

Practical Booklet 12

Separating DNA using electrophoresis

Cambridge International AS & A Level Biology 9700

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Introduction

Practical work is an essential part of science. Scientists use evidence gained from prior observations and experiments to build models and theories. Their predictions are tested with practical work to check that they are consistent with the behaviour of the real world. Learners who are well trained and experienced in practical skills will be more confident in their own abilities. The skills developed through practical work provide a good foundation for those wishing to pursue science further, as well as for those entering employment or a non-science career.

The science syllabuses address practical skills that contribute to the overall understanding of scientific methodology. Learners should be able to:

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

The practical skills established at AS Level are extended further in the full A Level. Learners will need to have practised basic skills from the AS Level experiments before using these skills to tackle the more demanding A Level exercises. Although A Level practical skills are assessed by a timetabled written paper, the best preparation for this paper is through extensive hands-on experience in the laboratory.

The example experiments suggested here can form the basis of a well-structured scheme of practical work for the teaching of AS and A Level science. The experiments have been carefully selected to reinforce theory and to develop learners' practical skills. The syllabus, scheme of work and past papers also provide a useful guide to the type of practical skills that learners might be expected to develop further. About 20% of teaching time should be allocated to practical work (not including the time spent observing teacher demonstrations), so this set of experiments provides only the starting point for a much more extensive scheme of practical work.

Guidance for teachers

Aim

To separate dyes by electrophoresis and to use this to develop an understanding of the principles by which DNA can be separated. Dyes which have different charges are used to simulate DNA so that they move different distances along an agarose gel, imitating the actual events in DNA separation.

Outcomes

Syllabus section 19.1 (d)

Skills included in the practical

A Level skills	How learners develop the skills
Evaluation	Evaluate which variables would need to be standardised when carrying out the procedure using DNA fragments Discuss how they may affect the accuracy and reliability of the results
Conclusions	Explain how gel electrophoresis is used to separate DNA fragments, using their scientific knowledge and linking it to their results

This practical provides an opportunity to build on essential skills introduced at AS Level.

AS Level skills	How learners develop the skills
MMO collection	Collect qualitative results
PDO recording	Record qualitative results appropriately

Method

- Learners need to be introduced to the idea of electrophoresis as a technique that is used to separate molecules by a combination of their charge and molecular size. It is often confused with chromatography, so it is helpful to demonstrate how to set up the electrophoresis apparatus while discussing how it works. As the electrophoresis gel needs to run for at least 90 minutes the practical will need to be conducted in two halves. Once a gel has 'run' it can be removed from the tank and stored in a container, covered by buffer, in a refrigerator.
- There is a supporting surface, usually agarose gel, on which the mixture of molecules to be separated is placed. The gel is placed in a tank and covered by a conducting buffer, which makes sure that an electrical current passes through the gel. Electrodes are immersed in the buffer at each end of the gel and a current passed through.
- DNA is a negatively charged molecule so it moves towards the anode (positive electrode). The agarose gel is a very complex molecular network with many narrow twisted passages. It can be thought of as a 3-dimensional sieve. Small molecules move quickly through these passages, but large ones move more slowly. This provides an opportunity to revisit AS knowledge about the structure of DNA and understand why it is negatively charged.
- DNA can be extracted from cells and cut into fragments using enzymes called restriction enzymes or restriction endonucleases. These enzymes cut DNA at specific points called restriction sites that occur throughout DNA molecules, so DNA fragments of different sizes are formed. This can be demonstrated using a ribbon or string with marks at irregular intervals to represent the restriction sites.

Guidance for teachers, *continued*

- The mixture of DNA fragments is placed at the cathode (negative electrode) end of an agarose gel. This end of the gel has wells into which DNA samples can be placed. When the current is passed through, the DNA fragments move towards the anode and separate according to their size. The DNA fragments are often added to glycerol so they sink into the wells more easily and a tracking dye is used to see how far the DNA fragments have moved and prevent them running off the end of the gel.
- DNA is colourless, so the gel has to be stained with a dye that binds to DNA, to see where each of the DNA fragments has reached. The DNA shows up as a band and is sometimes called 'visualisation'.
- Learners should then use electrophoresis to separate a mixture of dyes. These dyes represent DNA molecules. To do this separation the learners must:
 - Make an agarose gel by dissolving agarose powder in TBE buffer. This is done by adding the agarose to the buffer and heating using a Bunsen burner or heater until the powder dissolves. If a microwave is available then the mixture can be heated for 30 seconds and then for 10 seconds at a time until the agarose dissolves. When the agarose dissolves it becomes transparent.
 - Leave the agarose to cool to between 55–60 °C. This takes 6–8 minutes.
 - Prepare the electrophoresis tank by putting in casting gates and a comb for the wells.
 - When the agarose is cool, pour it carefully into the tank, between the two casting gates and leave to set. This takes about 15 minutes and provides an opportunity to explain how the agarose acts as a sieve through which molecules have to move.
 - When the gel has set, remove the casting gates and comb very carefully so the gel does not tear.
 - Pour TBA buffer into the tank until the surface of the gel is covered.
 - Use a micropipette and tips or capillary tubes to load dyes into the wells. If equipment is limited 2 or 3 learners can share a gel. The dyes can be provided separately and as mixtures, so that the idea of markers can be discussed when the results are interpreted.
 - Place the electrodes at the correct ends of the tank so that the positive electrode is at the same end as the wells loaded with dye.
 - Connect the battery pack and leave to run for a minimum of 90 minutes and a maximum of 180 minutes.
- It is possible to buy kits that use actual DNA samples, but these can be very expensive.

Guidance for teachers, *continued*

- Learners need to understand that for a complete DNA fingerprint there are six stages. They also need to understand that the dyes represent what happens to DNA during electrophoresis.
 1. Isolation of DNA (DNA extraction).
 2. Cutting DNA into fragments using restriction enzymes.
 3. Gel electrophoresis to sort DNA fragments by size.
 4. DNA denaturation (to make DNA single stranded).
 5. Southern blot onto nitrocellulose paper – this transfers the single stranded DNA onto a permanent medium (the gel is not permanent).
 6. Fluorescent probes are added to the Southern blot and base pair with the single stranded DNA on it. This is called hybridisation. The places where these specific probes bond can then be seen.

Results

1. Learners should draw a diagram of the completed gel and label the position of the dyes.
2. Explain why the dyes have separated differently.
3. Discuss what would happen if actual DNA fragments had been separated. This provides an opportunity to introduce the idea of DNA fingerprinting as a way of visualising the sequence of base pairs in a DNA sample and identifying to whom it belongs. To 'map' the entire sequence of an individual's DNA would be too difficult, so specific sections of DNA are used, which can be obtained using restriction enzymes. These specific sections are known as variable number tandem repeats (VNTRs) and are inherited genetically. The VNTR of every individual is different, so when two samples are compared it is possible to tell the difference between the DNA of two different people. It would also be possible to identify individuals who were related to one another.

Interpretation and evaluation

1. Learners should discuss the method they have carried out and decide which variables are likely to affect the reliability of results when used for an actual DNA sample. These variables should be listed and suggestions made about how they should be standardised.
2. For DNA fingerprinting the learner should also consider what errors could occur in the procedure and how this could affect the results.

Information for technicians

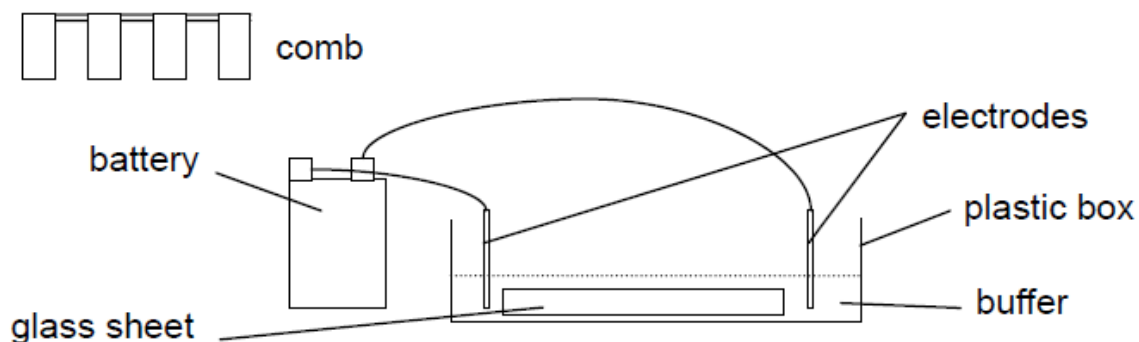
Each learner will require:

- 75 cm³ TBE buffer [H]
- fluorescein (see note on dyes below) [H]
- methylene blue (see note on dyes below) [H]
- crystal violet (see note on dyes below) [H][N]
- orange G (see note on dyes below) [H]
- 0.35 g agarose powder
- one micropipette
- micropipette tips – one for each dye used. Capillary tubes can be used if micropipettes are not available.
- one Bunsen burner, tripod and gauze or access to a microwave
- one conical flask
- either one gel electrophoresis tank with battery pack, electrodes, wires, and clips or a commercial electrophoresis kit.

Additional instructions

- Once prepared, agarose gel has a short shelf life and should be discarded after removal from the electrophoresis tank.
- TBE buffer is effective to a minimum of a 10% working concentration and has a shelf life of approximately 12 months.
- Possible variables to control include: temperature, pH, concentration of TBE buffer, volume of TBE buffer, electrical charge through the gel.
- It is convenient to make more of the reagents than is required in order to give sufficient quantities for accurate measurements.
- Very small quantities of dye solutions are needed. 1 cm³ is sufficient for 3 or 4 learners. These can be provided in a number of ways, either separately or in various mixtures. Other dyes that might be used are bromophenol blue and bromocresol green. Alternatively, red food colouring, blue food colouring, green food colouring, yellow food colouring can be used. The dye samples should be provided in small specimen tubes, labelled **A**, **B**, **C**, **D**, etc.
- It is expected that an electrophoresis kit will be available. Each kit usually has its own individual way of connecting a battery pack but will include instructions. For a practical that incorporates more aspects of DNA fingerprinting, including cutting DNA with restriction enzymes for example, the purchase of a kit that includes all the necessary materials is advised. A number of companies produce kits with instructions for practical activities.
- If electrophoresis kits are not available, they can be made using plastic boxes as buffer tanks, glass sheets for supporting a gel, a pair of electrodes (platinum is preferred although carbon will work) and a 9 V battery. A thin plastic sheet can be cut to form a comb.

Information for technicians, *continued*



Hazard symbols

C = corrosive substance

H = harmful or irritating substance

N = harmful to the environment

F = highly flammable substance

O = oxidising substance

T = toxic substance

Worksheet

Aim

To separate dyes by electrophoresis and to use this to develop an understanding of the principles by which DNA can be separated. Dyes which have different charges are used to simulate DNA so that they move different distances along an agarose gel, imitating the actual events in DNA separation.

Method

Preparing and pouring the agarose

1. Add 0.35 g of agarose powder to 35 cm³ of TBE buffer in a conical flask.
2. Heat the mixture over a Bunsen burner using a tripod and gauze, swirling the flask occasionally to prevent any lumps forming. If a microwave is available, heat for 30 seconds, then in units of 10 seconds until the agarose dissolves.
3. Agarose becomes transparent when it boils. When it reaches this stage, remove from the heat and leave to cool for 6-8 minutes or until the temperature of the agarose is between 55 °C and 60 °C.
4. Whilst the gel is cooling, prepare the electrophoresis tank by inserting the casting gates and comb.
5. When the gel has cooled sufficiently to the desired temperature it can be carefully poured into the tank, between the two casting gates.
6. Leave the gel to set for at least 15 minutes.

Loading the gel

7. Once the gel is set, carefully remove the casting gates.
8. Very gently remove the comb, taking care not to rip the gel. The comb should have introduced 'wells' into the gel for loading the dyes.
9. Pour 40 cm³ TBE buffer into the tank. This should completely cover the surface of the gel.
10. Fill a micropipette or capillary tube with dye **A**.
11. Position the pipette or capillary inside the mouth of the first well in the gel and dispense the dye into it.
12. Load dye **B** into the next well in the same way.
13. Repeat the loading procedure for each of the dyes available.
14. Connect the battery pack. The negative electrode should be at the same end as the wells loaded with dye.
15. After a minimum of 1.5 hours and a maximum of 3 hours, disconnect the battery pack and record the results.

Worksheet, *continued*

Results

1. Draw a diagram to represent the final positions of the dyes on your gel.
2. Explain why the dyes have separated differently.
3. Discuss what would happen if actual DNA fragments had been separated.

Interpretation and evaluation

Evaluate the method, considering the following:

1. Which variables are likely to affect the reliability of results when using a sample of DNA?
Suggest how these variables should be standardised.
2. When carrying out DNA fingerprinting, list the errors which could occur in the procedure and how they could affect the reliability of the results.

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