

### Practical 7 - R(g) Electrophoresis as a separation process







This practical focuses on – **Using complex procedures and apparatus, evaluation**

#### Intended learning outcomes

by the end of this practical and its write-up you should be able to:

- Set up and run a gel electrophoresis tank as a model of DNA fingerprinting
- Be able to use new apparatus correctly
- Understand the process of DNA fingerprinting and its usefulness in biotechnology

#### Safety Information

	You should wear eye protection throughout this practical. The coloured dyes used will stain skin or clothes.
	TBE buffer is <b>harmful</b> .
	Fluorescein is an <b>irritant</b> . It will stain skin or clothes.
	Methylene Blue is <b>harmful</b> . It will stain skin or clothes.
 	Crystal violet is <b>harmful</b> and <b>dangerous to the environment</b> . It will stain skin or clothes.

- Electrophoresis is a technique used for separating molecules by charge.
- The molecules to be separated are placed on a supporting surface, usually agarose gel and immersed in a conducting buffer. The buffer ensures a current passes through the gel.
- A potential difference is applied and the molecules move towards the electrodes according to their charge.
- The agarose gel has a structure like a sieve. Small molecules move quickly through it but larger ones are hindered by the gel.
- DNA is a negatively charged molecule so it moves towards the anode (positive electrode).
- All human DNA has the same basic chemical structure.
- However, each human's individual DNA is unique due to the sequence of the base pairs.
- DNA fingerprinting is a way of visualising the sequence of base pairs in a DNA sample, thus identifying whom it belongs to.
- To 'map' the entire sequence of an individual's DNA would be too great an

undertaking; so instead, specific sections of DNA are used, which can be obtained by using restriction enzymes.

- These specific sections (known as variable number tandem repeats – VNTRs, inherited genetically) are known to vary enormously between individuals so when two samples are compared it would be 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.
- The process of DNA fingerprinting consists of 6 stages, of which you will be carrying out stage 3, using dyes to substitute for DNA:
  - 1 Isolation of DNA (DNA extraction), which you may have carried out in another practical
  - 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 Hybridisation with a radioactive or fluorescent probes. Radioactive DNA bases are introduced to the southern blot and so bond with the single stranded DNA on it. The places where these specific probes bond can then be visualised.

You will carry out the gel electrophoresis stage of DNA fingerprinting, using coloured dyes to represent DNA samples.

- Read the information above.
- Draw a labelled diagram of a working electrophoresis tank.
- List the variables that should be controlled.
- Outline how each variable might be controlled.

### Method

#### Preparing and pouring the agarose

- 1 Add 0.35 g of agarose powder to 35 cm<sup>3</sup> 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. A microwave can be used, in which case heat for 30 seconds, then in 10 seconds increments 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 poured, carefully, into the tank, between the two casting gates.
- 6 Leave the gel to set for at least 15 minutes.

### Loading the gel

- 1 Once the gel is set, carefully remove the casting gates.
- 2 Very gently remove the comb, taking care not to rip the gel. The comb should have introduced 'wells' into the gel, which will be utilised later.
- 3 Pour 40 cm<sup>3</sup> TBE buffer into the tank. This should completely cover the surface of the gel.
- 4 Fill a micropipette or capillary tube with dye A.
- 5 Position the pipette inside the mouth of the first well in the gel and dispense the dye into it.
- 6 Load dye B into the next well in the same way.
- 7 Repeat the loading procedure for each of the six dyes available.
- 8 Connect the battery pack. The positive electrode should be at the same end as the wells loaded with dye.
- 9 After a minimum of 1.5 hours and a maximum of 3 hours, disconnect the battery pack and interpret the results

### Write-up

- Draw a diagram to represent the final positions of the dyes on your gel.
- Draw conclusions, considering the following; the meaning of the results, what the results might be able to tell us if real DNA fragments had been used, scientific explanations of results and conclusions.
- Make an evaluation, considering the following; the limitations of the methods used, the confidence with which conclusions can be drawn, the adaptations that would have to be made for this method to be a reliable tool for forensic investigations or paternity tests.

**Lesson plan****Electrophoresis as a separation process****Context**

A demonstration of electrophoresis set in the context of the 9700 syllabus. It is anticipated that students will have completed an AS practical course so that they will have good basic practical skills. It is also anticipated that students will have been given prior learning opportunities so will be able to handle the tasks required of this practical.

**Key aims of lesson**

This practical is designed to develop the skill with which students use techniques and apparatus, and to boost confidence in learning to use unfamiliar apparatus.

**Intended learning outcomes**

By the end of this practical and its write-up students should be able to:

- Set up and run a gel electrophoresis tank as a model of DNA fingerprinting
- Be able to use new apparatus correctly
- Understand the process of DNA fingerprinting and its usefulness in biotechnology

**Resources required**

White board or flipchart and suitable pens or blackboard and chalks

Practical materials specified on the Technical Information sheet

Either gel electrophoresis tanks with battery packs / electrode material, batteries, wire and clips or a commercial Electrophoresis kit.

Some spare copies of the student worksheet

**Planned activities** (timings can be altered to suit shorter or longer lessons).

It is possible that the time for the gels to run may overlap into another lesson. In this case the student activity can be split and the gels prepared in the previous lesson. The gels can be stored in a container or plastic bag for 24 hours. Alternatively gels can be pre-prepared and used after the technique is demonstrated

<b>Timings/ minutes</b>	<b>Teacher / Student Activities</b>
end of previous lesson	<b>Preparation</b> – students given some theory on DNA fingerprinting or instructed to research on topic for homework.
0-4	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson - teacher led oral presentation
4-8	<b>Context</b> – class discussion on application of DNA fingerprinting. Teacher to summarise overall process of DNA fingerprinting on board (possibly in flow-chart format).
8-12	<b>Introduction to method</b> – teacher demonstration of setting up electrophoresis tank. Teacher demonstration of using micropipette, pouring gel and connecting power supply.

12 -50	<b>Carrying out the practical</b> – students work through the method in groups and tidy away apparatus whilst waiting for gels to ‘run’. Students can make a sketch of the apparatus and list the variables and how they are controlled whilst the teacher circulates. Students may also begin an evaluation of method before gels have finished ‘running’.
50-60	<b>Drawing together the threads</b> – students observe the completed gels and sketch. Teacher-led class discussion on what has been learned, the results and their meaning. Teacher led introduction to write-up, which may be finished as homework if necessary.

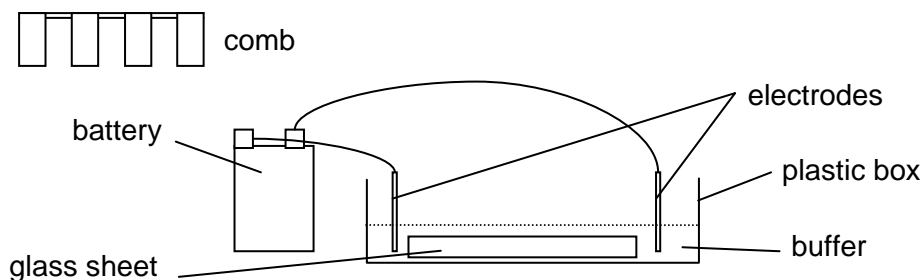
#### Useful information

- 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

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. A number of companies produce kits with instructions for practical activities.

### Technical Information - Electrophoresis as a separation process

- The list of apparatus is given per student, but this may be adapted if students are to work in pairs or groups.
- It is convenient to make more of the reagents than is required in order to give sufficient quantities for accurate measurements.
  - 1 Agarose powder – 0.35 g per student
  - 2 TBE buffer – 75 cm<sup>3</sup> per student
  - 3 Electrophoresis tank, including casting gates, comb and battery pack – 1 per student
  - 4 Micropipette – 1 per student (with a clean tip for each dye to be used)
  - 5 Bunsen burner, tripod and gauze – 1 per student OR access to a microwave
  - 6 Conical flask, flat-bottomed – 1 per student
  - 7 Coloured dye samples labelled A,B,C,D,E,and F
- Dyes that might be used: bromophenol blue, methylene blue, bromocresol green, crystal violet, fluorescein, Orange G , red food colouring, blue food colouring, green food colouring, yellow food colouring. A mixture of dye may also be used.
  1. 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.
  2. 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 volt battery. A thin plastic sheet can be cut to form a comb.





3. Most good electrophoresis kits include dyes which can be used for this practical and also micropipettes.
4. If micropipettes are not available, capillary tubes may be used instead.
5. Suppliers of kits for electrophoresis and DNA fingerprinting:
  - [www.ncbe.reading.ac.uk](http://www.ncbe.reading.ac.uk) – at the time of writing £50 will get you 8 low voltage electrophoresis kits. Price list is a pdf and international order details are on <http://www.ncbe.reading.ac.uk/NCBE/MATERIALS/ordering.html>
  - Bio-Rad –electrophoresis kits and DNA fingerprinting – [www.bio-rad.com/](http://www.bio-rad.com/) and click on the Life Science education tab
  - <http://wardsci.com/product.asp?pn=365164>
  - [http://www.sdr.com.au/electrophoresis\\_horizontal.html](http://www.sdr.com.au/electrophoresis_horizontal.html)

[http://sciencekit.com/category.asp\\_Q\\_c\\_E\\_432358](http://sciencekit.com/category.asp_Q_c_E_432358)



<http://www.bioteach.ubc.ca/TeachingResources/DoingScience/MacgyverProjShirazuEtalMaintext.pdf>

Safety Precautions/Risks.

TBE buffer = H 

Fluorescein = H (irritant) 

Methylene Blue = H 

Crystal violet = H, N  

A risk assessment should be carried out as a matter of course.