Science Inquiry Skills 1.7 – Using agarose gel electrophoresis

Introduction

Gel electrophoresis is a laboratory method that is used to separate very small volumes of mixtures of molecules e.g. proteins and DNA, according to their size and charge.

Minute quantities of mixtures are provided by using a special pipette called a micro-pipette. Molecules to be separated are moved by an electric field through a gel that contains small spaces between molecules. The gel is submerged in a liquid called an electrophoretic buffer to facilitate the movement of electric current, and thus the movement of molecules to be separated. Small molecules travel further through the pores away from the negative end (or cathode) of the gel than larger ones do. One commonly used gel is made using a polysaccharide called agarose.

Part A Setting up the agarose gel

Aim

To learn how to set up an agarose gel.

Materials (per group or for a class demonstration)

- Electrophoresis gel tank or chamber with lid and power leads attached
- Suitable power supply – this may be used to run two tanks at once
- Gel tray containing pre-prepared agarose gel (1% agarose in electrophoresis buffer) and a well former (or comb)
- 250 mL electrophoresis buffer solution (TAE - Tris Acetate EDTA)
- Black card

Method

1. Place the electrophoresis tank on the bench at your work station in a position away from the edge of the bench. Refer to *Figure 1*.
2. Put the lid on (with power leads attached) and then remove it from the tank to become familiar with how it feels to attach it correctly; it is important to be able to do this carefully and with a minimum of vibration (so as to not disturb the gel in Part B).
3. Collect the agarose gel containing the well former (or comb).
4. Remove the masking tape or 'dams' at the ends of the gel tray.
5. Place the tray down on the bench and gently remove the well former as shown by your teacher – this will expose the wells for use in Part B.

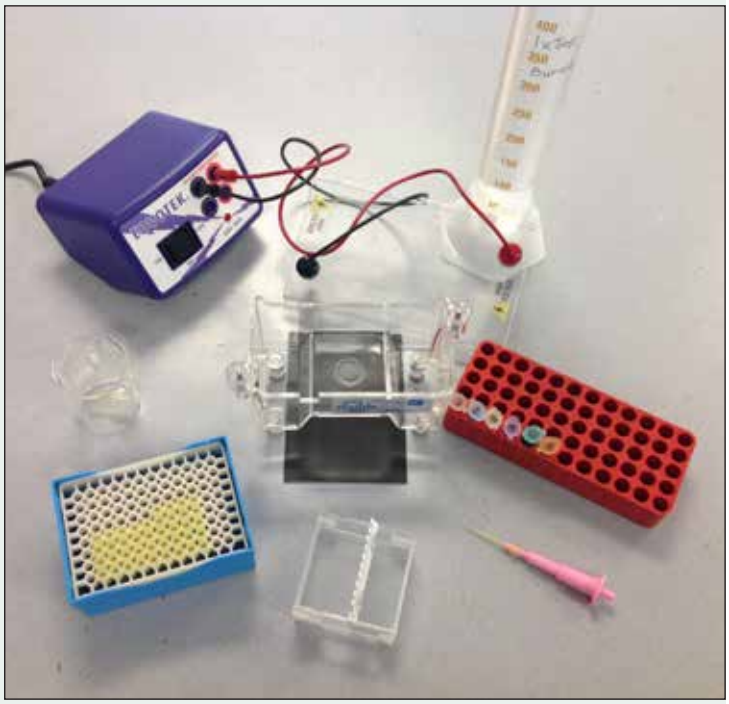

Figure 1 The equipment

6. Place the gel tray into the electrophoresis tank.
7. Carefully fill the electrophoresis tank with buffer until the gel is completely submerged. (There should be no gel above the surface of the buffer). If this is done too quickly or roughly the gel may lift off the tray making loading samples into the wells very difficult.
8. Place the black card under the tank to make the wells easier to see.

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Part B Running the gel

Aim

To use the provided dye samples to demonstrate separation of different molecules by electrophoresis.

Materials (per group or for demonstration)

- 100 mL beaker containing about 50 mL of distilled water
- Micro-pipette and tips
- 100 mL beaker for used tips
- Dye samples in micro-tubes

Method

1. Before you load the dye samples into the gel, practice how to use a micropipette using a small beaker of distilled water as shown by your teacher.
2. Using the micropipettes and dye samples provided, load the samples one at a time into the wells as shown by your teacher.
3. Place the lid onto the electrophoresis tank being very careful to not disturb the gel.
4. Connect the power leads of the lid to the power supply.
5. Turn the power supply to the voltage recommended by your teacher and leave the gel to run – it will probably take 10-20 minutes for clear separation to be achieved. Ensure the tank is not moved in any way and keep a close watch while the gel it is running to make sure that your samples do not end up running off the end of the gel (refer to *Figure 2*)
6. Once the electrophoresis is completed, turn off the power supply, and carefully remove the lid from the tank.
7. Gently remove the gel tray and gel from the tank. Be careful to keep the gel tray horizontal or the gel may slide off.
8. Place the gel tray on the bench and observe the results of the electrophoresis.
9. Take a photograph of the gel and the separated dye to keep a record of your results. This is important as once the electric current is removed (when the power supply is turned off) the dyes will diffuse into the gel and 'the bands' will become less distinct. Refer to *Figure 3*.

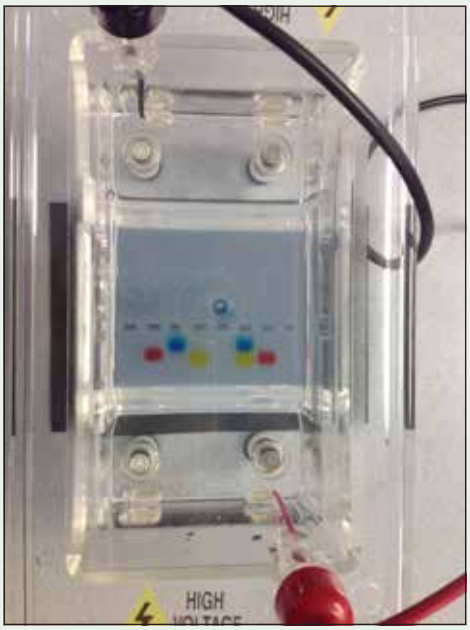

Figure 2 The process

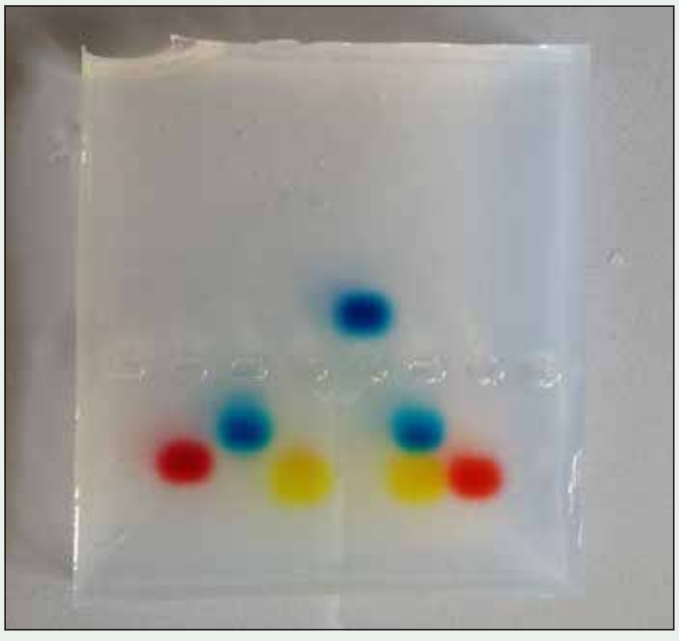

Figure 3 The product

Science Inquiry Skills 1.7 – Using agarose gel electrophoresis (cont.)

Discussion

1. The agarose gel needs to be completely submerged in an electrophoresis buffer solution. Why is an electrophoresis buffer solution needed for electrophoresis to be successful ?

2. What is a micropipette, and why does one need to be used in agarose gel electrophoresis?

3. Electrophoresis is a separation technique that relies on an electric field. What is an electric field, and why is one needed in electrophoresis?

4. The agarose gel acts as a type of tiny filter. What is meant by this statement?

5. Were all the samples of dyes negatively charged? How do you know?

6. Evaluate the electrophoresis procedure carried out and its effect on the quality of the data collected.

Helpful Online RESOURCES about using agarose gel

To visit a website on this topic use this URL or QRC:
<https://www.youtube.com/watch?v=vq759wKCCUQ>

Helpful Online RESOURCES to simulate an agarose gel

To visit a website on this topic use this URL or QRC:
<http://learn.genetics.utah.edu/content/labs/gel/>

SIS 1.7 Laboratory Notes

This activity provides a hands-on opportunity to set up an agarose gel and use it to separate a mixture of dyes using electrophoresis.

Setting up the agarose gel

Materials

- **Suppliers of electrophoresis gel tanks include:**

Southern Biological	www.southernbiological.com
Bio-Tek Services Pty Ltd	www.biotek.com.au
Bio-Rad Laboratories	www.bio-rad.com

- **Examples of gel tank costs – these include gel tray and combs:**

Southern Biological	$182 ex. GST.
Biotek	$299 ex. GST.
Bio-Rad	$390 ex. GST.

- **Suppliers of suitable power supplies (that will run two gel tanks) include:**

Southern Biological.	$498 ex GST.
Biotek	$375 ex GST.
Bio rad	also have an equivalent (cost available on request).

- Agarose is supplied in powder form, then prepared for gels as required – usually 1% in the electrophoresis buffer being used.
- Combs come in different sizes, to produce varying numbers and sizes of wells, as needed.
- Electrophoresis buffers include TAE. Usually supplied as a 50X stock concentrate, thus diluted prior to use. Approx. $35 for 500mL.
- Small pieces of black card can be placed on the bench under the electrophoresis tank when loading the samples into the wells – this makes the wells more visible.

Running the gel

Materials

- Micropipettes at fixed volumes approx. $30 each. Note that they require disposable tips. Appropriate tips may be obtained from *Livingstone International* approx. $25/1000.
- Dye samples that come with gel tanks obtained from the suppliers above may already contain loading buffer - this also includes a dense liquid such as glycerol to keep the samples in the wells as they are micropipetted, and to prevent them diffusing into the electrophoresis buffer. If in-school samples are made up, a loading buffer will be needed.
- Microtubes are needed to dispense dye samples into prior to student use. A rack of some kind is also needed to hold them. One option is to place a block of polystyrene foam into a small container and push the microtubes into it to hold them securely upright.

Additional information

The gel tanks generally come with detailed methods and explanations, and the websites have large amounts of supporting information on techniques and preparation. Bio-Tek for instance produces an Instruction Manual called *Classroom Biotechnology: Agarose Gel Electrophoresis Using Dyes*. Masking tape can be used to seal the ends of the gel casting trays prior to pouring agarose and is usually much easier to work with than the 'dams' supplied with the trays. Flinders University offers a workshop called Forensic Biology that includes running a gel electrophoresis in a contemporary forensic science context.