Cambridge Assessment International Education

Teaching Pack

Investigating how gel electrophoresis is used to separate DNA fragments of different lengths

Cambridge International AS & A Level

Biology 9700

Version 1

Cambridge Pathway

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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:

Briefing lesson (1 hour*)
This lesson reinforces the key concepts, relevant skills, knowledge and understanding linked to the experiment.

↓

Planning lesson (1 hour*)
This lesson focuses on planning an experiment.
It ends with a demonstration video of the experiment.

↓

Lab lesson (1 hour*)
This lesson focuses on carrying out the experiment including the collection and recording of observations, measurements and estimates.

↓

Debriefing lesson (1 hour*)
This lesson focuses on the analysis and interpretation of data.
This includes making conclusions, evaluating methods and the quality of data and how improvements could be made.

** 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 how gel electrophoresis is used to separate DNA fragments of different lengths

This *Teaching Pack* focuses on the procedure by which gel electrophoresis is used to separate DNA fragments of different lengths.

Gel electrophoresis is an important method used to separate DNA fragments of different lengths. In this experiment, you will follow instructions to carry out gel electrophoresis to identify the genotype of different people for a specific gene.

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

- 19.1 Principles of genetic technology

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.

- 6.1 Structure of nucleic acids and replication of DNA
- 19.1 Principles of genetic technology

Briefing lesson: Biological barcodes

Resources	• Teacher Instructions 1 • Worksheets A – D • Computer display or overhead projector
Learning objectives	By the end of the lesson: • **all learners** will be able to state that gel electrophoresis is used to analyse nucleic acids, and to distinguish between the alleles of a gene. • **most learners** will be able to describe how gel electrophoresis is used to analyse nucleic acids, and to distinguish between the alleles of a gene. • **some learners** will be able to explain why gel electrophoresis is used to analyse nucleic acids, and to distinguish between the alleles of a gene.

Timings	Activity
15 min	**Starter/Introduction** Share Worksheet A with learners. This describes a fictitious newspaper article regarding the use of DNA technology in a criminal investigation. It 'sets the scene' to provide learners with an example of the real-world use of the concept they are about to study. After they have read the article, instruct learners to engage in an 'ideas hothouse' to answer the questions listed. Learners initially work in pairs to brainstorm key words related to the five discussions, and then after 2–3 minutes, the pairs join together into 4s and then 8s to engage in deeper analysis. Learners record any new key words that arise during these wider discussions. Ask one or two learners from each group to then write their key words on the class board and then host a class discussion to join together the key terms into a mind map to help learners make meaning of them. Finally, learners copy down the mind map with things 'I knew' in green and things that 'are new' in red. This can be referred to at the end of the lesson to show learners how much progress they have made.
40 min	**Main lesson** Refer to Teacher Instructions 1 for this activity. This is a task called 'jigsaw reveal.' A diagram of the equipment required for gel electrophoresis is obscured by nine jigsaw pieces. Inform learners that during this series of lessons they will have the opportunity to explore the technique of gel electrophoresis. Provide Worksheet B, which contains the fully-obscured jigsaw. Display this on the board and ask one learner to choose a numbered jigsaw piece at random. Display the image with this piece removed. Challenge learners to infer what has been revealed, and the function of this section of the equipment. If the revealed image is difficult to interpret, provide learners with

	an opportunity to discuss their ideas as you circulate to provide prompts in order to guide them towards the correct answer. Encourage learners to make rough notes on the jigsaw piece on Worksheet B. Finally, provide Worksheet C. This includes an unlabelled diagram of the image in Teacher Instructions 1. Challenge learners to use their rough notes to label the different parts of the equipment with their functions.
	**Plenary** Provide Worksheet D, which requires learners to analyse the question, '*how is a barcode similar to the results of gel electrophoresis?*' This is a good way to focus learners on developing their higher-order thinking skills to make sense of the meaning of the terms they have encountered, rather than simply recall them. To scaffold this activity for some learners, provide the first and final sentences of the answers. Collect learners' work as they leave the room, so that their understanding can be formatively assessed in advance of the *Planning lesson.*

Planning lesson: Electrophoresis training circus

Resources	• Teacher Instructions 2 • Worksheets E – G • Computer display or overhead projector • Warm water • Electrophoresis tank • Casting tray, rubber end caps, and sample comb • Blue food colouring • Micropipette (0-50 μl) with tips • D.C. power supply • Micropipette • Small plastic tray • Visualisation tray or white torch • Paper towels • Gloves • Eye protection

Learning objectives	By the end of the lesson: • **all learners** will be able to describe how to carry out gel electrophoresis in order to distinguish between the alleles of a gene. • **most learners** will be able to describe how to carry out gel electrophoresis in order to distinguish between the alleles of a gene. • **some learners** will be able to describe how to carry out gel electrophoresis in order to distinguish between the alleles of a gene.

Timings	Activity
5 min	**Starter/Introduction** Refresh learners' knowledge of key terms related to gel electrophoresis by hosting a brief quiz using Teacher Instructions 2. Provide Worksheet E, a multiple-choice answer selector, to each learner. Learners can 'vote' for their choice of answer by holding up the piece of paper they think identifies the correct answer to each of a series of multiple-choice questions on the board. As a class, discuss which answers are wrong and why, in order to develop a common understanding. This activity encourages pair and whole-class discussion, and reinforces key terminology and concept from the *Briefing lesson*.
45 min	**Main lesson** Inform learners that they are going to undertake a series of activities that will develop understanding and skills related to the process of gel electrophoresis. If your school has one or several gel electrophoresis kits and associated equipment, the following task involves a 'circus' in which four 'stations' are arranged around the room. Leaners work in pairs or small groups to undertake the four tasks that require them to practice the steps involved in gel electrophoresis. If your school does not

	possess this equipment, the following activity can be adjusted to focus learners on researching the various steps using their course textbook or the internet. Set up the workstations and provide Worksheet F to your learners. Circulate the room as learners work in pairs or small groups to conduct the tasks listed at each station. Allow learners ten minutes to work at each station and sound an alarm or similar when the time is up. For the fourth activity, learners will need copies of Worksheet G. Many students will find it challenging to carry out these activities properly to begin with. It can be helpful to pair more confident learners with those needing more help and employ them as demonstrators. Both 'learner' and 'demonstrator' benefit, the 'demonstrator' by reinforcing their communication skills and developing communication skills, and the 'learner' by having more detailed and personalised help. During the activities, reinforce the names of the different parts of the equipment by displaying on the board a labelled diagram of the equipment (the Answers to Worksheet C may be used for this purpose). Insist that learners use the terms during the lesson in their discussions. This helps learners become familiar with the terms.
	**Plenary** This activity introduces many of the skills that learners will require during this topic. Encourage learners to reflect on their experiences and make some recommendations for the *Lab lesson*. These could be of the structure '*The next time I do … I must remember to …*' If there is time, ask learners to write these on post-it notes, which they must stick to the class board or wall. Ideally, leave these sticky notes in place until the *Lab lesson*.

Lab lesson: Getting practical

Resources	• Agarose gel • Electrophoresis tank • Casting tray, rubber end caps, and sample comb • DNA sample • Micropipette (0-50 μl) with tips • Tracking dye • DNA staining reagents • D.C. power supply • Micropipette • TAE buffer solution • Small plastic tray • Visualisation tray or white torch • Paper towels • Gloves • Eye protection

Learning objectives	By the end of the lesson: • **all learners** will undertake an investigation using gel electrophoresis. • **most learners** will be able to explain the basis of an investigation using gel electrophoresis. • **some learners** will suggest how an investigation using gel electrophoresis can be improved.

Timings	Activity
10 min	**Starter/Introduction** Ask learners to compare their answers to Worksheet F from the *Planning lesson*. Engage learners in a 'think, pair, share' activity with a partner for a few minutes, and then choose learners at random to offer their answers to inform a class discussion.
45 min	**Main lesson** Inform learners that they will now undertake the practical activity/ you will now host a demonstration. Ask learners to use their completed Worksheet F from the *Planning lesson* to help them. Because an hour is required to run the electrophoresis, and then additional time required to stain and observe results, it isn't possible for learners to complete the full experiment in a standard hour long lesson. In this case, learners can get their experiment running, then staining (depending on the method used) and observing results takes place in the next lesson, or you could pre-prepare stained samples that learners can use to complete the experiment (task 4 in Worksheet F). **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.

Plenary

Host an 'open board' to allow learners to list key words related to the method which illustrate some of the difficulties they encountered. These may include 'volume,' 'precise' and 'inaccurate.' Ask learners to record these key terms and in advance of next lesson, prepare a brief summary of the problems and how they could be overcome.

Teacher notes

Watch the video (teacher version) and read these notes.

Each group will require:

- Agarose gel
- Electrophoresis tank
- Casting tray, rubber end caps, and sample comb
- DNA sample
- Micropipette (0-50μl) with tips
- Tracking dye
- DNA staining reagents
- D.C. power supply
- Micropipette
- TAE buffer solution
- Small plastic tray
- Visualisation tray or white torch
- Paper towels
- Gloves
- Eye protection

Safety

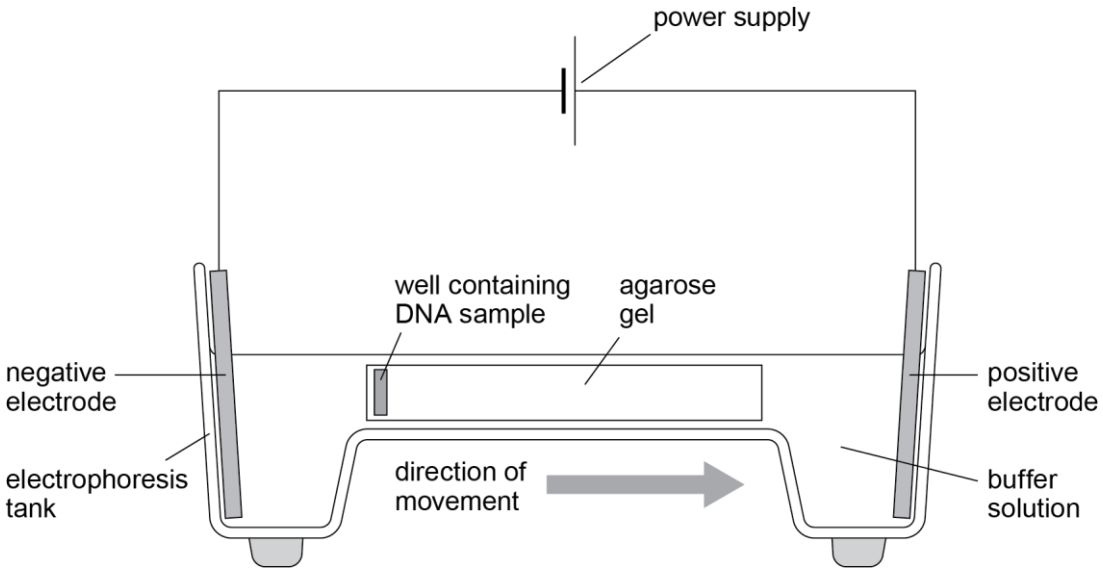
The information in the table below is a summary of the key points you should consider before undertaking this experiment with your learners.

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

Substance	Hazard	First aid
Agarose	Burns	Flood burnt area with water for at least 10 minutes. For serious injuries see a doctor.
DNA from laboratory suppliers	 Biohazard	**Skin or clothing:** Remove soiled clothing; wash skin thoroughly with soap and running water. **Spilt on the floor, bench, etc.:** For spills of cultures, place paper towels over the spill, pour disinfectant (e.g., Virkon) on top and leave for at least 15 min. Bleach is usually suitable in the home. **(You must do a risk assessment for any disinfectant or bleach used.)**
Tracking dye	Low Hazard	**In the eyes:** Flood the eye with gently-running tap water for 10 min. If discomfort persists, see a doctor. **Swallowed:** Do no more than wash out

Substance	Hazard	First aid
		the mouth with water. Do not induce vomiting. See a doctor if necessary. **Spilt on the skin or clothing:** Remove contaminated clothing; wash skin thoroughly with (antibacterial) hand soap and running water. **Spilt on the floor, bench, etc.:** Clean the area thoroughly using an appropriate disinfectant (**you must do a risk assessment for any disinfectant used**).
Gel electrophoresis	 Electric shock	**If in casualty is in contact with live electricity supply:** Break contact by switching off or removing the plug. If this is not possible, use a wooden broom handle or wear rubber gloves to pull the casualty clear. See a doctor. If the casualty is unconscious, check that airways are clear and that the casualty is breathing and has a pulse. If so, place the casualty in the 'recovery position'. If a pulse is found but the casualty is not breathing, artificial ventilation is necessary. If no pulse is found and the casualty is not breathing, cardio-pulmonary resuscitation is necessary.

Experiment set-up



Teacher method

This is your version of the method for this experiment that accompanies the *Teacher walkthrough* video.

Do not share this method with learners.

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 2–4 learners) or how you will host the demonstration
- the amount of equipment/chemicals required

Experiment

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

Steps	Notes
1. Prepare an agarose gel by dissolving the agarose powder in hot water, and pouring the mixture into a casting tray.	*Use a microwave to heat the agarose solution. Take care to avoid scalding.*
2. A sample comb is placed into the tray before the mixture is poured in.	*This provides a series of wells to which the DNA samples will be added later.*
3. Once it has solidified, the sample comb is removed from the gel and the gel is placed into the electrophoresis tank.	*The end of the gel that has the wells is placed closest to the negative electrode (cathode) which has a black surface.*
4. The electrophoresis tank is then filled with buffer solution. Enough buffer solution is added to completely submerge the gel.	*The buffer solution has a high concentration of ions.*
5. The DNA samples are prepared. The DNA is mixed with a small volume of tracking dye, usually blue in colour.	*If there is more than one DNA sample, these should be placed in the order in which they will be added to the gel. The tracking dye indicates the position of DNA in the gel during electrophoresis.*
6. A plastic tip is attached to the end of the micropipette.	*An airtight seal is ensured by pressing down firmly.*
7. The micropipette is held in a vertical positon and the plunger is pressed to the first stop.	*Air equal to the volume of the setting is displaced.*

8. The tip is immersed in the sample of DNA and the plunger is released to the rest position.	*Allow a moment for the liquid to be sucked up into the tip. This will equal the volume specified by the micropipette (in this experiment, this is 35 μl).*
9. The micropipette is then held against the hand to guide the tip into the well at an angle.	*Place the tip just a few millimetres into the well, to avoid puncturing the bottom of the well.*
10. The plunger is pressed to the first stop, before waiting a second, and then pressed again to expel the full volume of liquid.	*Take care to avoid pressing the plunger any further, which would bubble air into the well and disturb the sample.*
11. Repeat this process until all samples have been added to the gel. Once all the samples have been added to the wells in the gel, the lid is placed onto the electrophoresis tank.	*Replace the tips between loading, so that the DNA samples are not mixed.*
12. The power supply is connected to the tank using the leads.	*Ensure that the polarity of the power supply matches that of the electrophoresis machine: black to black and red to red.*
13. Set the voltage to 75 V and switch the power supply on.	*Bubbles should start to appear to indicate that the charge is flowing.*
14. Let the process run for around 60 minutes and switch off the power supply.	*As time passes, the position of the DNA and tracking dye will move closer to the positive pole (anode).*
15. Remove the gel from the tank and place in a small tray containing buffer solution.	*The gel may be kept in this buffer solution overnight but it should not be allowed to dehydrate.*
16. Add an appropriate substance to the solution to stain the DNA.	*DNA is colourless so it is only after staining the DNA that a pattern of bands can be seen. These represent fragments of DNA of different sizes.*

Clean-up

After the experiment learners should:

- clean all glassware
- tidy up their work space
- ensure any spillages have been mopped up
- return all equipment and any unused chemicals to you.

Debriefing lesson: Extrapolating electrophoresis

Resources	• Worksheets H and I
Learning objectives	By the end of the lesson: • **all learners** will be able to state some uses of gel electrophoresis. • **most learners** will be able to describe how gel electrophoresis can be used. • **some learners** will be able to explain some of the problems that are associated with the use of gel electrophoresis.

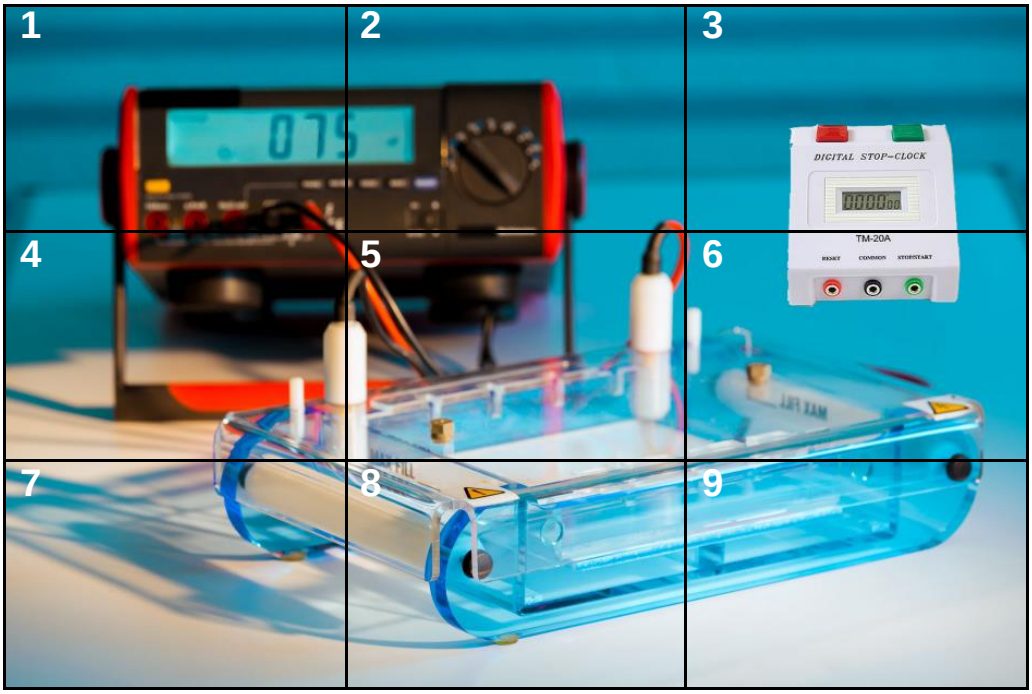
Timings	Activity
10 min	**Starter/Introduction** At the end of the *Lab lesson*, learners were asked to list key words related to the method which illustrate some of the difficulties they encountered. Allow learners to review the post-it notes and consider the recommendations of others, and highlight common mistakes that learners made. Alternatively, give learners 2–3 minutes to discuss with a partner one aspect of the method that presented an obstacle during the last lesson. Choose one learner at random (or ask for a volunteer) and ask for a contribution to get the class discussion started. Ask the other learners in the class '*Who else encountered this problem?*' and elicit various solutions that were used to overcome it.
30 min	**Main lesson** Refer learners to Worksheet H, which provides a written text that summarises the experience of a class that has undertaken a similar practical investigation into the sickle cell anaemia investigation. However, there are 5–10 conceptual and terminology errors. Encourage learners to spot and circle as many mistakes as possible, and offer corrections in the margin. Provide learners with 5 minutes to compare and contrast their work with that of a partner, and add any points they missed. This activity could be made into a competition, with the first pair of learners who identifies all mistakes deemed the winner.
20 min	**Plenary** Provide learners with Worksheet I and challenge them to work on this task individually for 10 minutes. This contains a series of synoptic questions that can be used to formatively assess learners' understanding of the method of gel electrophoresis and the analysis of DNA using this method. If there is time, provide the answers to this worksheet and host an exercise in which learners peer assess their work. Otherwise, collect learners' work and formatively assess it in advance of the next lesson.

Worksheets and answers

	Worksheet	Answers
For use in *Briefing lesson*:		
Teacher instructions 1: Jigsaw reveal	**18**	
A: Solving crime with DNA	**31**	**44**
B: Gel electrophoresis equipment 1	**33**	
C: Gel electrophoresis equipment 2	**34**	
D: Biological barcodes	**35**	**45**
For use in *Planning lesson*:		
Teacher instructions 2: Multiple choice questions	**28**	
E: Multiple choice answer sheet	**36**	
F: Method circus	**37**	**46**
G: Sample results	**41**	
For use in *Lab lesson*:		
F: Method circus	**37**	**46**
For use in *Evaluation lesson*:		
H: Critiquing a report	**42**	**47**
I: Applying knowledge	**43**	**48**

Teacher instructions 1: Jigsaw reveal

Use the following ten pages in the *Briefing lesson*. Refer to the instructions provided to host this activity.



As different jigsaw pieces are removed, encourage learners to record the key points from the discussion on Worksheet B, on top of the blank jigsaw pieces. Suggestions may include:

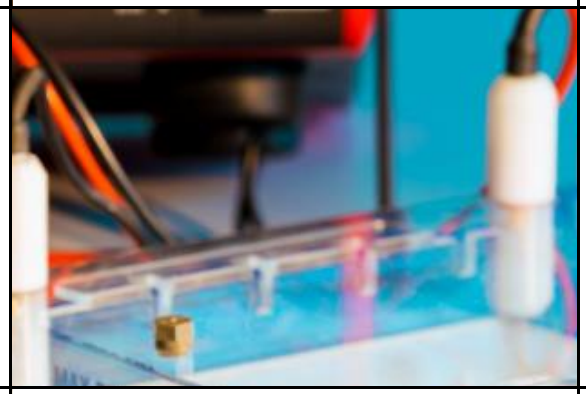
1. Two wires connected to a power supply.
2. The voltage of the power supply set at 75 V.
3. A stopwatch measures the time of the investigation.
4. A gel, made of agarose, is in the tank.
5. The gel has wells into which the DNA and loading (tracking) buffer is placed.
6. The tank is filled with electrophoresis buffer.
7. The positive electrode (anode) is connected to the tank furthest away from the gel wells.
8. The safety instructions are followed: this experiment uses electricity and hazardous chemicals.
9. The negative electrode (cathode) is connected to the tank closest to the gel wells.

		 DIGITAL STOP-CLOCK

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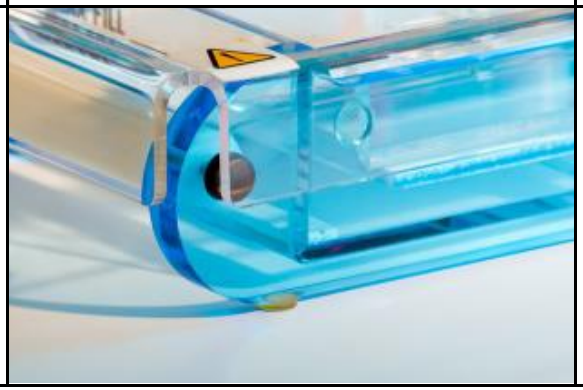
		

Teacher instructions 2: Multiple choice questions

Here are three example multiple choice questions about gel electrophoresis for use in the *Briefing lesson* starter. The correct answers are underlined. Read the text below in bold and consider displaying the figures, large enough for everyone to see. Learners will need to discuss their thoughts with a partner for 30 seconds before submitting their answer.

In the garden pea, *Pisum sativum*, seeds can either have a wrinkled skin or a smooth skin. Wrinkled peas have a recessive mutation caused by the insertion of DNA into the allele. Two heterozygous pea plants were crossed. Two of the offspring (m and n) had the DNA for this gene removed and separated by gel electrophoresis, as summarised in Figure 1. The results are shown in Figure 2.

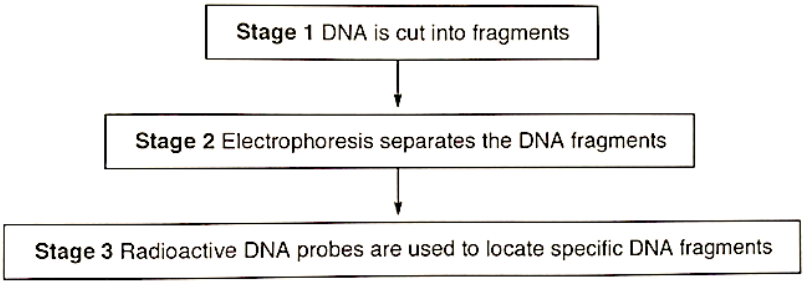


Figure 1.

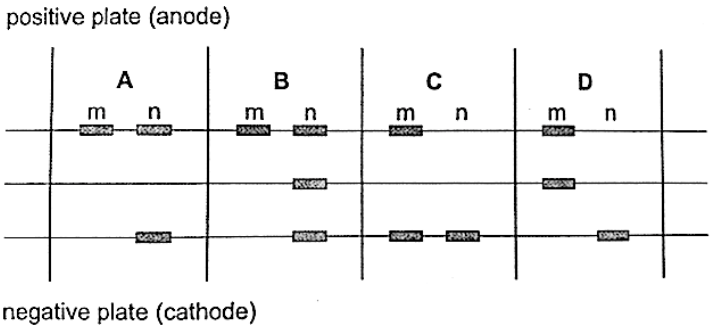


Figure 2.

1. What is the name of the enzyme used in Stage 1?

   A. DNA polymerase
   B. DNA ligase
   C. DNA helicase
   D. Restriction endonuclease

2. Identify the properties of the probes used in Stage 3.

   A. Radioactive with complementary sequence to the DNA fragments
   B. Radioactive with non-complementary sequence to the DNA fragments
   C. Non-radioactive with complementary sequence to the DNA fragments
   D. Non-radioactive with non-complementary sequence to the DNA fragments

3. Which pattern of bands shows a wrinkled phenotype and a smooth phenotype?

   A B C D

Figure 1.

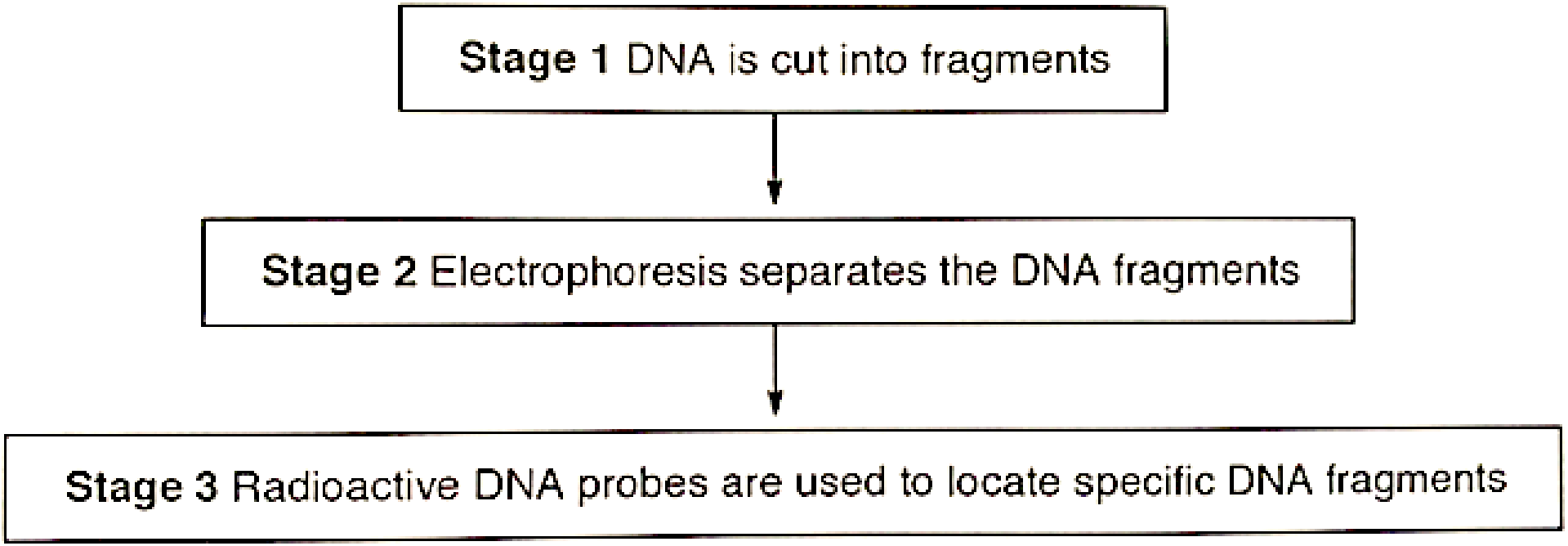
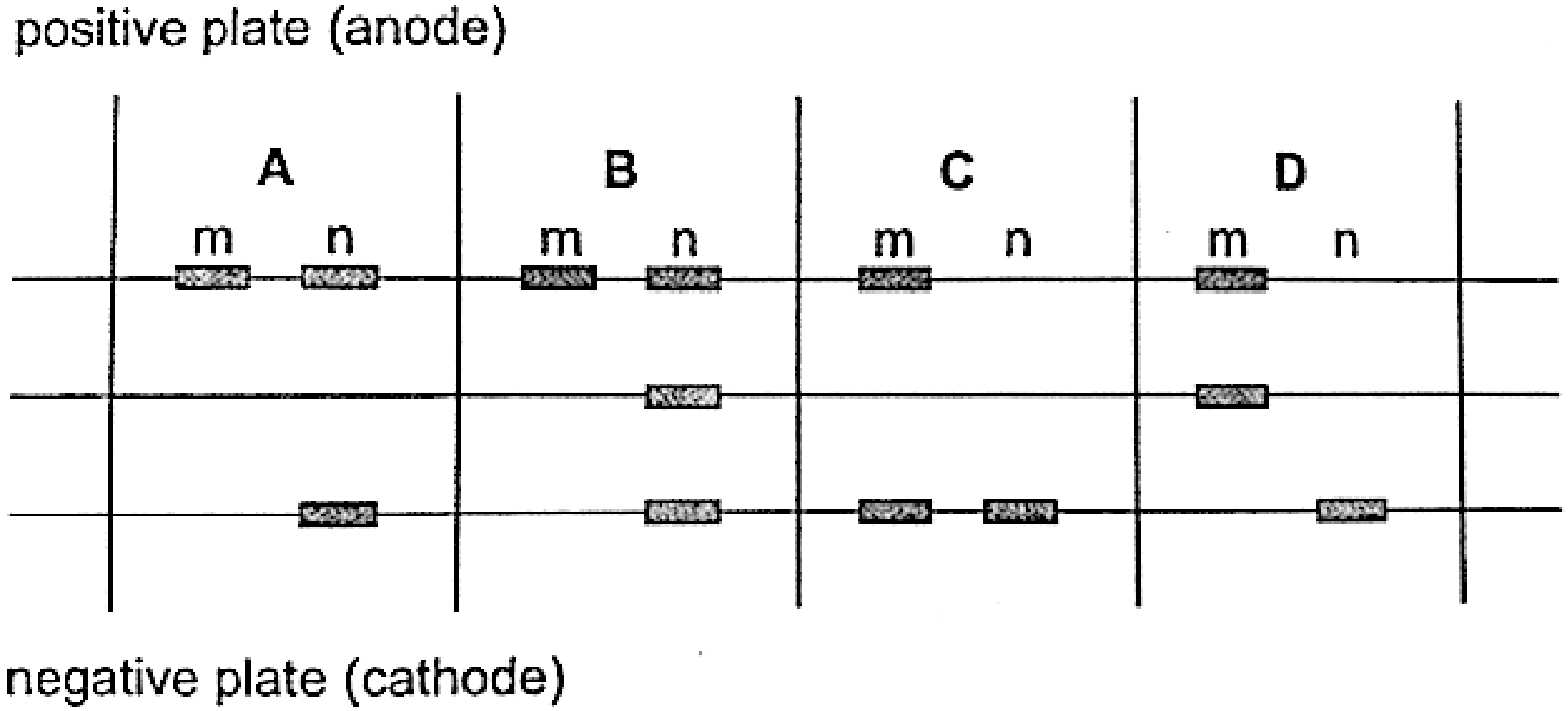

Stage 1 DNA is cut into fragments
Stage 2 Electrophoresis separates the DNA fragments
Stage 3 Radioactive DNA probes are used to locate specific DNA fragments

Figure 2.


positive plate (anode)
A
B
C
D
m
n
m
n
m
n
m
n
negative plate (cathode)

Worksheet A: Solving crime with DNA

THE DAILY NEWS

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Solving crime with DNA

A phone call is made to the police. Neighbours say that there was a loud disturbance in a house. When they arrive, police officers find it is empty. However, they find blood on the floor and a knife with blood on the blade. The knife is collected as evidence for forensic analysis.

In the laboratory, a scientist removes a small sample of blood and used it to collect the DNA for analysis. Just a tiny quantity of blood from this knife is enough to help to identify the victim and perhaps the perpetrator of the crime. The DNA is extracted from white blood cells. To extract the DNA, the forensic scientist first treats the cells with a reagent that causes them to break open. Any remaining protein in the sample is digested using a protease and then washed away to leave pure DNA. It is likely that the sample of DNA will be too small to analyse, so the quantity is increased by using DNA polymerase to replicate key regions of the DNA.

This procedure uses solutions of the four nucleotides that are used in semi-conservative replication of DNA. This results in multiple copies of key regions of DNA that can be used to form a genetic profile. The profiles of the people who left their blood on the knife are compared against with profiles held in the police DNA database to see if there is a match. If so, the chances of a positive match are high.

DNA profiling is an important tool that can be used for other purposes in addition to forensic

Worksheet A: Solving crime with DNA *continued*

To help you with your discussions, record key words from your studies in the table below.

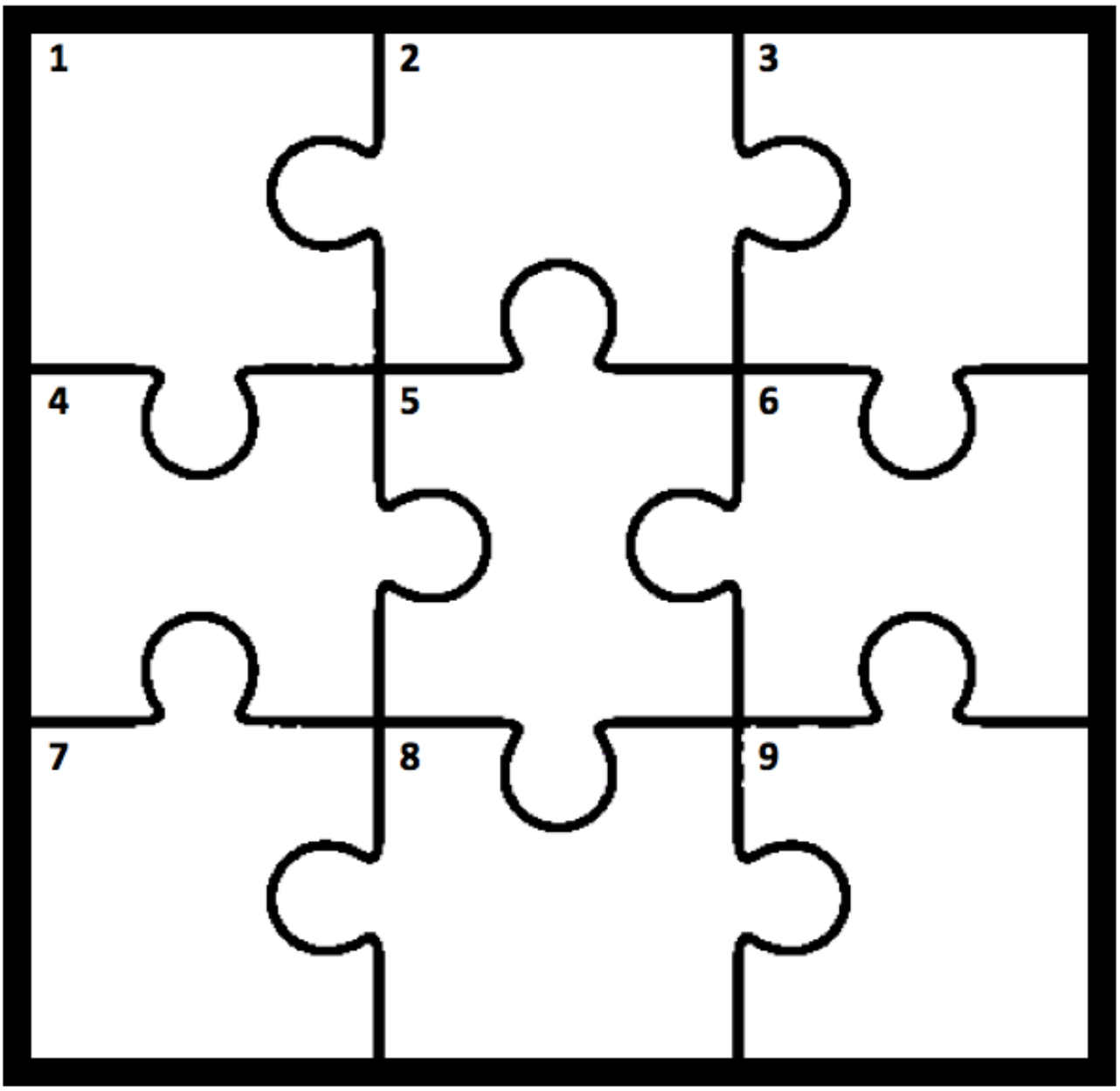
Discussion topics

1. '*The knife is… handed on for forensic analysis*.'
2. '*Just a tiny quantity of blood from this knife is enough*.'
3. '*The DNA is extracted from white blood cells*.'
4. '*Any remaining protein in the sample is digested using a protease*.'
5. '*DNA profiling is an important tool that can be used for other purposes in addition to forensic analysis*.'

discussion	key words to use in your discussion	key words that arise <u>during</u> your discussion
1		
2		
3		
4		
5		

Worksheet B: Gel electrophoresis equipment 1

As your teacher reveals the different jigsaw pieces, record your observations in the missing piece.



Extra notes

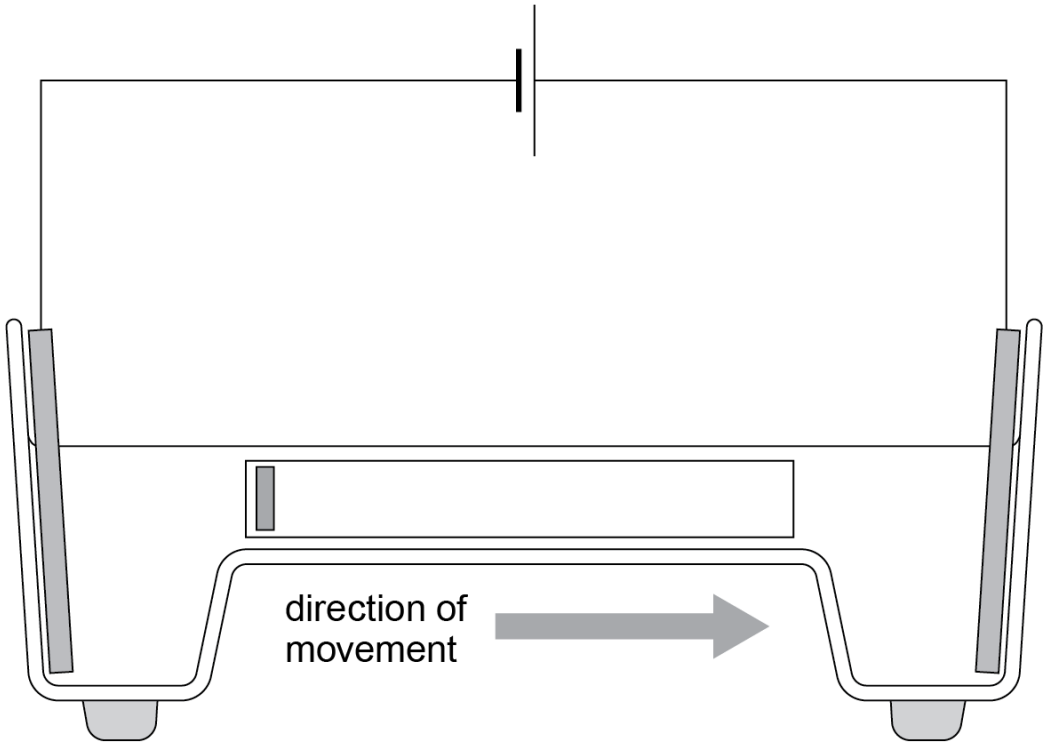
……

……

……

Worksheet C: Gel electrophoresis equipment 2

Refer to your rough notes on **Worksheet B** to help you label the diagram below. Draw arrows to the different parts of the machine and briefly summarise their purpose.



Worksheet D: Biological barcodes

Genetic profiles (fingerprints) can be produced using gel electrophoresis. Some people liken genetic profiles to 'barcodes' that are found on items purchased from shops. Barcodes can be read by a scanner and this tells the shop assistant what item is being purchased.

1. Give two ways in which the appearance and use of barcode accurately resembles those of a genetic profile (fingerprint). [2 marks]

1.
……

2.
……

2. Outline how gel electrophoresis separates DNA fragments. [5 marks]

……

……

……

……

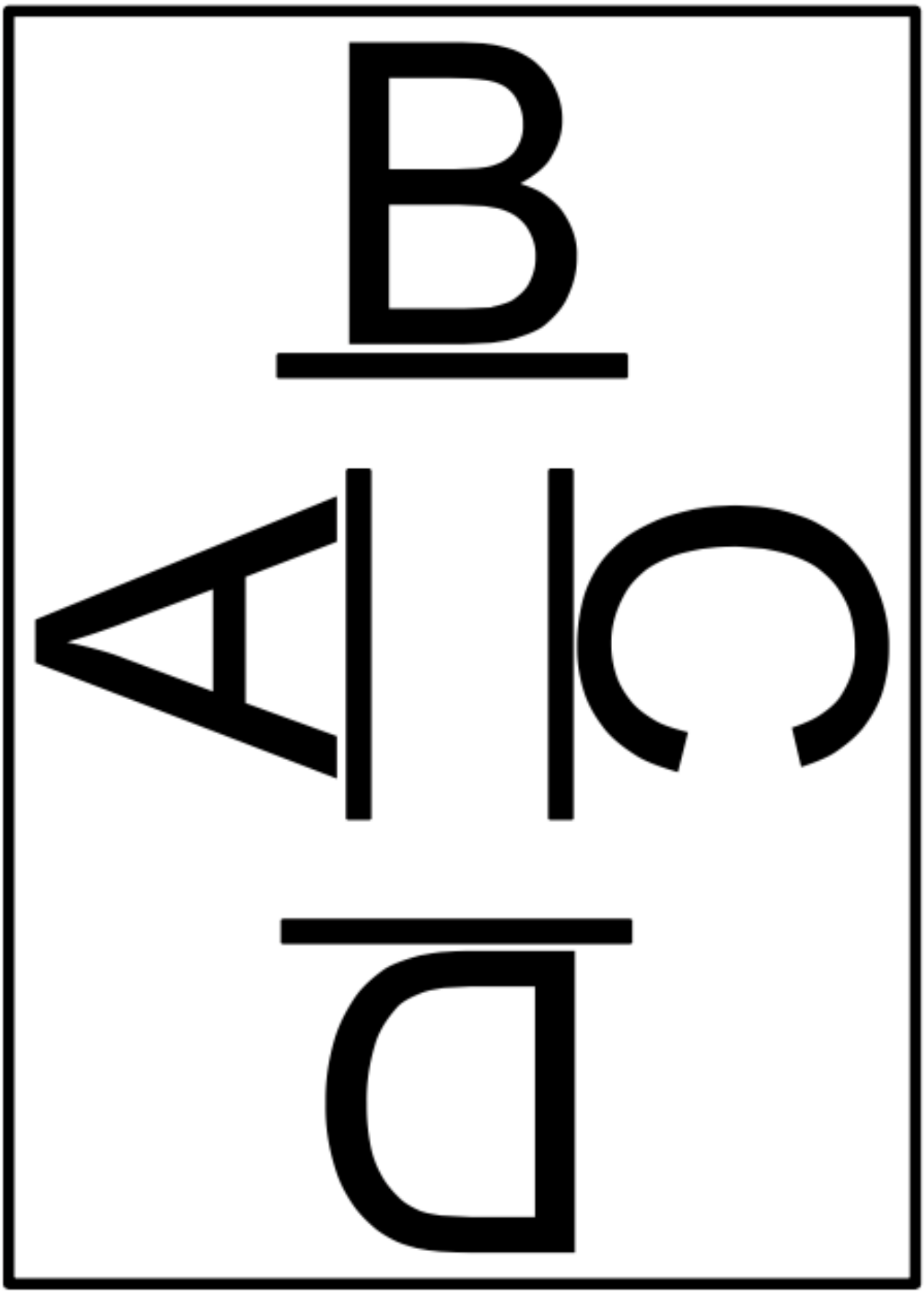
……

3. Suggest why, as in barcodes, some bands will appear thicker than others. [2 marks]

……

……

Worksheet E: Multiple choice answer sheet



Worksheet F: Method circus

During this activity, you will practice the skills required to undertake gel electrophoresis. Your teacher will provide you with 10 minutes to engage with each of four different tasks.

Follow the instructions below and make a note of any problems you encounter for a discussion afterwards.

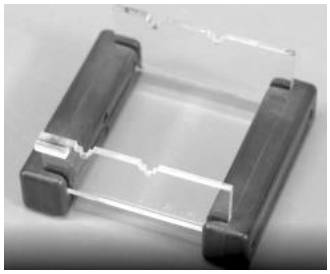
TASK 1. Making an agarose gel (10 minutes)

Instructions

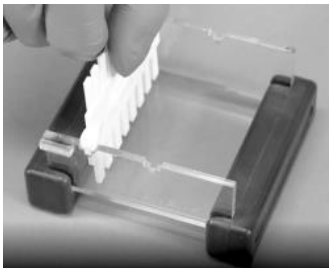
Note that you will not use the molten agarose gel in this practice task (you will use warm water).

1. Set up the casting tray by placing two end stoppers at either side.
2. Place a sample comb into the casting tray.
3. Pour warm water from the conical flask into the casting tray.

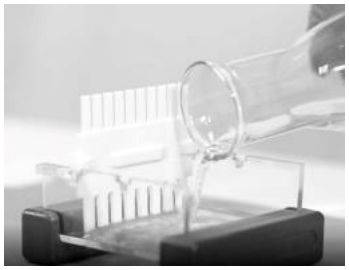
1.



2.



3.



When you have undertaken this task, pour the water back into the conical flask and disassemble the casting tray in preparation for the next group.

Questions to consider

1. Why must the sample comb be removed carefully only after the gel has set?
2. Why is it important that the agarose gel solution is of a particular concentration?

Problems encountered and expected safety precautions:

……

……

……

Worksheet F: Method circus *continued*

TASK 2. Setting up the gel electrophoresis tank (10 minutes)

Instructions

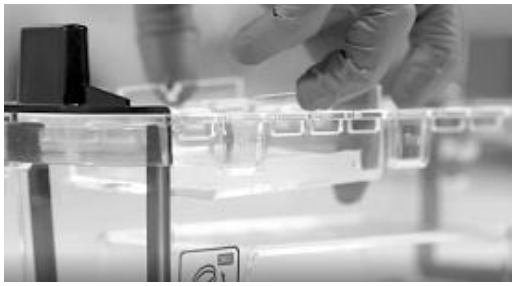
Note that you will not use the electrophoresis buffer in in this practice task (you will use warm water).

- Remove the two end stoppers from the casting tray that contains the gel and place it into the electrophoresis tank. The end of the gel with wells should be placed closest to the cathode, which has a black surface.
- Fill the electrophoresis tank with the buffer solution, so that the gel is completely submerged.

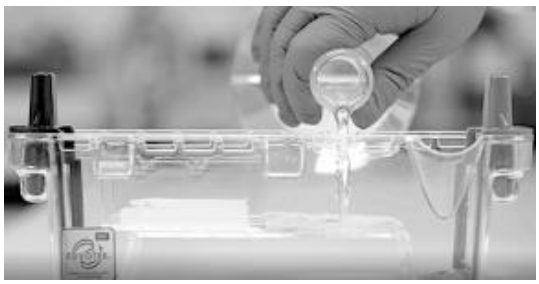
Questions to consider

1. Describe the role of the buffer solution in the gel electrophoresis protocol.
2. Why is the end of the gel that has the wells placed next to the cathode?

1.



2.



Problems encountered and expected safety precautions:

……

……

……

Worksheet F: Method circus *continued*

TASK 3. Using a micropipette to measure small volumes (10 minutes)

Instructions

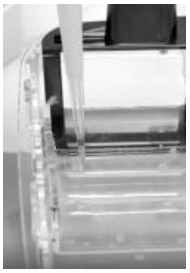
Note that you will not use DNA samples in this practice task (you will use a food colouring).

1. Attach a plastic tip to the end of the micropipette, and achieve an airtight seal is achieved by pressing down firmly.
2. Hold the micropipette in a vertical position and press down the plunger to the first stop, which has been set to 35 μl.
3. Immerse the tip into the sample of DNA and release the plunger into the rest position.
4. Hold the micropipette against the other hand to guide the tip into the well at an angle. Place the tip a few millimetres into the well and then press the plunger to the first stop and then the second stop.

1. 

2. 

3. 

4. 

Questions to consider

1. Describe the role of the loading (tracking) dye in the gel electrophoresis protocol.
2. Convert 35 μl in standard form as cm^3.
3. Why is it important to avoid pressing too hard on the plunger and bubbling air into the well?
4. Why is it important to replace/ wash the tips between loading of different samples?

Problems encountered and expected safety precautions:

……

……

……

Worksheet F: Method circus *continued*

TASK 4. Interpreting the results of gel electrophoresis (10 minutes)

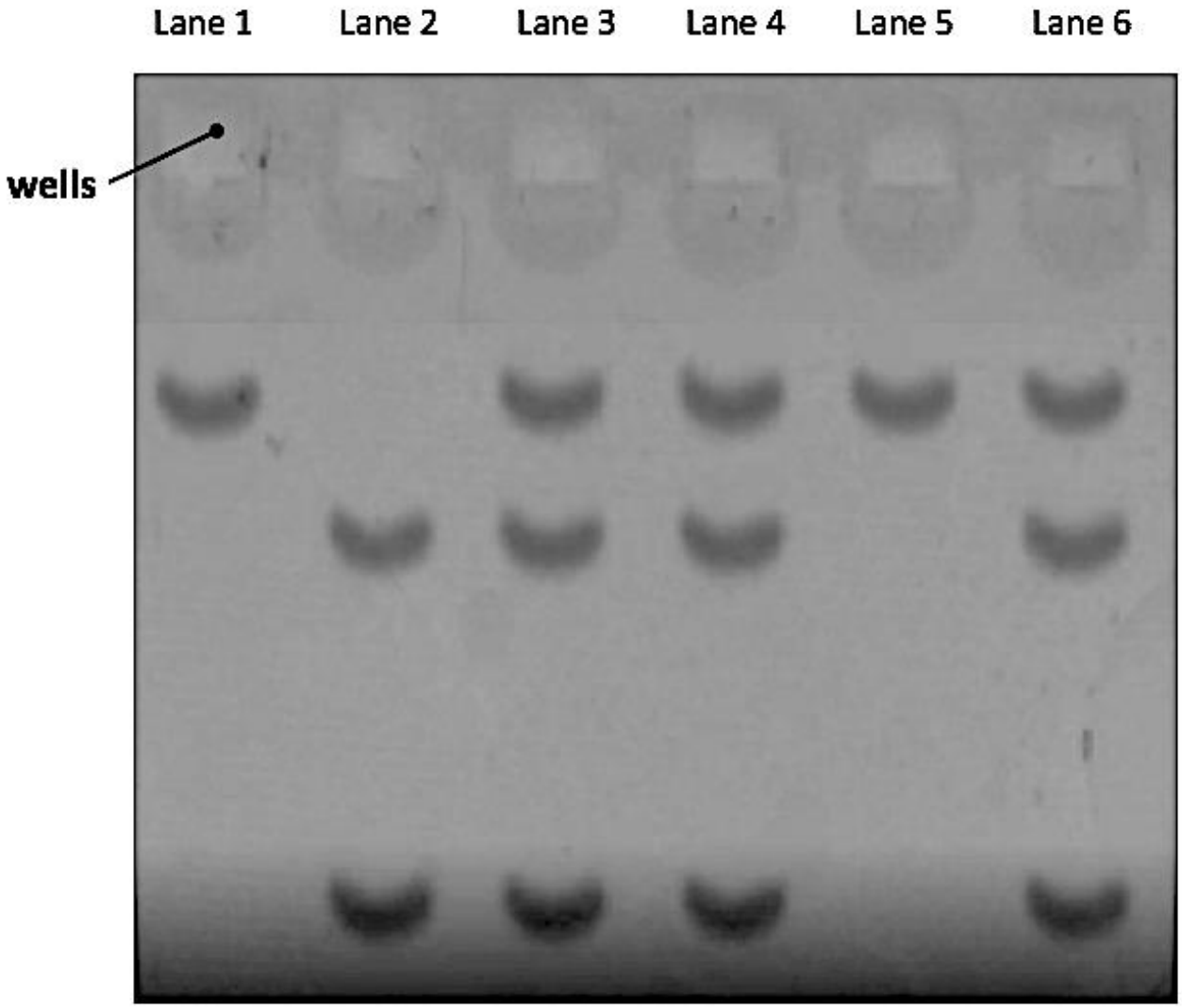
You will investigate the inheritance of the recessive genetic disorder sickle cell anaemia. Refer to **Worksheet G** provided by your teacher for some sample data.

Questions to consider

1. Worksheet G shows a photograph of the band pattern produced after gel electrophoresis of samples of DNA from six different people. Add labels to the diagram to indicate the position of the anode and cathode, and which DNA fragments are the largest and the smallest.
2. State two factors that would need to be standardised in this investigation to enable a valid comparison of the lanes to be made.
3. The table below shows the identity of the individuals whose DNA was analysed in the first three lanes. The samples were incubated with a specific restriction endonuclease before they were analysed. Using this information, determine the genotype of the father, mother and child **and** suggest the effect of the mutation on the DNA fragments that causes sickle cell anaemia.
4. Describe the different circumstances in which this genetic screening for the sickle cell allele, HbS, might be used.

Lane 1	Allele sample from a person with Sickle cell anemia
Lane 2	Allele sample from a healthy non-carrier
Lane 3	Allele sample from a carrier of Sickle cell anemia
Lane 4	Mother's alleles
Lane 5	Child's alleles
Lane 6	Father's alleles

Worksheet G: Sample results



Worksheet H: Critiquing a report

The plan below was written by a student in response to the following question:

"*How could gel electrophoresis be used to investigate whether a DNA sample left at a crime scene belongs to a suspect?*"

Circle any parts of the student's plan that you would change and add sections that are missing. Add annotations in the margins to explain how you would do things differently – and why.

"Prepare an agarose gel by dissolving agarose powder in cold water, and pour the mixture into a casting tray. A sample comb is forced into the gel once the gel has solidified. The gel is then placed into the electrophoresis tank, with the wells closest to the anode. Buffer solution is added to the tank so that the gel is half-submerged.

The DNA from the crime scene and the DNA from the suspect are mixed together. A plastic tip is attached to the end of the micropipette and the plunger is pressed to the first stop and released to suck up a volume of DNA. The tip is immersed in the sample of DNA and the plunger is released to the rest position. The micropipette is then held above the gel and the liquid is dropped in from a height of several millimetres.

Place the lid onto the electrophoresis tank and attach the power supply using the leads. Set the voltage to 750 V and switch the power supply on. Let the process run for around 5 minutes and switch off the power supply. Remove the gel from the tank and place in a small tray containing buffer solution and analyse the results."

Worksheet I: Applying knowledge

Short tandem repeats (STRs) are regions of non-coding DNA in which a sequence of two to five bases is repeated, for example: CAGCAGCAGCAGCAGCAGCAG. The number of these repeats, and hence the length of the STR, differs in different individuals. These different lengths can be determined by gel electrophoresis.

Ivory obtained from elephants is a valuable resource in many countries. Many elephants are listed as an endangered species by CITES (*Convention on International Trade in Endangered Species of Wild Fauna and Flora*). However, despite a ban on the trade in elephant products, ivory is still sold by poachers. It is estimated that only a small fraction of this ivory is seized by the authorities.

1. Explain why the PCR is carried out on the DNA from the ivory.

……

……

2. Outline the process of genetic fingerprinting using STRs that could be used to test this seized ivory. [4 marks]

……

……

……

……

3. Explain how the genetic fingerprints of the seized ivory could be used to confirm that it originated from elephants in South Africa. [3 marks]

……

……

……

4. DNA can be obtained from the faeces of wild elephants or their blood. Suggest two advantages of using faeces, rather than blood samples, to obtain DNA from elephants.

……

……

Worksheet A: Answers

Expected words that will arise during learners' discussions may include:

Discussion 1
- Blood
- Cells
- Sample

Discussion 2
- PCR
- Amplify
- Primers
- DNA polymerase

Discussion 3
- Chromosomes
- Nucleus
- Genetic material

Discussion 4
- Enzyme
- Digestion
- Hydrolysis

Discussion 5
- Paternity testing
- Evolution studies
- Identification

Worksheet D: Answers

QUESTION 1: low demand

Both the barcode and genetic profile:

1. consist of a series of bands.
2. consist of bands that are arranged in a vertical pattern.
3. consist of bands that are of different thicknesses.
4. consist of a similar number of bands.

2 marks *maximum*

QUESTION 2: intermediate demand

DNA fragments are separated according to length or mass. Because the phosphate groups of DNA have a negative charge, the DNA fragments move to the anode (positive electrode). The agarose gel impedes the movement of DNA fragments. Therefore, shorter, lighter, fragments move faster and therefore further in a given time than longer (heavier) fragments.

5 marks *maximum*

QUESTION 3: high demand

Humans have two alleles for most genes. If they have the same allele (are homozygous) then the lengths of the DNA fragments will be identical and will show as a thicker band on the DNA profile.

2 marks *maximum*

Worksheet F: Answers

TASK 1

1. Removing the sample comb from the gel before it is set will produce wells that are not uniform in size and shape.
2. The concentration of the gel will affect the degree of impedance (resistance) to the movement of DNA fragments. Too concentrated, and the DNA fragments will not move very quickly and so the time taken for the process would be too long. Too dilute, and the DNA fragments will move very quickly and they will not separate enough to be distinguished.

TASK 2

1. The buffer solution contains ions that act as an electrolyte. These conduct the current supplied by the power supply and this allows the DNA fragments to move.
2. The DNA samples are put into the wells of the gel. Therefore, the DNA will move from these wells towards the anode (positive pole), which must be placed furthest from them so that they move through the full length of the gel.

TASK 3

1. DNA is invisible, so the loading (tracking) dye provides an indication of the distance travelled by the DNA through the gel.
2. 35 μl = 0.035 ml = 0.0035 cm^3
3. If air is bubbled into the well after the DNA sample has been added, the sample may be forced out of the well and into the electrophoresis buffer. The DNA will therefore not move through the gel.
4. Replacing or washing the tips used to load the DNA into the gel minimises the opportunity for cross-contamination, which means that DNA of one sample is placed into the well of another sample.

TASK 4

1. The label for the anode should be placed at the bottom of the gel (far away from the wells); that for the cathode at the top (next to the wells). The largest fragments are closest to the cathode, and the smallest are furthest away.
2. Factors that would need to be standardised in this investigation include: the time at which…
3. The genotype of the father and mother are heterozygous, because they have three bands. Two of these bands are formed by the restriction of the normal allele (as shown by Lane 2) and one of these bands corresponds to the mutant allele (as shown by Lane 1). It is likely that the mutation that causes sickle cell anaemia changes the sequence of a DNA site recognised by the restriction enzyme, because it is not able to cut the DNA fragment that contains this allele.
4. This allows for genetic counselling of a family with a history of sickle cell anaemia. A carrier / heterozygote could be identified before marriage or before conceiving a child. It is also possible to opt for a therapeutic termination of a recessive homozygous child *in utero*, or prepare for the birth of a child with the disorder.

Worksheet H: Answers

Expected corrects that will arise during learners' discussions may include:

PARAGRAPH 1

- Agarose powder is dissolved in **hot** water.
- The sample comb is placed into the molten agarose gel **before** it sets, and is removed once the gel has solidified.
- The gel is placed into the tank with the wells closest to the **cathode**.
- Buffer solution is added to the tank so that the gel is **completely** submerged.

PARAGRAPH 2

- The DNA from the crime scene and the DNA from the suspect are added to **separate** wells in the gel.
- The plastic tip of the micropipette is placed **into** the well as the liquid is added.

PARAGRAPH 3

- The voltage is set to **75 V** and the process is allowed to run for **60 minutes**.
- A method is required to **stain** the gel so that the DNA fragments are made visible.

Worksheet I: Answers

1. PCR amplifies the DNA by making many copies of the molecule present. This is because there are very few DNA molecules in the ivory.

2. Using gel electrophoresis, DNA fragments are separated according to length or mass. Shorter, lighter, fragments move faster and therefore further in a given time than longer (heavier) fragments. Because the length of the STRs differ between different individuals, the lengths of the fragments will be different and will move different distances along the gel. A characteristic band pattern will therefore exist for an individual.

3. Compare the characteristic band pattern produced by gel electrophoresis for the seized ivory and for elephants from South Africa. If the band patterns are of a very similar or identical appearance, then there is a high chance that the ivory originated from elephants from South Africa.

4. Obtaining blood samples presents a risk to the scientist and could cause injury or disease to the elephants, which may also be difficult to find or capture.

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