Cambridge Assessment International Education

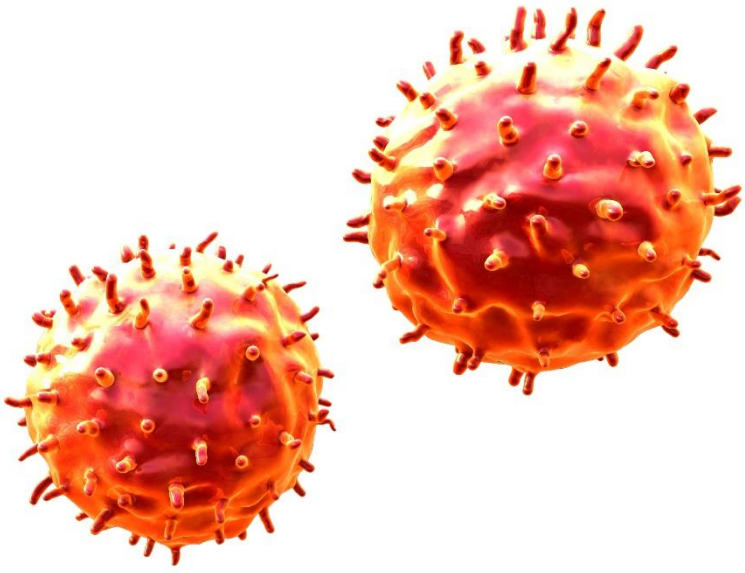
# Teaching Pack

## Extracting DNA from split peas

## Cambridge IGCSE™

## Biology 0610

This *Teaching Pack* can also be used with the following syllabuses:
- Cambridge IGCSE™ (9–1) Biology **0970**
- Cambridge IGCSE™ Biology (US) **0438**
- Cambridge IGCSE™ Co-ordinated Sciences (Double Award) **0654**
- Cambridge IGCSE™ (9–1) Co-ordinated Sciences (Double Award) **0973**
- Cambridge O Level Biology **5090**



Version 2.0

Cambridge Pathway

# Contents



**Icons used in this pack:**

**Briefing lesson**

**Lab Option 1: run the experiment**

**Lab Option 2: virtual experiment**

**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 5 (Practical Test) or Paper 6 (Alternative to the Practical Test).***

There are two options for practising experimental skills. If you have laboratory facilities this pack will support you with the logistics of running the experiment. If you have limited access to experimental equipment and / or chemicals, this pack will help you to deliver a virtual experiment.

This is one of a range of *Teaching Packs*. Each pack is based on one experiment with a focus on specific experimental techniques. The packs can be used in any order to suit your teaching sequence.

The structure is as follows:

| **Briefing lesson (1 hour*)** |
|---|
| This lesson introduces the focus experimental skills to be developed. It also introduces any content needed for your learners to understand the experiment being carried out in the *Lab lesson*. |

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| **Lab lesson (1 hour*)** | |
|---|---|
| **Option 1 – run the experiment** | **Option 2 – virtual experiment** |
| This lesson allows the experiment to be run with your learners, providing an opportunity to practise the experimental skills introduced in the *Briefing lesson*. | This lesson allows your learners to complete a virtual experiment, providing an opportunity to practise the experimental skills introduced in the *Briefing lesson*. |

↓

| **Debriefing lesson (1 hour*)** |
|---|
| This lesson consolidates and builds on the progress learners have made. In some cases, it will also provide the opportunity to practise extended writing skills. |

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

In this *Teaching Pack* you will find the lesson plans, worksheets for learners and teacher resource sheets you will need to successfully complete this experiment.

# **Experiment:** Extracting DNA from split peas

This *Teaching Pack* focuses on an extraction of DNA from split peas.

DNA is the biological molecule that makes up our genes and chromosomes. It is the material we inherit from our parents, which gives us many of our characteristics. In this experiment, you will extract DNA from split peas.

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

- 1.3 Features of organisms
- 4.1 Biological molecules
- 17.1 Chromosomes, genes and proteins

The experiment covers the following experimental skills, adapted from **AO3: Experimental skills and investigations** (see syllabus for assessment objectives):

- safely use techniques, apparatus and materials
- following a sequence of instructions
- plan experiments and investigations
- evaluate methods and suggest possible improvements.

## Prior knowledge

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

- 1.3 Features of organisms
- 4.1 Biological molecules
- 17.1 Chromosomes, genes and proteins

## Going forward

The knowledge and skills gained from this experiment will be useful for when you teach learners about any of the above-listed syllabus content, as well as variation and selection, mitosis, meiosis, adaptive features and genetic engineering.

# **Briefing lesson:** Making observations and planning

| | |
|---|---|
| **Resources** | • Worksheets A, B, C and D |
| **Learning objectives** | By the end of the lesson:<br>• ***all*** learners should be able to identify the hazards associated with an experiment.<br>• ***most*** learners should be able to suggest reasoned precautions to reduce the risk of each hazard.<br>• ***some*** learners will be able to suggest safer alternatives to particular methods. |

| Timings | Activity |
|---|---|
| 10 min | **Starter/introduction**<br>Ask learners to write some safety rules for working in the lab. Allow 2 minutes of individual thinking and 2 minutes of paired discussion. Go over ideas from each pair in order to create a set of rules that can be written for the whole class to see. These should include tying back long hair; standing to do practical work; tucking bags and stools under the desk; no running; and no eating or drinking. Prompt learners to also consider that another key point is listening and following instructions; this is often missed when discussing safety in the lab. |
| 15 min | **Main lesson**<br>Give learners Worksheet A, which asks them to consider the safety hazards of three different experiments. They then reflect on if the safety rules they devised in the starter are sufficient to cover each experiment. If they are not, what else do they need to consider in order to carry out the experiments safely? Discuss as a class to consolidate ideas and add to the class list. |
| 15 min | Introduce the idea of a 'risk assessment'. Explain that this is carried out before starting an experiment to identify all the potential risks and ways to reduce them. It also means that responses to problems can be planned beforehand so that if something does go wrong, everyone is prepared in terms of what to do. Arrange learners in pairs and ask them to carry out a risk assessment for heating water using a Bunsen burner. Worksheet B provides prompts for what they need to consider. For those who need support, use page 2 of Worksheet B and encourage learners to consider each piece of equipment in turn. |
| 10 min | Give learners Worksheet C, which is a completed risk assessment for heating the water. Learners peer-mark another pair's risk assessment using the worksheet. They give the other pair feedback in the form of two positive points, and one way to improve. Discuss as a class any points that were missed and why they are important. These might include that the equipment becomes hot and should be left to cool before handling to avoid burns; the water becomes hot and can scald; care should be taken when using glassware as there is the potential for breakages leading to cuts. All learners should at least recognise that the Bunsen burner can ignite materials. |
| 10 min | **Plenary**<br>Learners use Worksheet D to consider ways to avoid hazards by using alternative methods or equipment. Discuss the suggestions as a class. Consider if there are any hazards associated with the suggested alternatives. Which hazard is more acceptable in terms of the risk involved? |

# **Lab lesson:** Option 1 – run the experiment

| | |
|---|---|
| **Resources** | • Worksheets E, F, G and H<br>• *Teacher walkthrough* video, *Teacher notes, Teacher method*<br>• Equipment as outlined in the *Teacher method* |
| **Learning objectives** | By the end of the lesson:<br>• ***all*** learners should be able to identify two hazards and the correct precautions specific to this experiment.<br>• ***most*** learners should be able to follow a series of instructions to collect DNA.<br>• ***some*** learners will be able to explain why each chemical is added and the implications of working in the incorrect order. |

| Timings | Activity |
|---|---|
| 10 min | **Starter/introduction**<br><br>Briefly introduce the learners to the experiment for extracting DNA from split peas. Have the equipment out for them to see, and give them each Worksheet F (experiment set-up) and Worksheet G (risk assessment template). Ask them to complete a risk assessment. Draw their attention to the blender. Discuss ideas as a class; learners add in any hazards they have missed. You can steer learners in the right direction using the suggested answers. |
| 15 min<br><br>30 min | **Main lesson**<br><br>Arrange learners into groups of 2–4. Give each group Worksheet H and ask them to put the steps of the method for the experiment into the correct order. They will also need to decide the purpose of each step, this should help them with their ordering. Explain the role of the salt, washing up liquid, pineapple juice / protease and ethanol, and the need for the cold temperatures.<br><br>Discuss as a class the consequences of getting the steps in the wrong order; why is it important to do the steps in the order given? (So that the DNA is released from the nucleus into solution, otherwise it will still be locked inside the cells and left in the filter during filtration.) For example, if salt was not added at the start, then fewer cells would have been broken open, reducing the volume of DNA available and making it more difficult to extract. (See suggested answers for more examples.) Agree the correct order as a class; make sure learners are clear on the reason each step is carried out in the order given.<br><br>Before starting, briefly remind them of the safety precautions they should take when using the blender and handling methylene blue.<br><br>**Safety**<br>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. Depending on your class, learners can be allowed to use the blender themselves under your supervision. Alternatively, you can demonstrate this step for the learners whilst they observe; they then collect a split pea solution from you and follow the method from step 7 onwards.<br><br>*Continues on next page …* |

| Timings | Activity |
|---|---|
| | **Main lesson continued …**<br><br>Learners should collect their equipment and start following the method on Worksheet E.<br><br>When most learners are at step 18, ask them to pause for a moment. Discuss why it's important to test that the white precipitate found was DNA. Explain that step 18 is a technique to test that they have collected DNA. Explain that if they do not get a blue precipitate then they have extracted protein instead of DNA. They can confirm the precipitate is a protein using protease; if the addition of protease causes the precipitate to break down, then it is protein.<br><br>Ask the learners to look at their results. Was DNA found? How do they know?<br><br>If some groups did not successfully extract DNA, discuss what might have gone wrong. Suggestions might include that not enough pineapple juice was added, or the pineapple juice didn't contain a high enough concentration of protease; not enough washing up liquid was added; or the samples weren't incubated for long enough with the protease or the washing up liquid. The temperatures weren't cold enough and the DNA degraded. The person doing the experiment didn't successfully extract the white precipitate from the boiling tube, so there wasn't anything in the Petri dish to test. |
|  | **Plenary**<br><br>Discuss as a class if their risk assessments were sufficient: did they have any issues? If so, what were they? Did they occur because they didn't follow their risk assessment or the instructions? Did they know how to respond? |

# Teacher notes

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

Each group will require:

- 100 $cm^3$ split peas
- 200 $cm^3$ ice-cold water
- ice water in a beaker (ice water-bath)
- table salt
- blender
- muslin cloth
- elastic band
- timer
- boiling tubes/test-tubes
- boiling tubes/test-tubes rack
- beaker × 2
- washing up liquid
- pineapple juice (or other source of protease)
- chilled ethanol (below 4°C)
- glass rods
- dropping pipettes
- glass Petri dish
- methylene blue

## Safety

The information in the table below is a summary of the key points you should consider before undertaking this experiment with your learners.

Some associated safety precautions include:

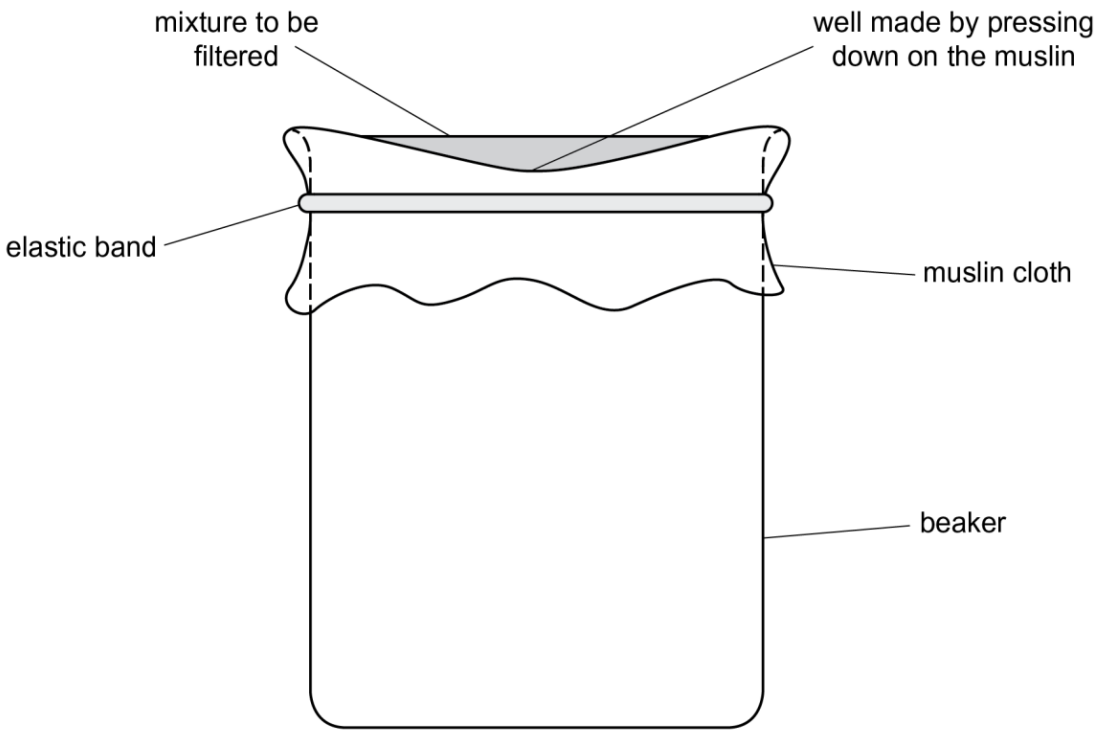
1. The lowest concentration possible of methylene blue should be used and eye protection should be worn.
2. Disposable gloves can be worn to reduce the risk of contact of methylene blue with the skin.
3. If the solvent used to make up the methylene blue is flammable, do not use near an open flame.
4. Learners should **not** make up the methylene solution themselves. When you or a technician make up the solution, do so in a fume cupboard to prevent powdered dyes and indicators escaping into the air. Solid / powdered methylene blue is harmful if it comes into contact with the skin, is ingested or is inhaled.
5. Pure ethanol is highly flammable and harmful; it must be kept away from naked flames.
6. Use as small a volume of ethanol as possible in a well-ventilated room; eye protection must be worn.
7. Make sure that a damp cloth, bench mat, fire blanket or other form of fire extinguisher is readily available when using pure ethanol.
8. Use of ethanol must be monitored, therefore count bottles out and in again.

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

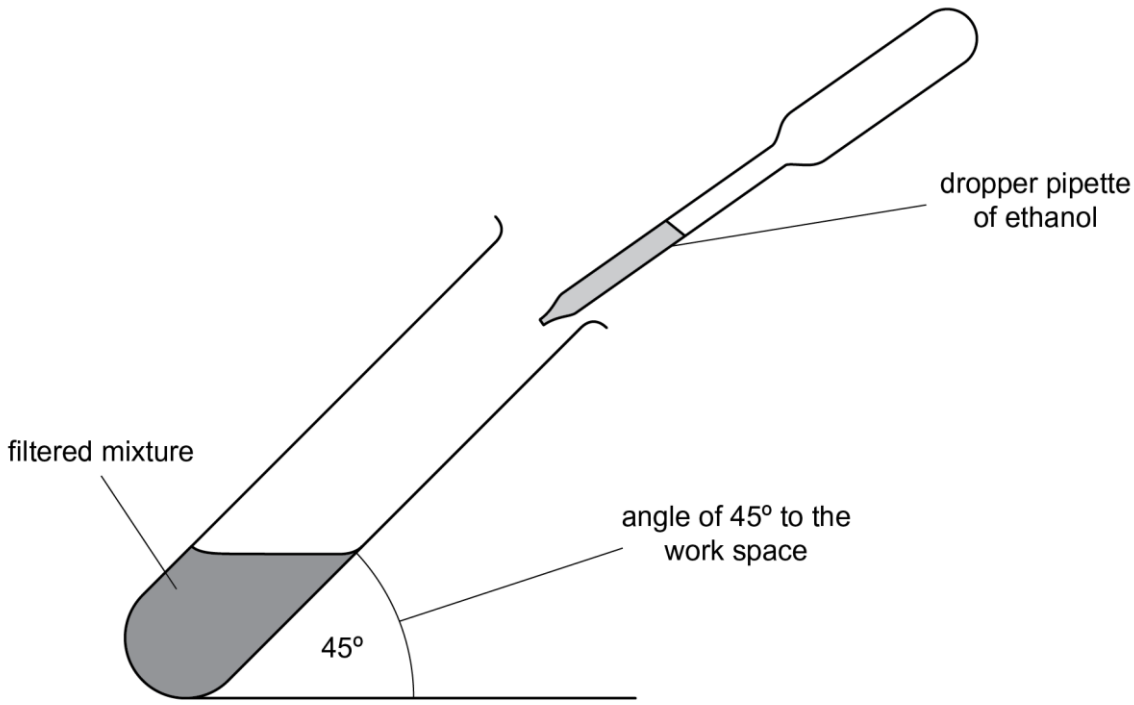
| Substance | Hazard | First aid |
|---|---|---|
| Methylene blue, solid | HARMFUL | **In the eye:** Flood the eye with gently-running tap water for at least 10 minutes. See a doctor.<br>**Swallowed:** Do no more than wash out the mouth with water. Do **not** induce vomiting. Sips of water may help cool the throat and help keep the airway open. See a doctor.<br>**Dust breathed in:** Remove the casualty to fresh air. See a doctor if breathing is difficult.<br>**Spilt on the skin or clothing:** Remove contaminated clothing. Wash off the skin with soap and plenty of water. Rinse contaminated clothing.<br>**Spilt on the floor, bench, etc.:** Scoop up solids (take care not to raise dust). Wipe up solution spills or any traces of solid with a damp cloth and rinse it well. |
| Methylene blue, dilute aqueous solution | LOW HAZARD | **In the eye:** Flood the eye with gently-running tap water for at least 10 minutes. See a doctor.<br>**Swallowed:** Do no more than wash out the mouth with water. Do **not** induce vomiting. Sips of water may help cool the throat and help keep the airway open. See a doctor.<br>**Dust breathed in:** Remove the casualty to fresh air. See a doctor if breathing is difficult.<br>**Spilt on the skin or clothing:** Remove contaminated clothing. Wash off the skin with soap and plenty of water. Rinse contaminated clothing.<br>**Spilt on the floor, bench, etc.:** Scoop up solids (take care not to raise dust). Wipe up solution spills or any traces of solid with a damp cloth and rinse it well. |
| Ethanol (pure) | MODERATE HAZARD<br>  <br>**GHS02 (*flammable* F)**<br>  <br>**GHS08 (*health hazard* HH)** | **In the eye:** Flood the eye with gently-running tap water for 15 min. See a doctor.<br>**Vapour breathed in:** remove the casualty to fresh air. Call a doctor if breathing is even slightly affected.<br>**Swallowed:** do no more than wash out the mouth with water. Do not induce vomiting. Sips of water may help cool the throat and help keep the airway open. See a doctor. Note: the casualty may show signs of drunkenness.<br>**Spilt on the skin or clothing:** remove contaminated clothing and rinse it. Wash the skin and clothing with plenty of water. If a large area is affected or blistering occurs, see a doctor.<br>**Spilt on the floor, bench, etc.:** Extinguish all Bunsen-burner flames. Wipe up small amounts with a cloth and rinse it well. For larger amounts, open all windows, cover with mineral absorbent (e.g. cat litter), scoop into a bucket and add water.<br>**Clothing catches on fire:** Push the casualty to the floor, roll the body or smother flames on clothing or the skin with a fire blanket or other material. Cool any burnt skin with gently-running tap water for 10 minutes.<br>**Other ethanol fires:** Allow fires in sinks, etc., to burn out. Fires at the top of test tubes, beakers, etc., should be smothered with a damp cloth or heat-proof mat. |

## Experiment set-up

**Filtering of the solution through muslin**




**Addition of ethanol to the sample**



# Teacher method

This is your version of the method for this experiment that accompanies the teacher video.

Do not share this method with learners. Give them Worksheet E.

**Before you begin**
Plan how you will group your learners during the experiment.

Think about:

- the number of groups you will need (group size 2–4 learners)
- the amount of equipment/chemicals required
- whether you will attempt to stain the DNA or not.

**Experiment**
Circulate during the experiment in case learners encounter any difficulties.

| Step | Notes |
|---|---|
| 1. Learners collect all the equipment they need from the front of the class. | *Make sure the ethanol is kept on ice throughout the experiment. Remind learners of the safety precautions.* |
| 2. Demonstrate or supervise the use of the kitchen blender. | *For your class, decide if it is safer for you to demonstrate and hand out split pea solution, or allow the learners to blend their own.* |
| 3. Learners put some of the mixture in a boiling tube. | *Learners should not consume the split peas or the resulting mixture!* |
| 4. Learners set up muslin filter by stretching the muslin cloth over the beaker and securing with an elastic band. They should gently push down in the middle of the muslin to create a well for the mixture. | |
| 5. Learners filter their mixture. The green solution they collect can be poured into a fresh boiling tube. | |
| 6. The boiling tube is placed into an ice water-bath. | *This can be made using ice cubes and a small amount of water in a beaker.* |

| Step | Notes |
|---|---|
| 7. Learners add washing up liquid and leave for 5–10 minutes. | |
| 8. Learners add a few drops of fresh pineapple juice. | |
| 9. Learners tilt the boiling tube to 45° and slowly add the chilled ethanol. Ethanol is added until the volume of ethanol is equal to the volume of the split-pea solution. | *The ethanol should float on top of the green solution as it is less dense than water. The angle means the ethanol immediately floats on top rather than mixing in and having to wait for it to settle out. Equal volume allows for sufficient DNA to be extracted. Remind learners of safety precautions.* |
| 10. Learners should observe a cloudy layer forming where the ethanol meets the split pea solution. Prompt learners to think about what this layer may be. | *DNA is soluble in water but salted DNA is insoluble in ethanol and white precipitate (clumps) of DNA will be visible where the water layer meets the ethanol.* |
| 11. Learners gently stir where the two layers meet with a glass rod to pull the DNA out of the split pea solution and into the ethanol, where it will precipitate. | |
| 12. Learners remove the DNA from the boiling tube and put into a Petri dish filled with ethanol using a dropping pipette. | *This makes it easier to test the precipitate to see if it is DNA.* |
| 13. Learners add methylene blue to the Petri dish of ethanol to stain the DNA. | *DNA absorbs methylene blue and becomes a blue colour. So, a blue precipitate is positive for DNA; if the precipitate does not go blue then it's likely to be protein (probably pectin). This can be tested by adding protease, which will break down the white precipitate if it is protein. Remind learners of the safety precautions.* |

**Clean-up**

After the experiment learners should:

- clean all glassware and tidy up their work space
- ensure any spillages have been mopped up; if the spillage is ethanol or methylene blue they must inform you immediately and you must clean it up
- return all equipment and any unused chemicals to you (you should count ethanol bottles out and in again).

**Teacher / technician disposal**

- to dispose of methylene blue, dilute below 1% solution and pour down a foul-water drain
- to dispose of ethanol, dilute to 5% solution and pour down a foul-water drain.

# **Lab lesson:** Option 2 – virtual experiment

| | |
|---|---|
| **Resources** | • Worksheets F, G and H<br>• *Virtual experiment* video |
| **Learning objectives** | By the end of the lesson:<br>• ***all*** learners should be able to identify two hazards and the correct precautions specific to this experiment.<br>• ***most*** learners should be able to describe the method for extracting DNA by ordering the instructions correctly.<br>• ***some*** learners will be able to explain why each chemical is added and the implications of working in the incorrect order. |

| Timings | Activity |
|---|---|
| 15 min | **Starter/introduction**<br><br>Explain that they are going to watch a video that demonstrates a technique for extracting DNA from split peas. Play the introduction of the video; it will automatically pause on the equipment shot. Give each learner Worksheet F (experiment set-up) and Worksheet G (risk assessment template). Ask them to complete a risk assessment for the experiment. Draw their attention to the blender. Click on the 'Equipment list' to reveal a written list of all the equipment.<br><br>Discuss ideas as a class; learners add in any hazards they have missed. You can steer learners in the right direction using the suggested answers for Worksheet G. |
| 15 min<br><br>20 min | **Main lesson**<br><br>Arrange learners into groups of 2–4. Give each group Worksheet H and ask them to put the steps of the method for the experiment into the correct order. They will also need to decide the purpose of each step, this should help them with their ordering. You might need to discuss or explain the role of the salt, washing up liquid, pineapple juice / protease and ethanol, and the need for the low temperatures.<br><br>Discuss as a class the consequences of getting the steps in the wrong order; why is it important to do the steps in the order given? (So that the DNA is released from the nucleus into solution, otherwise it will still be locked inside the cells and left in the filter during filtration.) For example, if salt was not added at the start, then fewer cells would have been broken open, reducing the volume of DNA available and making it more difficult to extract. (See suggested answers for more examples.) Agree the correct order as a class; make sure learners are clear on the reason each step is carried out in the order given. (At this stage, they do not have to have the correct order, as they will compare it to the order given in the video.)<br><br>Resume play on the video, which will show them the practical being carried out. Tell learners to make notes for each step including any explanations given, and to check these against the order they agreed on in Worksheet H. They should note down any required changes.<br><br>As you watch the video, you can click on the buttons to ask questions about the method as you go along if you want to (the video will pause when the buttons are pressed), or just allow it to play through. Learners can raise their hands to answer, or there can be a class discussion.<br>*Continues on next page …* |

| Timings | Activity |
|---|---|
| | **Main lesson continued …**<br><br>Discuss why it's important to test that the white precipitate found was DNA (the video will automatically pause). Ask the learners to look at the results on screen. Was DNA found? (No, because it's not possible to see blue precipitate in the liquid.) Discuss what might have gone wrong to cause this. Suggestions might include that not enough pineapple juice was added, or the pineapple juice didn't contain a high enough concentration of protease; not enough washing up liquid was added; or the samples weren't incubated for long enough with the protease or the washing up liquid. The temperatures weren't cold enough and the DNA degraded. The person doing the experiment didn't successfully extract the white precipitate from the boiling tube, so there wasn't anything in the Petri dish to test.<br><br>Watch the end of the video, then allow learners time to correct their order for Worksheet H, if required. As a class go through the method (answers are available for Worksheet H) and question any incorrect orders and discuss the implications of that particular step being out of sequence. Resolve any misconceptions. |
|  | **Plenary**<br><br>Discuss as a class if their risk assessments were sufficient: did they have any issues? If so, what were they? Did they occur because they didn't follow their risk assessment or the instructions? Did they know how to respond? |

# Debriefing lesson: Planning a related experiment

| | |
|---|---|
| **Resources** | • Learners' completed Worksheet H<br>• Worksheets I and J |
| **Learning objectives** | By the end of the lesson:<br>• ***all*** learners should be able to list the basic equipment needed to extract human DNA.<br>• ***most*** learners can justify their choice of equipment and give a basic method for extracting human DNA.<br>• ***some*** learners can use previous knowledge to plan in detail how to extract human DNA. |

| Timings | Activity |
|---|---|
| 5 min | **Starter/introduction**<br>Arrange learners into groups of 2–4; the groups should be different to those used in the *Lab lesson.* Ask learners to review and compare their results from the *Lab lesson*. Did they get the same outcome? If not, can they identify what the other groups did better/wrong? |
| 10 min | **Main lesson**<br>Ask, '*What is an evaluation?*'; '*What is it useful for?*' Learners have 2 minutes individually and then 2 minutes as a pair to discuss how to evaluate an experiment.<br><br>As a class, discuss what goes into an evaluation. Learners should agree that an evaluation is the process of identifying and explaining the strengths and weaknesses of the experiment (either the method, data or both) in relation to its purpose. In other words, the experiment is assessed for validity. A valid experiment is one in which only the independent variable is changed and all other variables are controlled; this means that you can be confident the collected data is due to the hypothesis being tested and not some other factor. As part of the evaluation, improvements for increasing the accuracy of the results are proposed with justifications. Ask them why it's important to evaluate a method. They should understand that it helps to inform the planning of future experiments. |
| 5 min | Ask learners to answer the questions on Worksheet I; this can be done individually, in pairs, groups or as a class. These questions should remind them about some of the key steps in the method and why they were carried out. |
| 15 min | Then, start a class evaluation of the method that they carried out / watched in the *Lab lesson*. Use the following prompts to guide the discussion.<br><br>*'What was the purpose of our experiment?'*<br>To extract DNA from split peas.<br><br>*'How did we collect the data from the experiment, i.e. how did we determine if we had been successful or not?'*<br>Methylene blue was used; if the precipitate was stained blue it was DNA.<br><br>*Continues on next page …* |

| Timings | Activity |
|---|---|
|  | **Main lesson continued …**<br><br>'*Was our technique of data collection valid?*'<br>The purpose of the experiment was to extract DNA, not to extract a given amount of DNA. Therefore, using methylene blue to carry out a qualitative test (DNA is / is not present) was sufficiently accurate for the purpose of the experiment.<br><br>Ask if there would be any benefit to looking at the DNA under a microscope. Would this add any accuracy to the data collected? (No, the methylene blue tells us if it's there or not.) Would anything be visible? Are the school microscopes powerful enough? Would the fact that the DNA is clumped mean it is more difficult to see?<br><br>Make sure learners do not confuse evaluating how data was collected with the method of obtaining the data; whilst the test at the end was valid, the method for extracting DNA in the first place might not have been.<br><br>*'What were the strengths of the method?*' Split peas are easy to obtain and there are no ethical issues related to extracting DNA from them. The method is not too time-consuming.<br><br>'*What are the weaknesses of the method?*' Some possible suggestions of problems might include:<br><br>Leaving the washing up liquid in for longer before adding pineapple juice (if the washing up liquid wasn't left for long enough, the nuclear membranes would remain intact and DNA would not be released into solution).<br><br>Blending the split peas for longer than 15–20 seconds (if the blending time wasn't sufficient it would have made it harder to get to the nuclei and hence the DNA of the cells).<br><br>A 'pinch' of salt isn't a precise amount; perhaps more was needed. Different learners will have different 'pinch' sizes, which might explain why some groups extracted DNA and others didn't.<br><br>It's possible that the water and ethanol weren't cold enough; the temperature of the water-baths and solutions was not kept constant and it wasn't measured.<br><br>Learners might have held the boiling tube such that the solution was warmed in the palm of their hands; using test-tube holders to transfer the tube would reduce heat transfer. Warmer temperatures would cause enzymes in the extracts to break down the DNA more quickly.<br><br>It is possible that not enough pineapple juice was added; a volume wasn't specified; or that whether or not it was shaken before use had an impact on the distribution of the protease within it; it's also possible that different brands of pineapple juice contain different concentrations of protease, perhaps there wasn't enough? This could mean less / not enough protease enzyme was added to break down protein around the DNA, reducing yield.<br><br>*Continues on next page …* |

| Timings | Activity |
|---|---|
| | **Main lesson continued …**<br><br>Learners should suggest suitable alternatives to resolve these issues, such as using electronic water-baths to control temperature; using a protease enzyme solution rather than pineapple juice; using more washing up liquid and incubating it for longer; handling the boiling tubes using test-tube holders; adding a measured volume of salt; blending for longer, etc.<br><br>Ask learners to consolidate their evaluation of the method by writing a plan to extract DNA from human cells using Worksheet J. If you're running out of time, learners only have to write one of the sections. These can be compiled to form a class plan.<br><br>Ask the learners to think of suitable sources, i.e. those that can be obtained ethically without causing any pain (blood, cheek cells, skin cells, hair cells). Explain that the cells can be obtained by scraping the inside of the cheek or by gargling with water and then spitting it out. This would not need filtering. Discuss briefly the safety implications of using human cells. Why isn't it a good idea to use blood cells? (from a safety point of view, the risk of transmitting an infection is high, but also red blood cells don't contain a nucleus!). There is still a biohazard risk associated with cheek cells but it is considerably less than blood. Another option is to use a piece of adhesive tape, put it on the wrist and pull, skin cells will be found on the tape. |
|  | **Plenary**<br><br>Learners share the plans they have written. Ask learners to peer-assess each other's plans. They should use what they have learned to consider how suitable the plans are for the aim of the experiment. |

# Worksheets and suggested answers

| | Worksheets | Suggested answers |
|---|---|---|
| **For use in the *Briefing lesson:*** | | |
| **A:** Identifying hazards | **20** | **37** |
| **B:** Heating water risk assessment | **21–22** | **—** |
| **C:** Exemplar risk assessment for heating water | **23** | **—** |
| **D:** Finding alternative methods | **24** | **38** |
| **For use in *Lab lesson: Option 1:*** | | |
| **E:** Method | **25–26** | **—** |
| **F:** Experiment set-up | **27** | **—** |
| **G:** Risk assessment template | **28–29** | **39** |
| **H:** Ordering the method | **30–31** | **40** |
| **For use in *Lab lesson: Option 2:*** | | |
| **F:** Experiment set-up | **27** | **—** |
| **G:** Risk assessment template | **28–29** | **39** |
| **H:** Ordering the method | **30–31** | **40** |
| **For use in the *Debriefing lesson:*** | | |
| **I:** The split pea method | **32–33** | **41** |
| **J:** Planning to extract DNA from humans | **34–36** | **—** |

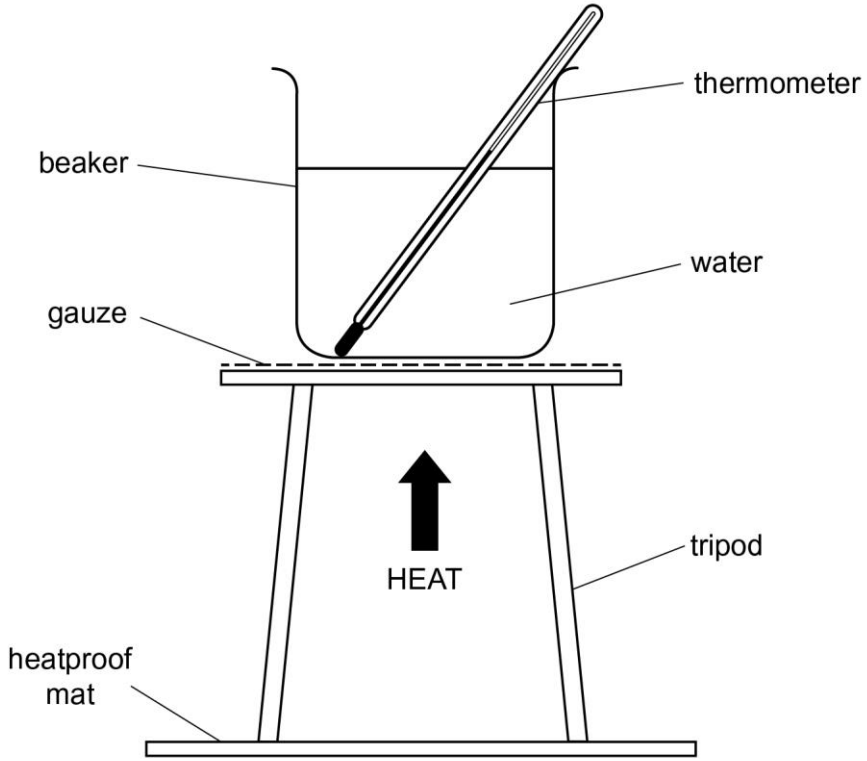
# **Worksheet A:** Identifying hazards

List the safety hazards, risks and precautions for each experiment.

| Experiment | Equipment | Chemicals used | Hazards, risks and precautions |
|---|---|---|---|
| Looking at stained onion cells using a microscope; the onion cells are prepared and then stained with iodine before viewing under a microscope. | Onion<br>Sharp knife<br>Forceps<br>Cutting board<br>Microscope slide<br>Cover slip<br>Microscope<br>Dilute iodine solution | Dilute iodine solution (mild skin / eye irritant) | |
| Calorimetry-testing crisps to determine which contain the most energy. Crisps are burnt and the energy they release used to heat water. The change in temperature is measured to see how much energy is released by the crisps. | Bunsen burner<br>Mounted needle<br>Heatproof mat<br>Mass balance<br>Boiling tubes<br>Spirit thermometer<br>Clamp and stand | Water | |
| Testing a leaf for starch: the leaf is boiled to break open the cells. Ethanol is used to remove chlorophyll and the iodine is used to test for starch (turns blue / black in the presence of starch). | Leaf<br>250 $cm^3$ glass beaker<br>Bunsen burner<br>Gauze<br>Heatproof mat<br>Tripod<br>Forceps<br>Ethanol<br>White tile<br>Dilute iodine solution | Water<br>Ethanol (highly flammable)<br>Dilute iodine solution (mild skin / eye irritant) | |

# **Worksheet B:** Heating water risk assessment

Write a risk assessment for heating a beaker of water in the laboratory using the equipment set-up shown below.




**Think about:**

1. What hazards there are in this experiment.
2. What problems the hazards could cause.
3. How you could reduce the risk of the issues occurring.
4. What to do if the issue happens.

| Hazard | Risk | Control measures (how to stay safe) | What to do in case of accident |
|---|---|---|---|
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# **Worksheet C:** Exemplar risk assessment for heating water

Here is an example of what your risk assessment should look like.

| Hazard | Risk | Control measures (how to stay safe) | What to do in case of accident |
|---|---|---|---|
| Bunsen burner | Hair, clothing or other material catches fire (ignites) | Tie back long hair.<br>Use safety flame when not heating.<br>Avoid reaching over flame.<br>Keep work area clear, especially of material such as paper that ignites easily. | Extinguish flames using a fire blanket or fire extinguisher.<br>Turn off gas.<br>Remove clothing if not stuck to burnt skin.<br>Follow your school's first aid advice for burns (for example, hold affected area under cool water for 20 minutes) and seek immediate medical assistance. |
| Bunsen burner | Burns from hot equipment | Be aware that equipment will get hot quickly.<br>Leave equipment to cool before handling.<br>Do not lean near the hot equipment.<br>Use tongs to move gauze, glassware or other items off the tripod if they have to be moved. | Remove clothing if not stuck to burnt skin.<br>Follow your school's first aid advice for burns (for example, hold affected area under cool water for 20 minutes) and seek immediate medical assistance. |
| Hot water | Scald from contact with skin | Leave water to cool before handling.<br>Do not lean near the hot equipment, especially whilst the water is boiling.<br>Wear eye protection and a lab coat. | Remove clothing if not stuck to burnt skin.<br>Follow your school's first aid advice for burns (for example, hold affected area under cool water for 20 minutes) and seek immediate medical assistance. |
| Using glass equipment | Cuts from broken glass | Wear eye protection.<br>Keep glass equipment away from the edge of the work area.<br>Clear up broken glass with dustpan and brush, not hands.<br>Dispose of broken glass in a broken glass bin. | Follow your school's first aid advice for treating cuts (for example, injured person applies clean and dry absorbent material to the cut with consistent pressure and elevates the cut above their head; if cut is to a lower limb, the injured person lies down and elevates the cut above heart level) and seek immediate medical assistance. |

**Important note:** When you are working in a lab, you must inform the teacher **immediately** if an accident occurs. Your teacher is responsible for carrying out the procedure for what to do in case of an accident, especially if putting out fire and clearing up broken glass.

# **Worksheet D:** Finding alternative methods

Think of an alternative method and / or equipment that can be used to make the step safer.

| Required step | Method | Alternative safer method |
|---|---|---|
| Hot water is needed to speed up a reaction involving Benedict's solution. | Use a Bunsen burner to heat the water. | |
| A highly flammable liquid needs to be heated to 40°C. | Use a Bunsen burner to directly heat the solution. | |
| Testing a sample of food for the presence of starch. | Use iodine liquid concentrate straight from the supplier. HINT: highly concentrated solutions of iodine can severely irritate and burn the skin and eyes, and irritate the lungs. | |
| Cutting a leaf off a plant. | Use a scalpel and white tile. | |
| Slicing an onion. | Hand out knives to all groups and have learners cut the onion themselves. | |
| The thermite reaction: adding aluminium to iron(III) oxide to produce molten iron. | All students in the class do this. HINT: the experiment produces a lot of heat energy, very quickly. | |
| Separate iron filings from sulfur powder. | Use a magnet and then pick filings off magnet after. HINT: iron filings are difficult to remove and if they get in the eye, they can be damaging. | |

# **Worksheet E:** Method

1. Collect your equipment.
2. Put 100 $cm^3$ of split peas into a blender.
3. Add 200 $cm^3$ of ice-cold water.
4. Add a pinch of salt.
5. Turn the blender on and blend for 15–20 seconds.

> *Only use the blender if you have been shown how to. Fingers or hands could be cut with the fast-moving blades. Do **not** put hands inside the blender, even when turned off.* ***Always keep hands clear of the blades.***

6. Allow the mixture to settle then pour it into a boiling tube.
7. Set up the muslin filter by stretching the muslin cloth over a 250 $cm^3$ glass beaker, secure with an elastic band and gently push in the middle to create a well for your mixture to sit in.
8. Add some of the mixture into the well and allow it to filter. Wait until all mixture is filtered.
9. Pour the filtered mixture from the beaker into a clean boiling tube.
10. Place the boiling tube in an ice-water bath.
11. Add 30$cm^3$ of washing up liquid and gently swirl to mix.
12. Leave to settle for at least 5 minutes. Use a stopwatch to time this.
13. After 5 minutes, add a few drops of pineapple juice; the pineapple juice contains the enzyme protease. Swirl gently and allow to settle.
14. Tilt the boiling tube to 45° and slowly add the chilled ethanol. Keep adding the ethanol until there is about the same volume as there is split pea solution.

> *The ethanol will form a layer on top of the solution since ethanol is less dense than water. The angle helps the layers to form naturally, without having to wait for the mixture to settle.*
>
> *Take care not to spill any ethanol on your skin; do not breathe in the fumes. Do **not** use near a naked flame.*

15. Observe the boiling tube closely.

> *What do you observe about the two liquids when you add the ethanol? Why does this happen?*

16. Gently move a glass rod around the white layer between the ethanol and the split pea solution.

*DNA is soluble in water. Salted DNA is insoluble in ethanol.*
*What do you think is in the white layer?*

17. Use a twirling and pulling motion to pull the strands of DNA out of the split pea solution and into the ethanol. You will see the DNA as a white precipitate.
18. Use a dropping pipette to remove the DNA and place in a Petri dish of chilled ethanol.
19. Add a couple of drops of methylene blue solution. If you have collected DNA, this will stain the DNA dark blue.

*Avoid direct contact of methylene blue with the skin or eyes; wear eye protection.*
*Gloves could also be worn to prevent getting stain on your hands.*

If the sample does not stain blue, then it is likely that the white precipitate collected is a protein rather than DNA. This can be tested by adding protease; if the white precipitate is protein, then the enzyme will break it down.

If DNA was not extracted, in which steps could something have gone wrong?

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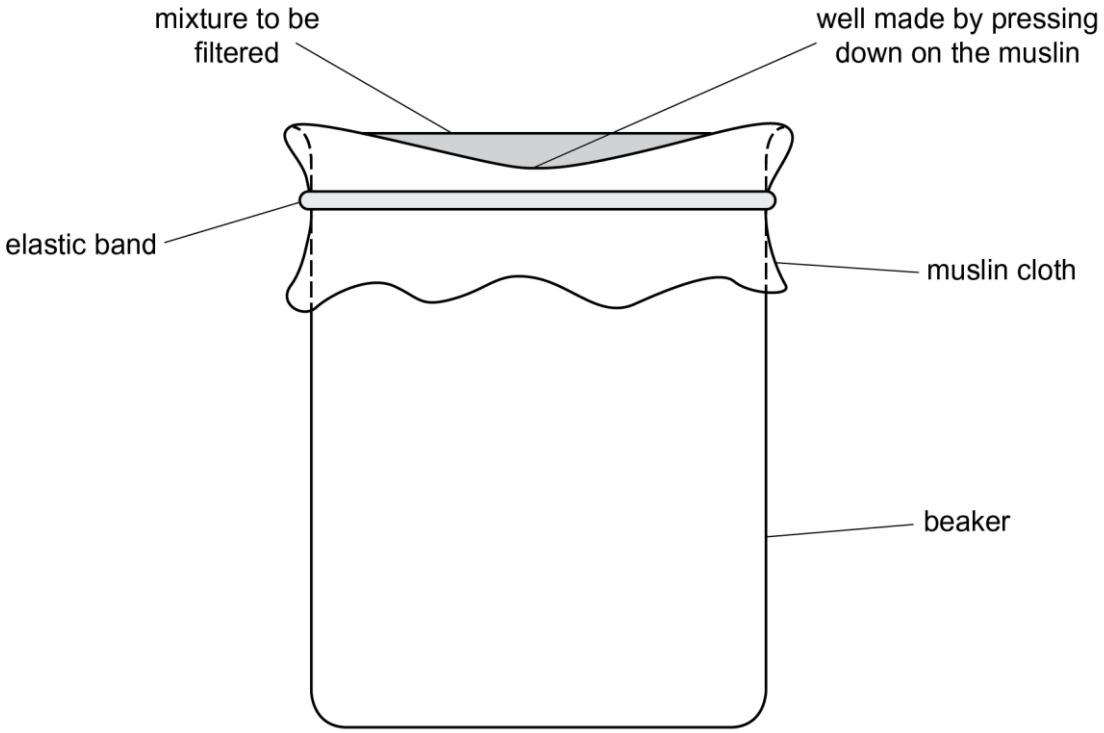
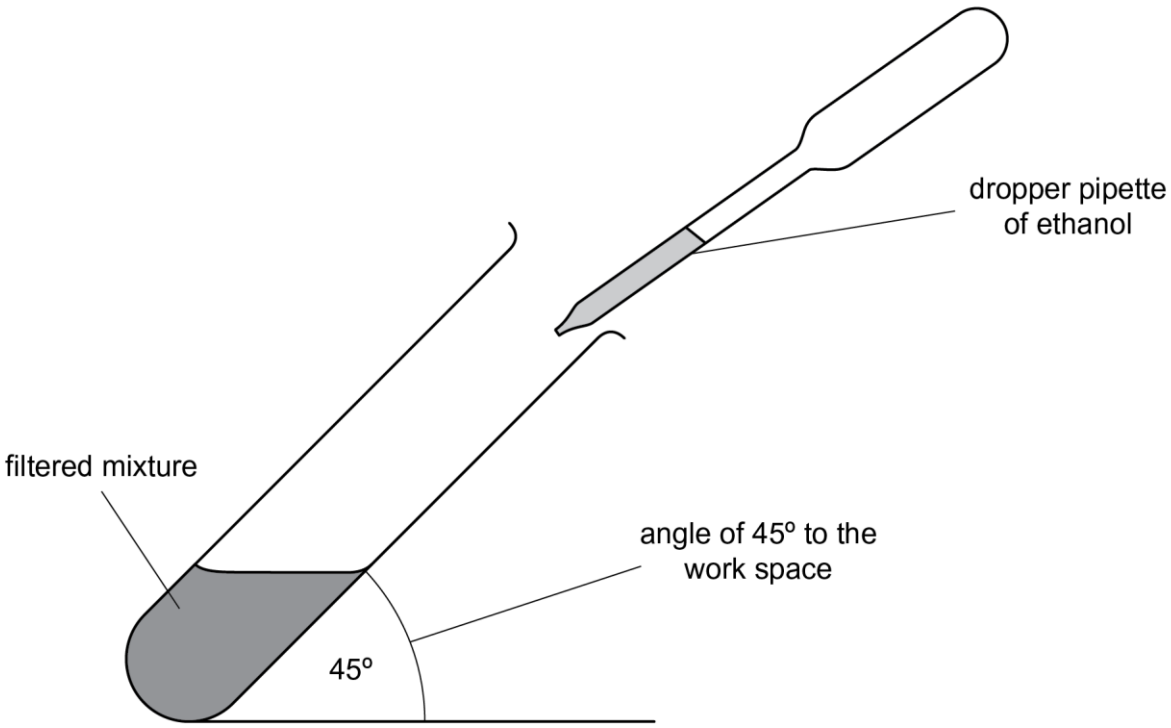
# **Worksheet F:** Experiment set-up

**Filtering of the solution through muslin**




**Addition of ethanol to the sample**



# **Worksheet G:** Risk assessment template

| Hazard | Risk | Control measures (how to stay safe) | What to do in case of accident |
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| Hazard | Risk | Control measures (how to stay safe) | What to do in case of accident |
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# **Worksheet H:** Ordering the method

The following instructions are steps in the technique to extract DNA from split peas. Cut out the steps and put them in the correct order on the next page. Then match the reason for the step.

| Steps | Reason |
|---|---|
| Add a pinch of salt. | The ethanol floats on the split pea solution as it is less dense; the angle helps the layers to settle more quickly. |
| Use a dropping pipette to remove the white precipitate (DNA). Place in a Petri dish of chilled ethanol. Add a few drops of methylene blue. | Blending separates the cells and starts to break them open, releasing the contents including the nucleus. |
| Pour the filtered mixture into a boiling tube and put in an ice water-bath. | DNA absorbs methylene blue and becomes a dark blue. This identifies the white precipitate as DNA and not protein. |
| Blend for 15–20 seconds. | Breaks cells apart and helps the DNA precipitate when it comes into contact with the ethanol. |
| Gently move a glass rod around the white layer between the ethanol and the split pea solution. | Filter paper would be too fine and the DNA would be left on the filter paper. |
| Add 200 $cm^3$ of ice-cold water. | This is needed to make a solution of the contents of the split pea cells. The cold temperature increases the yield of DNA by slowing down enzymes that would break down the DNA. |
| Put 100 $cm^3$ of split peas into a blender. | This breaks down any protein around the DNA. |
| Tilt the boiling tube to 45° and slowly add the chilled ethanol. Keep adding the ethanol until there is about the same volume as there is split pea solution. | Only a small amount of split pea mixture is needed. Filtering all of it would take too much time. |
| Add a few drops of pineapple juice / protease enzyme. Mix gently and allow to settle. | This breaks up the nuclear membranes, releasing the DNA into the solution. |
| Allow the mixture to settle then pour some of the mixture into a boiling tube. | The split peas provide the DNA to be extracted. |
| Pour the mixture through a muslin filter over a 250 $cm^3$ beaker. | Salted DNA is insoluble in ethanol. Where the split pea mixture comes into contact with the ethanol, the DNA in the mixture forms a white precipitate (clumps). |
| Add 30$cm^3$ of washing up liquid and gently swirl to mix. Leave for at least 5 minutes. | The cold temperature prevents enzymes from the broken cells breaking down the DNA. This increases the yield of DNA. |

 

| Steps | Reason |
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# **Worksheet I:** The split pea method

Answer the following questions in order to remind yourself of the reasons for each step in the method for extracting DNA from split peas.

(a) What is the safety hazard of using a blender? What precautions should be taken?

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(b) Why is it necessary to use ice-cold water? What is the pinch of salt for?

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(c) Why is a muslin filter used instead of a coffee filter and funnel?

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(d) What is the washing up liquid used for? Why do you need to leave the mixture for 5 minutes?

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(e) Why do you need to add protease?

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(f) Why does the ethanol need to be ice cold?

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(g) What did you observe about the two liquids when you add the ethanol? Why does this happen?

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(h) DNA is soluble in water. Salted DNA is insoluble in ethanol. What was in the white layer?

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(i) What are the safety hazards of using methylene blue? What precautions should you take?

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# **Worksheet J:** Planning to extract DNA from humans

Write a single paragraph for each section to create your plan.

**Section 1 – writing up your initial planning choices**

Explain where you plan to get the human DNA from and how you will extract it. **Example:**

*I am going to collect DNA from myself. I am going to extract the DNA from cheek cells using a cotton bud to swab the inside of my cheek; I will then swirl the bud in a solution of ice-cold water to remove the cells from the bud.*

**Section 2 – risk assessment**

Is there anything different about this extraction compared to using plant cells? Do you need to add anything new to your risk assessment? Complete a table for your experiment. The first row includes an example linked to the method above.

| Hazard | Risk | Control measures (how to stay safe) | What to do in case of accident |
|---|---|---|---|
| Human cheek cells are a biohazard. | Transmission of infection or diseases spread through saliva. | Dispose of the used cotton bud in a biohazard bin immediately and make sure no one else touches the sample. If having to handle someone else's sample, gloves should be worn. | Seek immediate first aid from trained medical personnel. |

 

**Section 3 – writing up your method**

Write your method as a series of instructions for someone else to follow. Make sure they are written in the correct order and contain enough detail. Refer back to Worksheet H to help you design your method. Decide which steps you will need to keep the same and which you will need to change.

**Example:**

1. Add 50 $cm^3$ of distilled water to a boiling tube.
2. Gently rub the inside of your cheek in an up and down motion, using a clean cotton bud. Cheek cells will collect on the end of the cotton bud.
3. Dip the cotton bud into the water and swirl it around. This should dislodge the cells and leave them in solution in the water.
4. Immediately dispose of the used cotton bud in a biohazard bin.

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**Section 4 – equipment list**

Use your method to help you write an equipment list. Justify your choices.

**Example:**

*Cotton bud – to collect cheek cells; collects cells without breaking the skin or causing harm.*
*Methylene blue – to test for presence of DNA; this stains DNA blue but will not stain protein blue, allowing for the positive identification of DNA.*

 

# **Worksheet A:** Suggested answers

| Experiment | Equipment | Chemicals used | Hazards, risks and precautions |
|---|---|---|---|
| Looking at stained onion cells using a microscope; the onion cells are prepared and then stained with iodine before viewing under a microscope. | Onion<br>Sharp knife<br>Forceps<br>Cutting board<br>Microscope slide<br>Cover slip<br>Microscope<br>Dilute iodine solution | Dilute iodine solution (mild skin / eye irritant) | **Sharp knife:** cuts; keep fingers away from blade; cut down onto cutting board; ensure they are counted out and in, and stored safely; make sure not put in washing up where someone could cut themselves.<br>**Microscope slide and cover slip:** made of very thin glass so easily broken, could cause cuts; ensure broken glass swept up using dustpan and brush, not picked up by hand, and disposed of properly in broken glass bin.<br>**Microscope**: heavy, if dropped on feet will cause damage; ensure carried correctly (two hands, with one underneath).<br>**Dilute iodine solution**: if dilute is low hazard but still a potential irritant to skin and eyes; wear eye protection; do not consume and avoid spilling; if spillage occurs or there is contact with skin or eyes, inform the teacher immediately. |
| Calorimetry-testing crisps to determine which contain the most energy. Crisps are burnt and the energy they release used to heat water. The change in temperature is measured to see how much energy is released by the crisps. | Bunsen burner<br>Mounted needle<br>Heatproof mat<br>Mass balance<br>Boiling tubes<br>Spirit thermometer<br>Clamp and stand | Water | **Bunsen burner**: possibility of fire and burn; long hair tied back; keep clear of flame; and keep flammable materials away.<br>**Hot equipment**: possibility of burns; ensure equipment is left to cool before touching; or move with tongs or heat-protective gloves if necessary.<br>**Mounted needle**: sharp, possibility of piercing skin; ensure handled correctly and carried around the room with needle pointing downwards; pierce food down onto heatproof mat, not by holding food in palm of hand.<br>**Boiling tubes**: made of glass, thin so easily broken; ensure broken glass swept up not picked up, and disposed of properly in broken glass bin; keep in a boiling-tube rack.<br>**Clamp and stand**: heavy, if dropped on feet will cause damage; ensure carried correctly (two hands, one underneath). |
| Testing a leaf for starch: the leaf is boiled to break open the cells. Ethanol is used to remove chlorophyll and the iodine is used to test for starch (turns blue / black in the presence of starch). | Leaf<br>250 $cm^3$ glass beaker<br>Bunsen burner<br>Gauze<br>Heatproof mat<br>Tripod<br>Forceps<br>Ethanol<br>White tile<br>Dilute iodine solution | Water<br>Ethanol (highly flammable)<br>Dilute iodine solution (mild skin / eye irritant) | **250 $cm^3$ glass beaker**: made of glass, could break; ensure broken glass swept up not picked up, and disposed of properly in broken glass bin; keep away from edge of the work area.<br>**Bunsen burner**: possibility of fire and burn; long hair tied back; keep clear of flame; and keep flammable materials away.<br>**Ethanol**: highly flammable, possibility of ignition and burns; keep clear of naked flame.<br>**Dilute iodine solution**: if dilute is low hazard but still a potential irritant to skin and eyes; wear eye protection; do not consume and avoid spilling; if spillage occurs or there is contact with skin or eyes, inform the teacher immediately.<br>**IMPORTANT**: in this experiment, the order is important; boiling the leaf can be done and the Bunsen turned off **before** getting out the ethanol; this dramatically reduces the risk of the ethanol igniting. |

# **Worksheet D:** Suggested answers

| Required step | Method | Alternative safer method |
|---|---|---|
| Hot water is needed to speed up a reaction involving Benedict's solution. | Use a Bunsen burner to heat the water. | Use a kettle to heat the water, or an electric water-bath. |
| A highly flammable liquid needs to be heated to 40°C. | Use a Bunsen burner to directly heat the solution. | Use an electric water-bath. Alternatively, you could create a water-bath by heating 100 $cm^3$ of water and placing a boiling tube of the solution into the hot water, so the hot water indirectly heats the solution. If the water-bath is heated using a Bunsen burner, then the Bunsen should be turned off **before** putting the boiling tube in. |
| Testing a sample of food for the presence of starch. | Use iodine liquid concentrate straight from the supplier. HINT: highly concentrated solutions of iodine can severely irritate and burn the skin and eyes and irritate the lungs. | Dilute the iodine so it poses less of a hazard. Dilute iodine is classed as low hazard and so only eye protection is needed. |
| Cutting a leaf off a plant. | Use a scalpel and white tile. | Use scissors. |
| Slicing an onion. | Hand out knives to all groups and have learners cut the onion themselves. | Have the teacher cut the onion, or have pre-sliced onion. |
| The thermite reaction: adding aluminium to iron(III) oxide to produce molten iron. | All students in the class do this. HINT: the experiment produces a lot of heat energy, very quickly. | The teacher can perform a demonstration so that there is only one source of heat and it's heavily controlled; or show the class the reaction on a video. |
| Separate iron filings from sulfur powder. | Use a magnet and then pick filings off magnet after. HINT: iron filings are difficult to remove and if they get in the eye, they can be damaging. | Wear gloves; wrap the magnet in cling film to separate the iron filings, they will still be attracted to the magnet; then unwrap the cling film and remove from the magnet; wrap the iron filings in the cling film; remove gloves and wash hands. |

# **Worksheet G:** Suggested answers

| Hazard | Risk | Control measures (how to stay safe) | What to do in case of accident |
|---|---|---|---|
| Ethanol | Highly flammable, could ignite and burn or cause clothing/hair to ignite. | Keep well away from naked flames.<br>Tie back long hair.<br>Use small amounts.<br>Keep bottle closed unless pouring. | Extinguish flames using fire blanket or fire extinguisher.<br>Remove clothing if not stuck to burnt skin.<br>Run burns under cool water for 20 minutes.<br>Seek immediate medical assistance. |
| Ethanol | Can cause a cough, headache, drowsiness or fatigue if inhaled; can lead to dark skin and redness or burning of the eyes; if ingested, it can lead to a burning sensation, headache, dizziness, confusion and even unconsciousness. | Wear eye protection.<br>Do not consume.<br>Use small amounts.<br>Keep bottle closed unless pouring.<br>Do not spill on skin. | If contact with the skin, wash with plenty of water; remove contaminated clothing and rinse well.<br>If gets in eyes, rinse with plenty of running tap water for at least 10 minutes and seek medical attention.<br>If ingested, do not induce vomiting; wash out mouth with water; sips of water might help cool the throat and help keep the airway open; seek medical attention.<br>If spilt, use a cloth to wipe up small amounts and rinse well; open all windows and cover with mineral absorbent (e.g., cat litter) if large volumes are spilt, and scoop into a bucket and add water. |
| Kitchen blender | Cuts from blade. | Keep hands well clear of blade, do not put into blender. | Injured person applies clean and dry absorbent material with consistent pressure to the cut and elevates the cut above their head.<br>Seek immediate medical assistance. |
| Glassware | Cuts | Wear eye protection.<br>Keep glass equipment away from the edge of the bench.<br>Clear up broken glass with dustpan and brush, not hands.<br>Dispose of broken glass in broken glass bin. | Injured person applies clean and dry absorbent material with consistent pressure to the cut and elevates the cut above their head; if cut is to lower limb, lie down and elevate cut above heart level.<br>Seek immediate medical assistance. |
| Methylene blue | Methylene blue solution is classed as a low hazard if it is made up in concentrations less than 1% by mass as a dilute aqueous solution. | Use 1% solution with water as the solvent.<br>Do not ingest or spill on skin.<br>Wear eye protection.<br>Wear gloves to prevent staining. | If gets in eyes, rinse with plenty of running tap water for at least 10 minutes and seek medical attention.<br>If gets on skin, wash off the skin with soap and plenty of water; remove and rinse contaminated clothing.<br>If ingested, do not induce vomiting; wash out mouth with water; sips of water might help cool the throat and help keep the airway open; seek medical attention.<br>If spilt, use a damp cloth to wipe up solution spills or any traces of solid, rinse well; scoop up solids making sure not to raise dust |

# Worksheet H: Answers

| Steps | Reason |
|---|---|
| Put 100 $cm^3$ of split peas into a blender. | The split peas provide the DNA to be extracted. |
| Add 200 $cm^3$ of ice-cold water. | This is needed to make a solution of the contents of the split pea cells. The cold temperature increases the yield of DNA by slowing down enzymes that would break down the DNA. |
| Add a pinch of salt. | Breaks cells apart and helps the DNA precipitate when it comes into contact with the ethanol. |
| Blend for 15–20 seconds. | Blending separates the cells and starts to break them open, releasing the contents including the nucleus. |
| Allow the mixture to settle then pour some of the mixture into a boiling tube. | Only a small amount of split pea mixture is needed. Filtering all of it would take too much time. |
| Pour the mixture through a muslin filter over a 250 $cm^3$ beaker. | Filter paper would be too fine and the DNA would be left on the filter paper. |
| Pour the filtered mixture into a boiling tube and put in an ice water-bath. | The cold temperature prevents enzymes from the broken cells breaking down the DNA. This increases the yield of DNA. |
| Add 30$cm^3$ of washing up liquid and gently swirl to mix. Leave for at least 5 minutes. | This breaks up the nuclear membranes, releasing the DNA into the solution. |
| Add a few drops of pineapple juice / protease enzyme. Mix gently and allow to settle. | This breaks down any protein around the DNA. |
| Tilt the boiling tube to 45° and slowly add the chilled ethanol. Keep adding the ethanol until there is about the same volume as there is split pea solution. | The ethanol floats on the split pea solution as it is less dense; the angle helps the layers to settle more quickly. |
| Gently move a glass rod around the white layer between the ethanol and the split pea solution. | Salted DNA is insoluble in ethanol. Where the split pea mixture comes into contact with the ethanol, the DNA in the mixture forms a white precipitate (clumps). |
| Use a dropping pipette to remove the white precipitate (DNA). Place in a Petri dish of chilled ethanol. Add a few drops of methylene blue. | DNA absorbs methylene blue and becomes a dark blue. This identifies the white precipitate as DNA and not protein. |

# **Worksheet I:** Suggested answers

(a) What is the safety hazard of using a blender? What precautions should be taken?

Fingers or hands could be cut with the fast-moving blades. Hands should not be put inside the blender, even when turned off; hands should be kept clear of the blades.

(b) Why is it necessary to use ice-cold water? What is the pinch of salt for?

When the cells are broken open, the DNA becomes exposed to enzymes in the cell that will break it down. The cold temperature reduces the rate of reaction catalysed by the enzymes, slowing the process and as a consequence, increasing the yield of DNA. Salt is used to help break open the cells and it also helps DNA to precipitate later.

(c) Why is a muslin filter used instead of a coffee filter and funnel?

The pores in filter paper are too fine and the DNA could get caught in the filter paper.

(d) What is the washing up liquid used for? Why do you need to leave the mixture for 5 minutes?

The washing up liquid breaks open the nuclear membrane to release the DNA. It needs to be left for 5 minutes in order to give time for it to take effect.

(e) Why do you need to add protease?

This enzyme breaks down any protein around the DNA, allowing the DNA to be free in the solution.

(f) What are the hazards of using ethanol? Why does it need to be ice cold?

Ethanol is highly flammable so could catch on fire and cause burning; it is also harmful if it comes into contact with the skin (causes dryness) or eyes (can lead to pain and burning). Ethanol should be kept away from naked flames, hair should be tied back, eye protection and a lab coat should be worn. It should only be used in small amounts and the container should be sealed when not in use. It should not be inhaled or ingested as this can lead to headaches, dizziness and confusion.

(g) What do you observe about the two liquids when you add the ethanol? Why does this happen?

The ethanol forms a layer on top of the split pea solution and a white layer forms at the interface of the two liquids. This is because ethanol is less dense than water and the split pea solution contains water.

(h) DNA is soluble in water. Salted DNA is insoluble in ethanol. What do you think is in the white layer?

The white layer is where DNA in the split pea solution has come into contact with ethanol; DNA is not soluble in ethanol and forms a white precipitate upon contact.

(i) What are the safety hazards of using methylene blue? What precautions should be taken?

Methylene blue solution is a low hazard if it is in a dilute solution, so as dilute a solution as possible is used and in small volumes. Eye protection and a lab coat are worn. It should not be ingested and direct contact with skin should be avoided. Gloves can be worn to avoid staining.

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