V	This would minimise mechanical damage to the cells, which may cause further pigment leakage
15	1	A	This light frequency will move effectively through the red-coloured solution and can be detected by the colorimeter
16	2	A	If the cuvette has writing on its side, this may interfere with the measurement of absorbance
17	1	A	This is necessary to provide a baseline reading against which all absorbance readings are calibrated
18	1	R	This will provide three readings from which an anomalous data point may be identified, and a reliable mean value could be calculated
19	2	A	This will provide a series of data values from which an obvious trend could be identified
20	2	R	This will reduce the time taken to conduct the investigation and yet still yield reliable data

Minimising sources of random errors (*this list is not exhaustive*)

- Use a cork borer to cut cylinders of beetroot (to increase the likelihood that they are of the same size).
- Repeating the collection of absorbance values for each solution three times (to identify anomalous readings).
- Use a dropping pipette to transfer the solutions from the boiling tubes to the cuvettes, in order to avoid touching the beetroot cylinder and adding pieces of tissue to the cuvette.

Minimising sources of systematic errors (*this list is not exhaustive*)

- Use a colorimeter to measure the intensity of the colour of the solution as absorbance (which reduces subjectivity in measurement compared with assessing it by eye).
- Measure the absorbance of distilled water before, and between, the measurement of absorbance values for the different solutions. This is to make sure the colorimeter is always calibrated correctly.
- Be careful to avoid inaccurate measurements due to parallax error (the imprecise measurement of the meniscus of a liquid) when measuring the volume of distilled water required for the separate boiling tubes. Be sure to read the meniscus of the liquid at eye level.

Safety precautions (*this list is not exhaustive*)

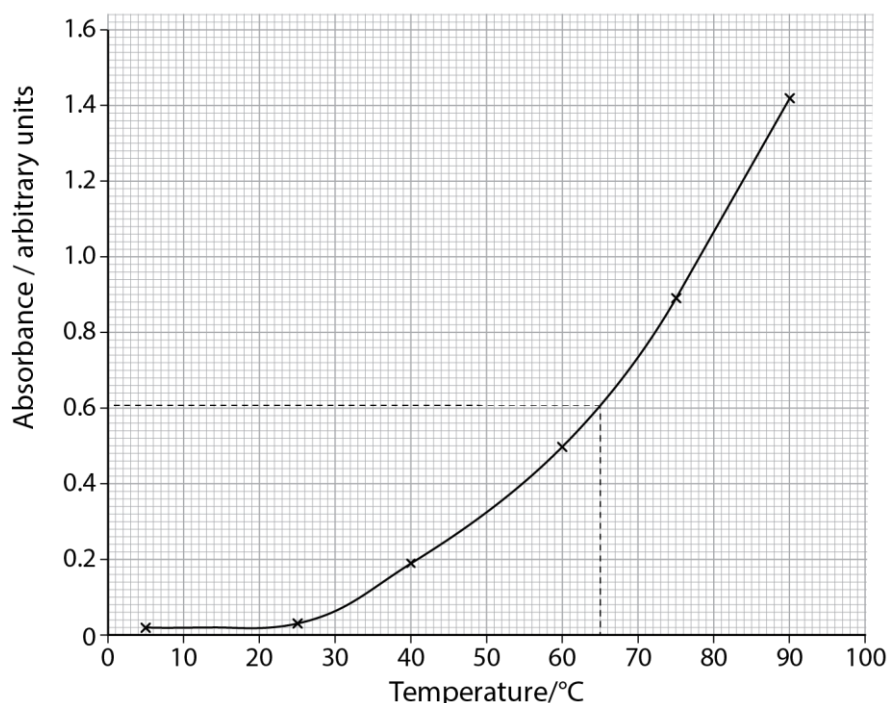
- Take care with the sharp knife; carry it clasped to a white tile, and when cutting, drive downwards and away from the body.
- Take care when working with the hot water baths and handling the boiling tubes that have been taken from them.



Worksheet G: Answers

The below are example answers based on the following example results.

Temperature / °C	Mean absorbance / arbitrary units
5	0.020
25	0.026
40	0.194
60	0.474
75	0.893
90	1.421



1. There is a general trend of increasing absorbance with increasing temperature. At temperatures below 30°C, the absorbance values are very low (less than 0.1), suggesting little or no leakage of pigment from the cells. Between temperatures 25°C and 40°C, there is a gradual increase in absorbance, then the absorbance increases more steeply after 40°C as shown by an increased gradient of the curve. The absorbance increases even more sharply after 65°C. For temperatures greater than 80°C, the change in absorbance is increasing sharply, as shown by the steep gradient.
2. The changes in absorbance from low to high can be observed by the naked eye as changes in intensity of red-purple colour of the solution, from very light at lower temperatures to dark at higher temperatures. The absorbance (and therefore colour intensity) of the surrounding liquid is proportional to the concentration of pigment that has been able to leave the beetroot cells, which correlates directly to the permeability of the beetroot cell membranes. Only a small concentration of pigment is able to leave the cells at low temperatures. This is because the kinetic energy of the pigment molecules and the molecules within the cell membrane is relatively low and the cell membrane acts as a barrier to contain the contents of the beetroot cells. At higher temperatures, the kinetic energy of the molecules in the cell membrane is greater and they move more relative to each other, increasing the size of the gaps between them. There is also an increase in the kinetic energy of the pigment molecules, so their rate of diffusion out of the cell increases and they begin to leave the cell at a greater rate, increasing

the concentration of the pigment cells in the surrounding solution and therefore increasing the absorbance and colour intensity. Beyond a particular temperature (approximately 65 °C), the kinetic energy of membrane proteins is so great that they denature, which, due to changes in their tertiary structure, results in large gaps forming in the membrane. At this point, large quantities of the pigment are able to diffuse out of the solution increasing the concentration of pigment in the surrounding solution and thus increasing the absorbance and colour intensity of the solution.

3. The actual value will depend on the graph plotted by the learner. It can be found by drawing a vertical line from the value of 65°C on the x-axis to the curve, and then a horizontal line from this point on the curve to the y-axis, then reading off the absorbance value on the y-axis.
4. The judgement here will depend on the reliability of the data collected by the class; if the plotted error bars are narrow and do not overlap, then there is likely to be a significant difference between these mean values. However, if there is an overlap between error bars, then the significance of any differences observed between the mean values of the data points is questionable.
5. The answer here will depend on the extent to which the error bars for given pairs of mean values' data points overlap; if they do, then the accuracy of the prediction made by interpolation could be questionable.
6. Possible answers may include the consistency of mass/volume between beetroot cylinders, the volume of water in the boiling tubes, and so on.
7. Some learners might have observed that the pigment concentrates around the surface of the beetroot cylinder, particularly at higher temperatures. This might prompt some learners to reason that this pigment fails to diffuse fully into the water in which it is suspended. This would have the effect of limiting the diffusion gradient between the tissue and the water. By agitating the boiling tube every 5 minutes or so, a steep diffusion gradient would be maintained, allowing further pigment to leave the tissue.



Worksheet H: Support for the posters

Below are summary notes on each of the checklist points in Worksheet H, to help guide the summary discussions that focus on modifying a practical task.

EXPECTED OUTCOME	
1	Learners should use a range of concentrations, appropriate to the method of dilution that they chose, that would give a series of results from which trends can be identified and on which predictions can be made.
2	Learners should decide on a number of concentrations that would enable a trend line to be drawn and on which predictions can be made.
3	Learners should describe how the 100% ethanol solution can be diluted using either the serial or simple (proportional) dilution method. In the serial dilution method, 9 cm ³ distilled water is topped up to 10 cm ³ with 100% ethanol to give a 10% solution. Then, 9 cm ³ distilled water is topped up to 10 cm ³ with the 10% ethanol solution to give a 1 % ethanol solution. This can be repeated to give 0.1% and 0.01% concentrations. In the simple (proportional) dilution method, a series of dilutions of ethanol of concentrations 20%, 40%, 60%, and 80% are prepared by diluting a specific volume of 100% ethanol with distilled water. For example, adding 2 cm ³ water to 8 cm ³ 100% ethanol will give a 10 cm ³ solution of 80% concentration.
4	Learners should describe how the temperature of the different ethanol concentrations should be consistent, using a thermostatically controlled water bath (set to remain constant at no more than 25°C). The mass/volume of the beetroot cylinders placed into the different concentrations should also be the same, as well as the time that they were allowed to incubate. If any of these factors were to also vary in addition to the concentration of the ethanol between the tubes, then no valid comparison could be made of the collected data.
5	A control experiment is required to show that it is the ethanol, rather than another factor, that has the measured effect on membrane permeability. In this investigation, the control investigation would involve placing a cylinder of beetroot, of identical mass/volume to the others, into a solution of 0% ethanol (100% distilled water) for the same amount of time to incubate, and at the same temperature. Learners should predict that there should be no detectable release of pigment in the control experiment.
6	Learners should describe how the intensity of the red colour in the solution will be measured in a similar way to the method that they employed in the practical lesson, by way of using a colorimeter set to transmit blue-green light and measure absorbance. They should explain how distilled water is used to provide a baseline level of absorbance between measured solutions, and carry out three replicates of each absorbance reading, to minimise the chance of systematic and random errors respectively and improve data reliability.
7	Learners should recognise that the use of a sharp knife represents a hazard and how risk can be minimised. In addition, ethanol is highly flammable, and so no naked flames should be brought near the laboratory equipment.
8	The learners should construct a table that includes a left-hand column for the concentration of ethanol (independent variable) and a right-hand column for the absorbance value as read from the colorimeter (dependent variable). Note that because the learners have been

EXPECTED OUTCOME	
	informed that they cannot pool class data in this investigation, they should have stated that they will measure (and included in their table space to record data for) the absorbance values for three separate beetroot cylinders placed into three separate boiling tubes of each concentration of ethanol. The right-hand column should therefore be divided into 4 sub-columns in order to provide space to record the three replicates (measured from each beetroot cylinder) from the colorimeter and to show the mean value.
9	The learners should predict that the relationship between ethanol concentration and membrane permeability will follow a similar trend to the relationship that applies between temperature and membrane permeability: a positive correlation. There may be some who recognise that protein channel denaturation may be more gradual, and hence there is a more uniform gradient to the line.
10	Learners should explain that they would find the lowest value of ethanol concentration on the x-axis that corresponds to the value of zero absorbance on the y-axis. Some learners may suggest that they would then carry out another investigation using a narrower range of ethanol values that concentrate on this section of the line, in order to make a more accurate estimate of the actual concentration of ethanol that does not cause pigment leakage.

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