

BIOTECHNOLOGY: Getting started with Gel Electrophoresis

Introduction

Welcome to our guide on using gel electrophoresis in the classroom. With the extensive range of equipment available from Southern Biological, gel electrophoresis can now be performed safely and easily by science students in school laboratories.



What is Gel Electrophoresis?

Gel electrophoresis is a method to separate molecules based on their size and electrical charge. 'Electro' refers to the use of electricity and 'phoresis' comes from the Greek verb 'phoros' meaning 'to carry'. Gel electrophoresis therefore means using electricity to carry molecules through a gel.

How Does Gel Electrophoresis Work?

The gel used in electrophoresis is made of a solution of agarose, a polysaccharide obtained from red algae. When dissolved in hot water and allowed to cool, the solution turns into a gel because long agarose molecules hydrogen bond together, creating a large, three-dimensional network trapping the water.

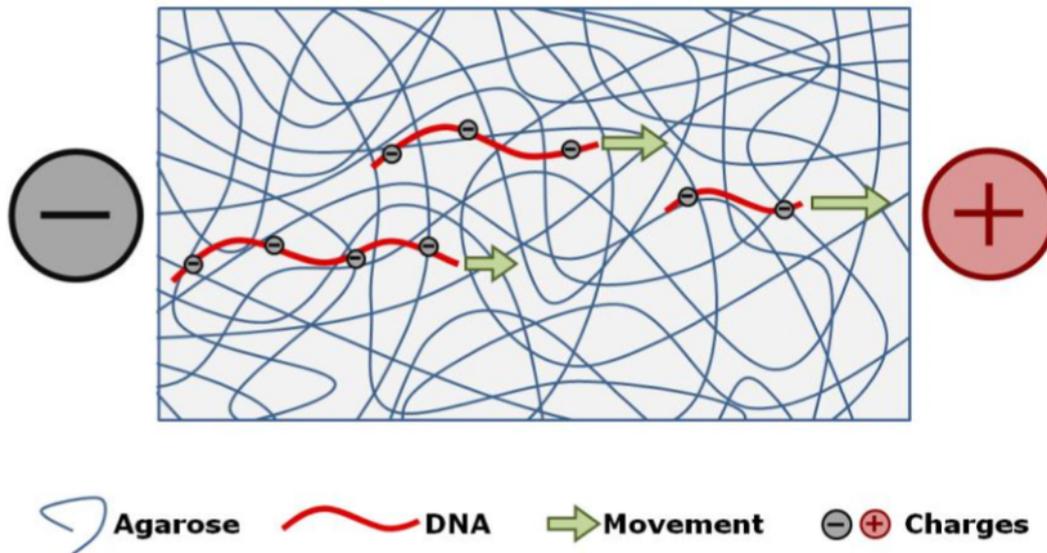
This gel can then be placed in an electrophoresis chamber, which along with a power supply, creates an electrical field through the gel. When a charged molecule is placed in this electric field, a force will act upon it. Negative charges will be pulled towards the positive side of the electric field and positive charges will be pulled to the negative side.

The speed at which a charged molecule will move through the gel depends on a number of factors. Firstly, stronger electric fields result in more electrical force and therefore a faster rate of molecular motion. Secondly, molecules with

stronger charges will move faster. Thirdly, a gel made from more concentrated agarose will create a denser network that will slow down moving molecules. Less concentrated gels conversely allow for faster rates.

However, perhaps the most important fact is that the size and shape of the molecule will also affect the speed. Small molecules will encounter less resistance moving through the network of agarose molecules and will therefore move more quickly. Large molecules will be more obstructed by the three-dimensional agarose gel network and will therefore move more slowly. It is this property that makes electrophoresis such a useful technique for separating components of mixtures.

By selecting the right combination of electric field strength and gel concentration, complex mixtures can be separated, the distances components have travelled can be analysed and individual components can even be collected for further analysis. Let's get started by looking at how to set up and use the equipment to perform electrophoresis. This guide will cover electrophoresis of dyes (a simpler experiment) and electrophoresis of DNA fragments (a more complex experiment).



Agarose Gel Network

Equipment

The Power Supply

We carry two varieties of electrophoresis power supplies: those that can run two simultaneous gels and those that can run four simultaneous gels. Each can be adjusted from 10 to 300V and has a built-in safety switch to limit the maximum current.



Power Supply

The Gel Electrophoresis Chamber

Each electrophoresis chamber Southern Biological sells is a complete unit consisting of:

- A tank fitted with platinum wire electrodes
- A lid with interlocking electrical connections and leads
- One or more trays in which to cast gels
- One or more combs to create wells in the gel

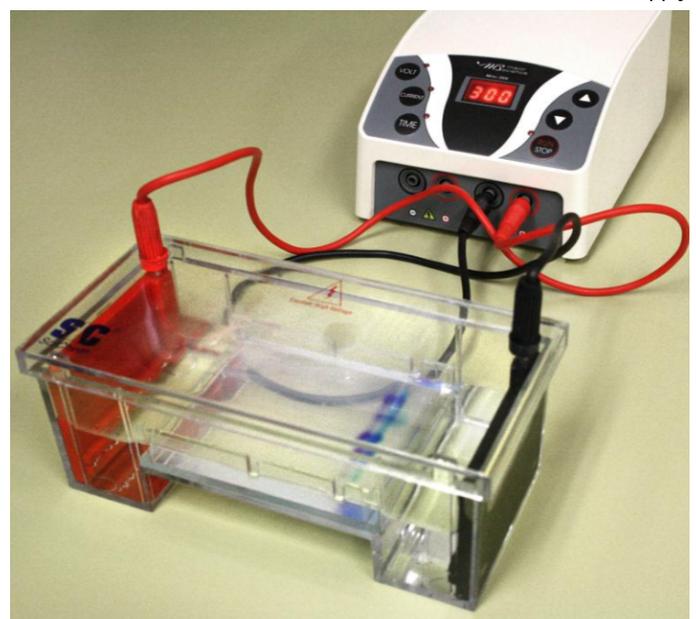
Other Necessary Materials

In addition to the power supply and electrophoresis chamber, you will need some additional materials to perform gel electrophoresis. For conducting electrophoresis of dyes, you will need:

- Micropipettes
- Micropipette tips
- Plain agar powder
- Sodium bicarbonate (NaHCO_3)
- Dye samples

For conducting electrophoresis of DNA, you will need:

- Micropipettes
- Micropipette tips
- Agarose powder
- Tris Borate EDTA (TBE) buffer
- Methylene blue stain
- Fragmented DNA with dye and loading buffer



Gel Electrophoresis Chamber



Dyes for Gel Electrophoresis

Procedure 1: Electrophoresis of Dyes

Overview

Using water-soluble dyes with electrophoresis provides a good introduction to the topic – the supplies are more affordable than when using DNA and the procedure is less complex. Conditions during electrophoresis do not have to be as carefully controlled as they would with DNA. Also, dyes have the advantage of being immediately visible whereas DNA requires staining to see.

Electrophoresis with dyes can be done as a stand-alone activity or as a precursor to electrophoresis with DNA. Using dyes before DNA allows students and teachers to practice techniques in a simpler experiment before the more complex procedure of DNA electrophoresis.

This list summarises the basic steps involved in conducting an experiment in the electrophoresis of dyes. Detailed instructions for each of these steps follows.

- 1 Prepare the sodium bicarbonate solution
- 2 Prepare the agar gel
- 3 Load the dye samples into the gel
- 4 Run the electrophoresis

Preparing the Sodium Bicarbonate Solution

A sodium bicarbonate solution will function as the electrolyte which will channel the electric field through

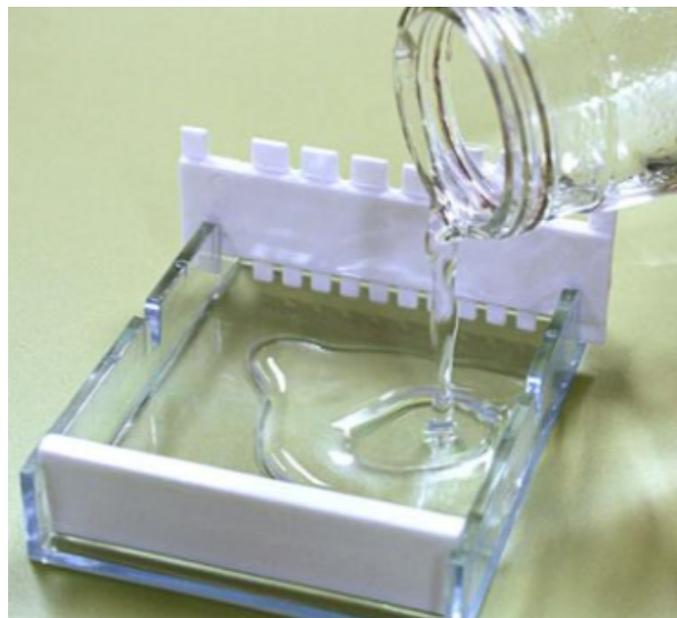
the gel. It will be used as an ingredient in creating the gel and it will also surround the gel during the electrophoresis. Prepare a 1% w/v stock solution by dissolving 1 g of NaHCO_3 in 100 mL of distilled water and dilute this down to a 0.1% w/v working solution as required.

SCIENCE:

- *The concentration of the electrolyte solution must be balanced (neither too weak nor too strong) in order to achieve ideal results.*
- *If too dilute, the solution will fail to channel the electric field created by the power supply. The field will therefore be spread out over a greater volume of space and weaker in the gel.*
- *If too strong, the solution can become too conductive and may discharge the electrical field generated by the power supply, again resulting in a weaker field in the gel. Even if the power supply is able to maintain the voltage, an overly conductive solution will carry a larger current and generate more heat, and this can negatively affect results.*



Microwaving the agar solution to a boil



Pouring the melted agar solution into the tray.

Preparing the Agar Gel

For electrophoresis of dyes, plain agar will make a suitable gel. It is not as refined as the agarose used for electrophoresis of DNA, but it is considerably more affordable.

To make a 1% w/v agar gel, add 100 mL of your 0.1% w/v NaHCO₃ buffer to a conical flask and sprinkle in 1 g of plain agar powder. Heat the solution to a boil in a microwave oven (use short bursts to avoid boiling over) and then allow it to cool to 60°C.

Seal each end of the casting tray with the supplied dams and place the comb into the position at the middle of the tray. Put the tray on a flat and level surface.

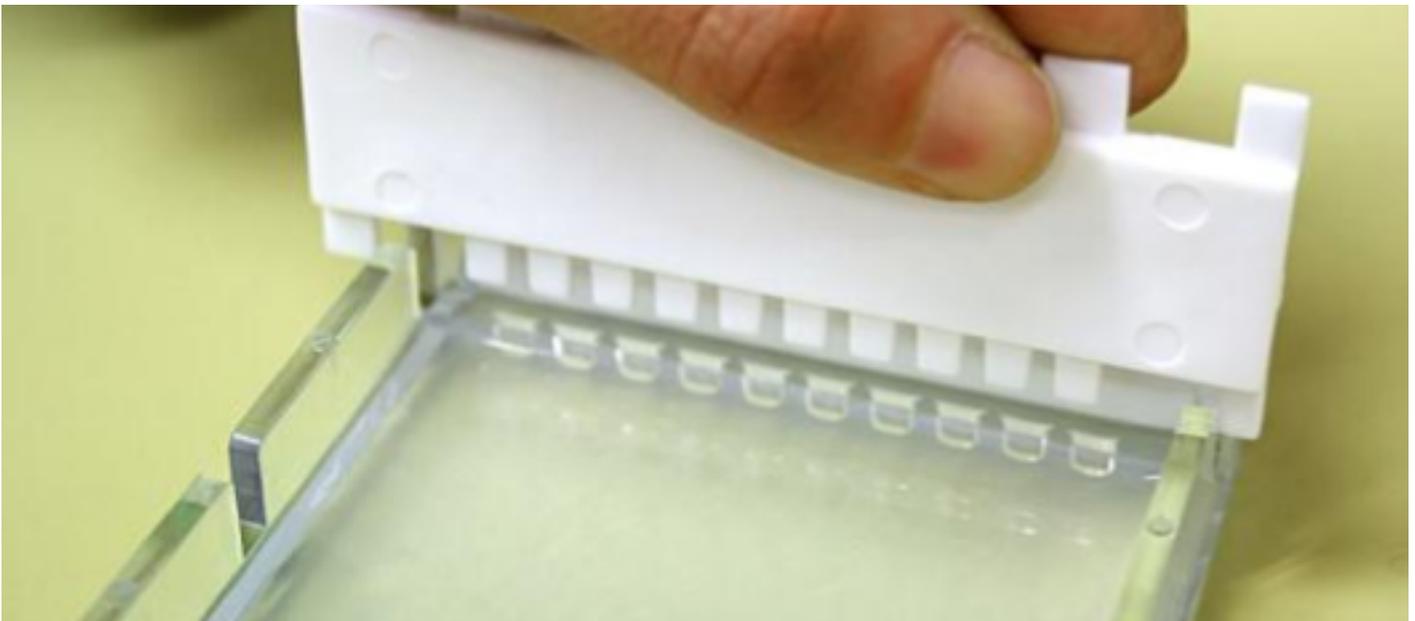
Carefully and slowly pour the cooled agar solution into the tray. Fill according to the requirements of the specific chamber you are using (consult the chamber's documentation).

TIPS:

- *A hot plate may be used for making larger quantities of agar solutions, but keep the mixture stirred at all times.*
- *For short term storage (up to several hours), the agar solution can be kept in a liquid state in a hot water bath at 55-60°C. Keep the container covered to minimise loss of moisture.*
- *For long term storage, excess gel can be sealed and kept in a heat resistant container for later use.*
- *Simply re-melt the gel for pouring.*
- *For your personal safety and to avoid cracking the gel casting tray, do not pour the agar before it has cooled to 55-60°C.*

SCIENCE:

- *Since the dyes that you will be using could have either a positive or a negative charge, they could migrate towards either end of the gel during electrophoresis. By placing the comb in the middle, you will allow room for movement in both directions. This contrasts with DNA which will only have a negative charge.*



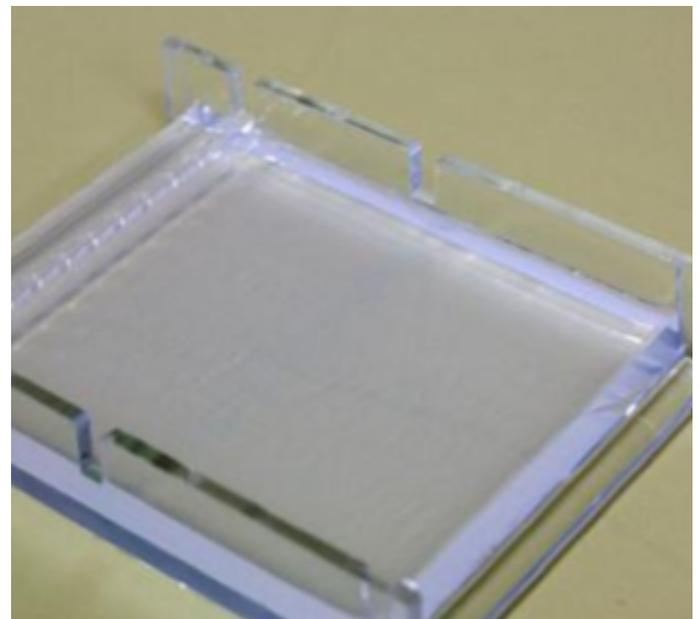
Removing the comb from the gel

Leave undisturbed until the gel has set, then carefully remove the comb and unseal the ends by removing the dams.

The gel can now be placed in the tank. Pour diluted (0.1%) NaHCO_3 buffer solution into the tank until it just covers the surface of the gel. Ensure there are no bubbles trapped in the wells.

TIPS:

- *Take care to keep the tray horizontal or you risk having the gel slide off once the ends have been unsealed.*
- *You can prepare gels up to a few days in advance. Simply wrap them in cling wrap and store in the refrigerator until required.*



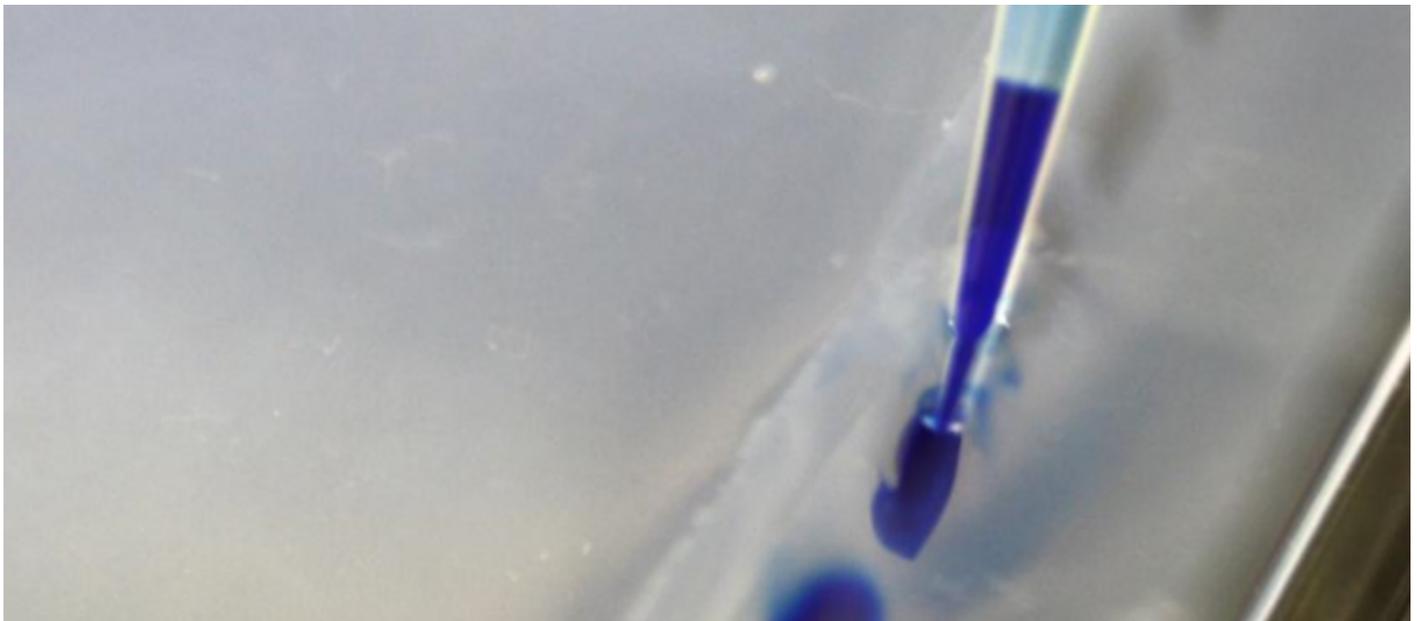
A gel ready to go into the chamber

SCIENCE:

- *The voltage of the power supply creates the electric field responsible for moving the charged molecules, and the conductivity of the buffer channels this electric field through the gel.*
- *If you add too much buffer to the tank, the electric field will be spread over a larger volume and will therefore be weaker and less uniform in the gel. This can cause slower DNA migration and band distortion.*



Pouring the NaHCO_3 buffer solution into the chamber



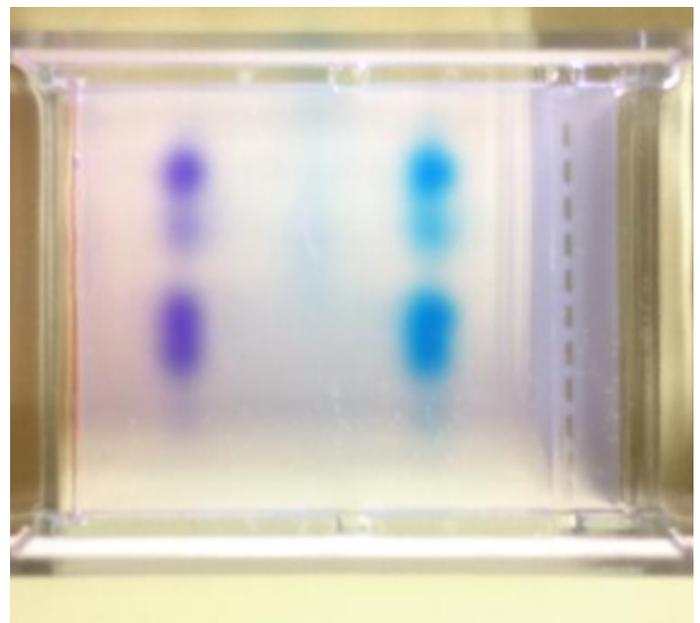
Using micropipette to load dyes

Loading the Dye Samples with a Micropipette

A variable-volume micropipette may be necessary if you are preparing your own samples, but if you are using Southern Biological's pre-mixed dye samples, you can use fixed-volume micropipettes to save costs.

- 1 Fit a clean tip to the barrel of the micropipette.
- 2 To draw up a sample, depress the plunger (depress fully on a fixed-volume micropipette; depress to the first stop on a variable-volume micropipette). Immerse the tip in the liquid to be collected. Use a slow and controlled action to release the plunger and draw the liquid into the tip.
- 3 To expel a sample, place the outlet of the pipette tip below the surface of the buffer and over the desired well in the gel. Use a slow and controlled action to depress the plunger fully and release the contents into the well.
- 4 Carefully withdraw the micropipette, holding the plunger in the down position until clear of the liquid.
- 5 Remove and discard the used micropipette tip.
- 6 Repeat these steps for each well until all samples have been loaded.

As an alternative to using the prepared dye solutions for electrophoresis, it is possible to use dyes from other sources. The dyes will need to be water-soluble, possess an ionic charge and be mixed with propylene glycol before being loaded into the wells.

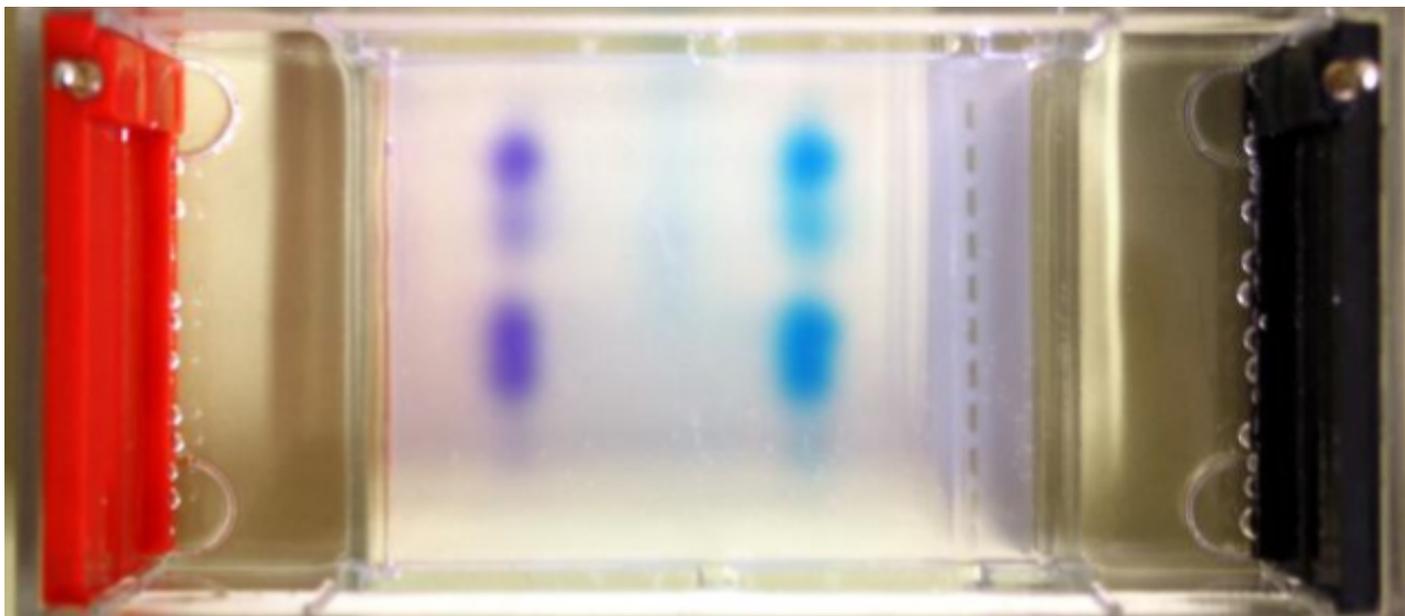


Loaded dye samples after Electrophoresis running

In order to use dyes from marker pens, apply the dyes to small pieces of filter paper and insert the pieces into the wells before the gel is covered with the buffer solution.

TIPS:

- *If you have fewer samples to run than there are wells in the gel, fill the central wells in preference to the wells at the edges.*
- *Use smaller rather than larger volumes for sharper bands. The standard combs supplied with Southern Biological's electrophoresis chambers allow for sample volumes of 10 μ L.*
- *The wells can be difficult to see on a light background. Place a piece of black card under the chamber to highlight the position of the wells.*



Completed electrophoresis run of dyes

Running the Electrophoresis

When the wells have been loaded and you are ready to begin, carefully fit the lid of the chamber onto the tank. Plug the leads into the power supply, choose a suitable voltage setting and turn on the power.

It is usual to start at the power supply's maximum voltage. This causes the samples to move quickly into the gel where they cannot be disturbed. At this voltage, the entire run will take approximately 30 minutes. You can alternatively reduce the voltage to slow the process.

Monitor the progress by observing the position of the dyes. Switch off the power when the dyes have been sufficiently separated. Remove the lid of the chamber and if the temperature of the buffer is comfortable to touch, lift the gel and tray out of the chamber. Carefully slide the gel into a shallow dish.

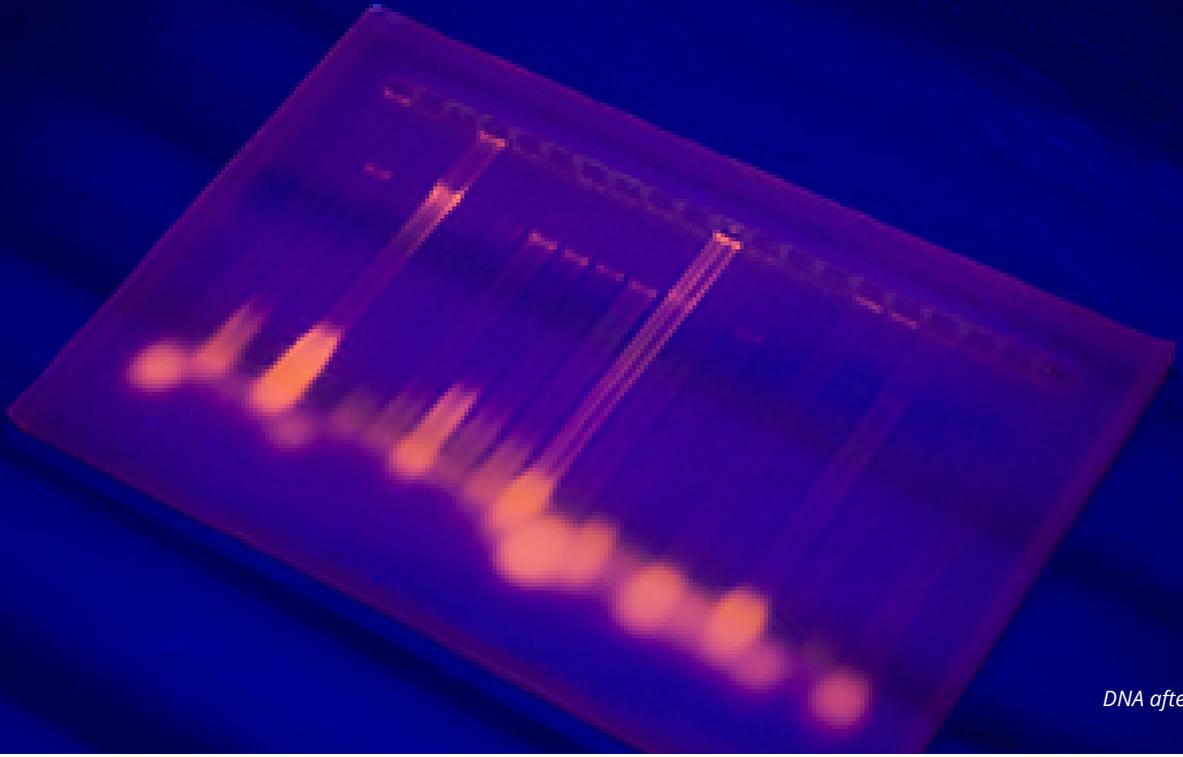
Due to their relatively small molecular weight, the dyes will continue to diffuse after the electrophoresis is finished. For this reason, make sure to take any measurements or photos shortly after the run is complete. By the following day, the bands of dye will not be clearly defined in the gel.

TIPS:

- *Never force the lid into position. If you encounter resistance, check that the plugs and sockets are aligned and try again.*
- *The power setting chosen will apply to all chambers connected to a particular power supply, but you can connect or disconnect a chamber without affecting the others. For safety, it is recommended that you briefly turn the power supply off when changing a connection.*
- *To ensure the unit is receiving power, check for the evolution of gas bubbles at the electrodes due to the electrolysis of the NaHCO_3 solution.*
- *The gel will be quite slippery at this point, so take care to keep the tray horizontal until you are ready to remove it.*
- *The buffer can be retained for reuse. It is suitable to use a buffer solution three times before it is discarded.*

SCIENCE:

- *The liquid in the chamber will warm up during the process as the electrical energy supplied by the power supply becomes heat. It is desirable to minimise this effect as heat can lead to convection currents that can blur and smear the bands of dye. One way to express electrical power, $P=V^2/R$, clearly shows that lower voltages will lead to lower power. Therefore, running your gel on a lower voltage over a longer period of time will result in less heat build-up and may give better results. However, very long runs can also result in low-quality bands, so the ideal voltage setting is usually an intermediate one.*



DNA after electrophoresis

Procedure 2: Electrophoresis of DNA

Overview

While dyes provide an easy introduction to the topic, a more common use of electrophoresis is to separate DNA fragments by length. Since electrophoresis of DNA is a more complex activity than electrophoresis of dyes, many prefer to use dyes first in order to practise the techniques.

This list summarises the basic steps involved in conducting an experiment in the electrophoresis of DNA. Detailed instructions for each of these steps follows.

- 1 Prepare the TBE buffer solution
- 2 Prepare the agarose gel
- 3 Load the DNA samples into the gel
- 4 Run the electrophoresis
- 5 Stain the DNA in the resulting gel

Preparing the TBE Buffer Solution

Tris-Borate-EDTA (TBE) buffer is the electrolyte which will channel the electric field through the gel. It will be used as an ingredient in creating the gel and it will also surround the gel during the electrophoresis.

TBE buffer is supplied as a 20x concentrate. Prepare diluted (1x) working solutions as required. Re-dissolve any solids that precipitate out during storage by heating in a water bath.

SCIENCE:

- *The composition of this buffer is designed to keep the pH slightly alkaline. This ensures that the DNA molecules stay in their deprotonated form which includes a negative charge on each phosphate group. Without that negative charge, DNA molecules would not be moved by an electric field and electrophoresis would not work.*
- *The electrolyte solution must be fairly dilute in order to minimise the conductivity of the solution. A more conductive solution will allow for greater current at a given voltage, and therefore more heat will be created. If the solution is too conductive, enough heat can build up to interfere with the results of the experiment. Additionally, if the resistance of the solution is too low, the power supply may be unable to maintain the set voltage, resulting in a weaker electrical field and slower electrophoresis.*



Preparing the agarose solution



Microwaving the agarose solution to a boil

Preparing the Agarose Gel

For electrophoresis of DNA, a gel made from refined agarose is necessary for good results.

To make a 0.8% w/v agarose gel, add 100 mL of your diluted (1x) TBE buffer to a conical flask and sprinkle in 0.8 g of agarose powder. Heat the solution to a boil in a microwave oven (use short bursts to avoid boiling over) and then allow it to cool to 60°C.

Seal each end of the casting tray with the supplied dams and place the comb into the position close to one end of the tray. Put the tray on a flat and level surface.

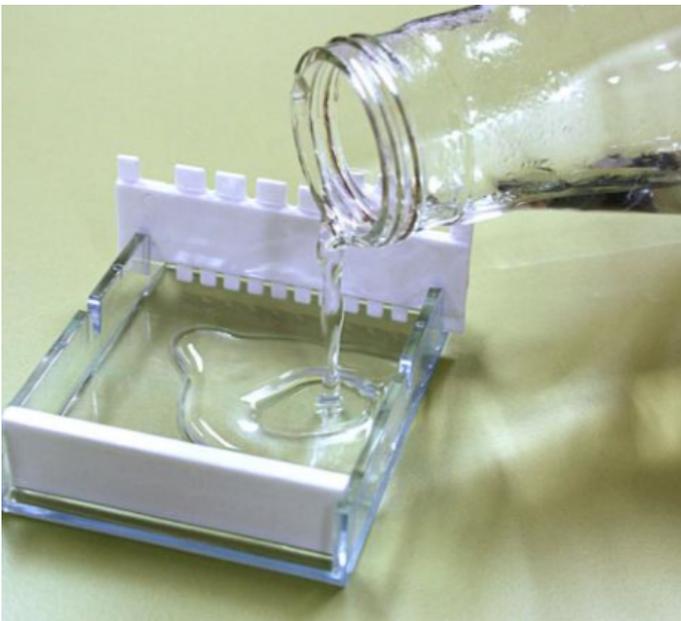
Carefully and slowly pour the cooled agarose solution into the tray. Fill according to the requirements of the specific chamber you are using (consult the chamber's documentation).

TIPS:

- *A hot plate may be used for making larger quantities of agarose solutions, but keep the mixture stirred at all times.*
- *For short term storage (up to several hours), the agarose solution can be kept in a liquid state in a hot water bath at 55-60°C. Keep the container covered to minimise loss of moisture.*
- *For long term storage, excess gel can be sealed and kept in a heat resistant container for later use. Simply re-melt the gel for pouring.*

SCIENCE:

- *Agarose is the polysaccharide in agar responsible for its ability to form a gel. Unrefined agar also contains another polysaccharide, agaropectin, which contains charged groups such as sulfates. When formed into a gel, these charged groups can cause a phenomenon known as electroendosmosis where water flows under the influence of electricity. This water flow can move the DNA in the gel and smear the bands, and this is why cleaner and more consistent results can be obtained by using refined agarose instead of unrefined agar.*
- *Since the DNA fragments will all have a negative charge, they will migrate towards the positive electrode during electrophoresis. By placing the comb at the end position, you will allow for the maximum distance for the DNA to travel. This contrasts with dyes which can have either a positive or negative charge and therefore may move in either direction.*



Pouring the melted agarose solution into the tray



Removing comb from the gel

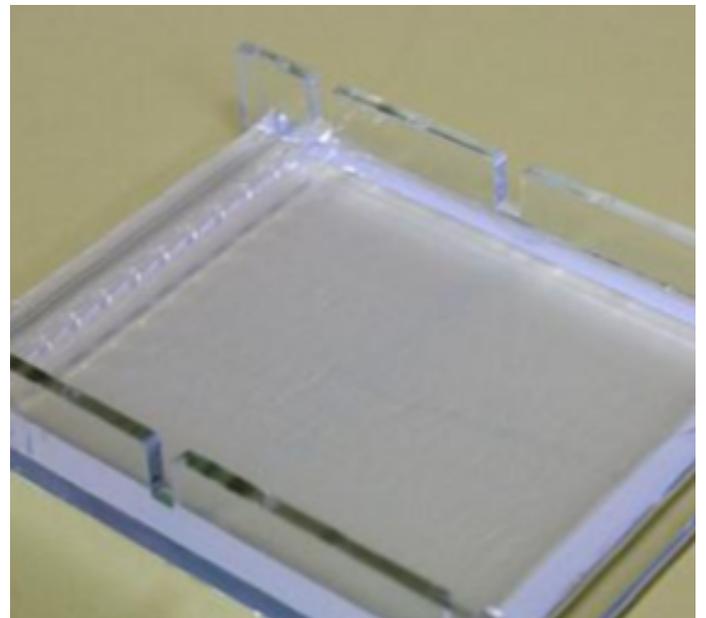
Leave undisturbed until the gel has set, then carefully remove the comb and unseal the ends by removing the dams. Removing the comb from the gel A gel ready to go into the chamber. The gel can now be placed in the tank with the wells oriented to the negative electrode. Pour diluted (1x) TBE buffer solution into the tank until it just covers the surface of the gel. Ensure there are no bubbles trapped in the wells.

TIPS:

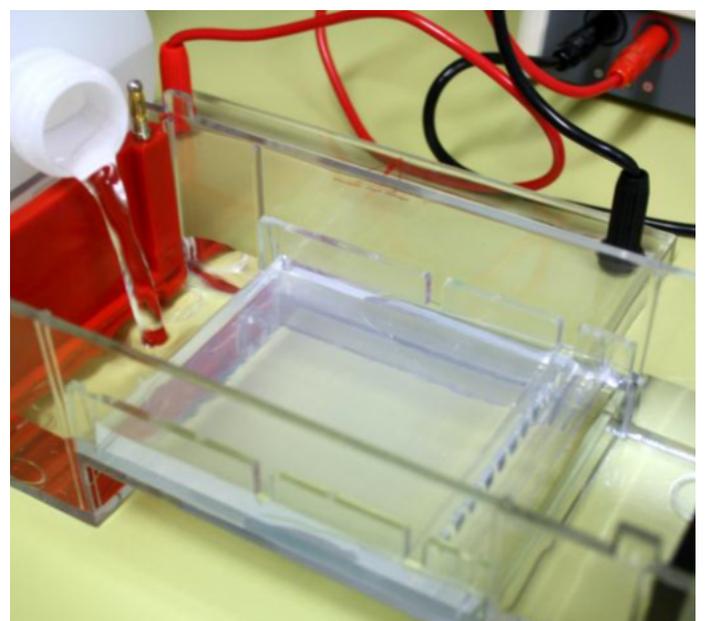
- *Avoid forming bubbles in the gel by gently pouring the agarose solution into the end of the tray away from the comb.*
- *Take care to keep the tray horizontal or you risk having the gel slide off once the ends have been unsealed.*
- *You can prepare gels up to a few days in advance. Simply wrap them in cling wrap and store in the refrigerator until required.*
- *Take care to orient the gel correctly. If a gel is run backwards, even for a short period, the samples are usually lost.*

SCIENCE:

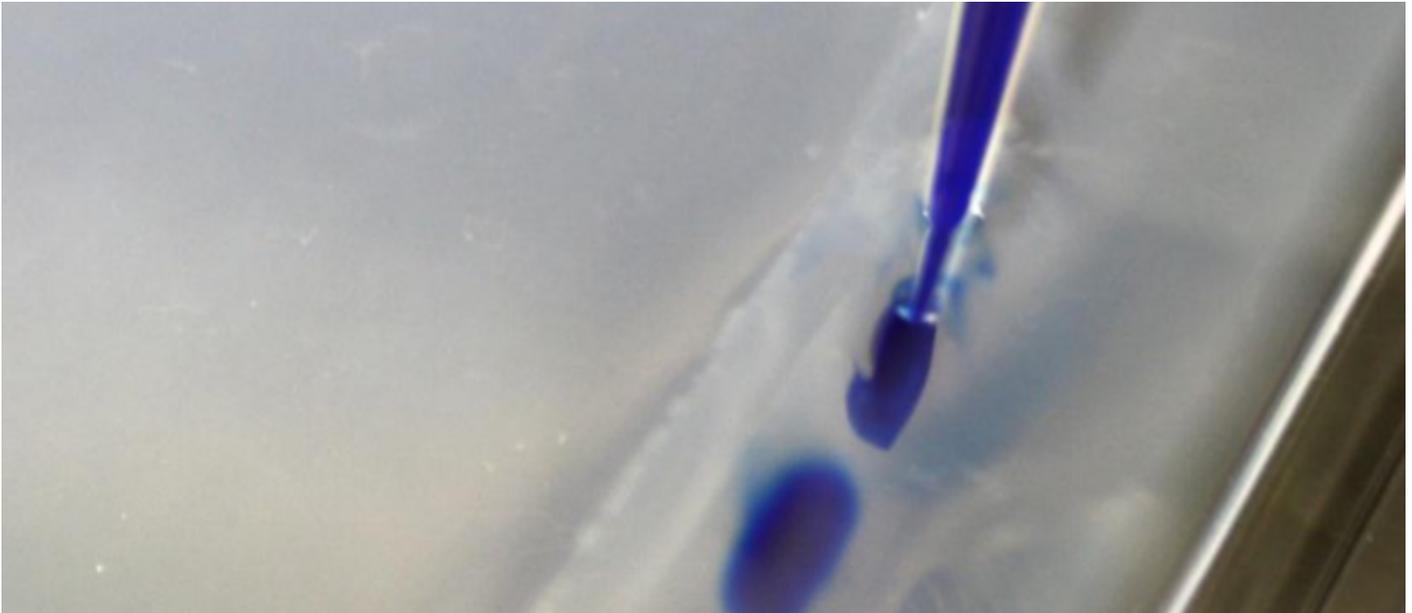
- *The voltage of the power supply creates the electric field responsible for moving the charged molecules, and the conductivity of the buffer channels this electric field through the gel.*
- *If you add too much buffer to the tank, the electric field will be spread over a larger volume and will therefore be weaker and less uniform in the gel. This can cause slower DNA migration and band distortion.*



A gel ready to go into the chamber



Pouring the TBE buffer solution into the chamber



Loading Dye samples using micropipette

Loading the DNA Samples with a Micropipette

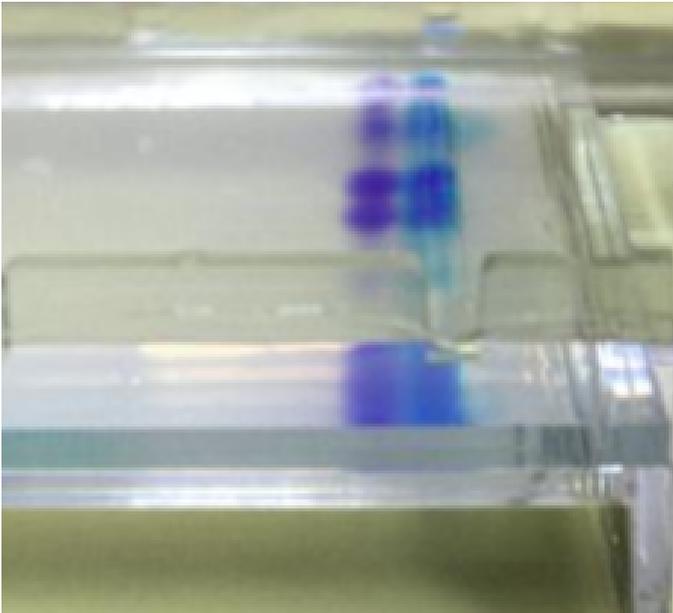
Loading the DNA Samples with a Micropipette If you have purchased your DNA samples from Southern Biological, they require no preparation before being loaded into the gel as they will already have loading buffer and dye mixed in.

Pouring the TBE buffer solution into the chamber A variable-volume micropipette may be necessary if you are preparing your own samples, but if you are using Southern Biological's pre-mixed DNA samples, you can use fixed-volume micropipettes to save costs.

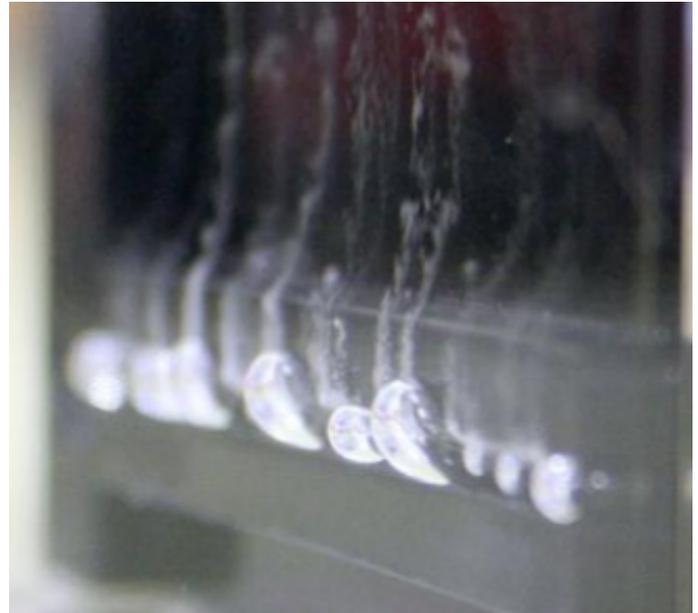
- 1 Fit a clean tip to the barrel of the micropipette.
- 2 To draw up a sample, depress the plunger (depress fully on a fixed-volume micropipette; depress to the first stop on a variable-volume micropipette). Immerse the tip in the liquid to be collected. Use a slow and controlled action to release the plunger and draw the liquid into the tip.
- 3 To expel a sample, place the outlet of the pipette tip below the surface of the buffer and over the desired well in the gel. Use a slow and controlled action to depress the plunger fully and release the contents into the well.
- 4 Carefully withdraw the micropipette, holding the plunger in the down position until clear of the liquid.
- 5 Remove and discard the used micropipette tip.
- 6 Repeat these steps for each well until all samples have been loaded.

TIPS:

- *If you have fewer samples to run than there are wells in the gel, fill the central wells in preference to the wells at the edges.*
- *Use smaller rather than larger volumes for sharper bands. The standard combs supplied with Southern Biological's electrophoresis chambers allow for sample volumes of 10 μ L.*
- *The wells can be difficult to see on a light background. Place a piece of black card under the chamber to highlight the position of the wells.*



Early in a DNA electrophoresis run



Bubbles forming at an electrode

Running the Electrophoresis

When the wells have been loaded and you are ready to begin, carefully fit the lid of the chamber onto the tank.

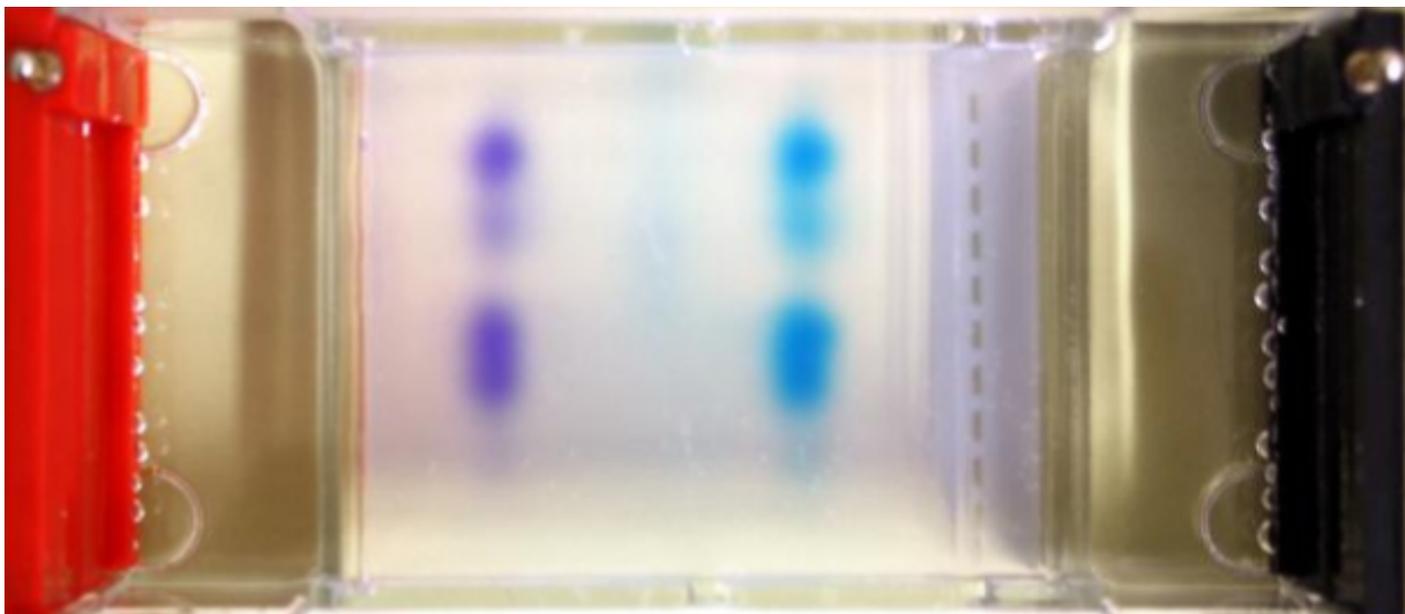
Plug the leads into the power supply, choose a suitable voltage setting and turn on the power. It is usual to start at the power supply's maximum voltage. This causes the samples to move quickly into the gel where they cannot be disturbed. At this voltage, the entire run will take approximately 30 minutes. You can alternatively reduce the voltage to slow the process.

TIPS:

- *The power setting chosen will apply to all chambers connected to a particular power supply, but you can connect or disconnect a chamber without affecting the others. For safety, it is recommended that you briefly turn the power supply off when changing a connection.*
- *To ensure the unit is receiving power, check for the evolution of gas bubbles at the electrodes due to the electrolysis of the buffer solution.*
- *Never force the lid into position. If you encounter resistance, check that the plugs and sockets are aligned and try again.*

SCIENCE:

- *The liquid in the chamber will warm up during the process as the electrical energy supplied by the power supply becomes heat. It is desirable to minimise this effect as heat can lead to convection currents that can blur and smear the bands of DNA. One way to express electrical power, $P=V^2/R$, clearly shows that lower voltages will lead to lower power. Therefore, running your gel on a lower voltage over a longer period of time will result in less heat build-up and may give better results. However, very long runs can also result in low-quality bands, so the ideal voltage setting is usually an intermediate one.*

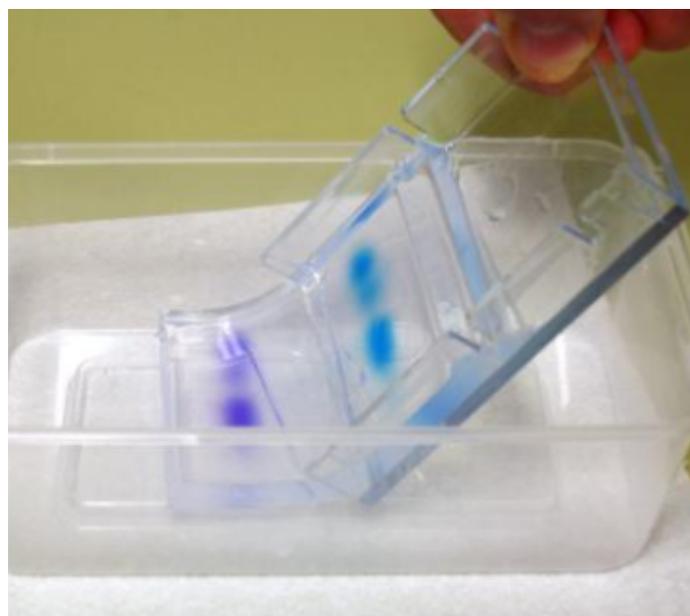


A completed electrophoresis run of DNA

Monitor the progress by observing the position of the loading dyes. There are two loading dyes in Southern Biological's DNA samples: bromophenol blue and xylene cyanol. The bromophenol blue is the leading dye and will travel at a similar rate to the smaller DNA fragments. The xylene cyanol is the trailing dye and will travel at a similar rate to the larger DNA fragments.

Switch off the power when the leading loading dye (bromophenol blue) is about three quarters of the way down the gel.

Remove the lid of the chamber and if the temperature of the buffer is comfortable to touch, lift the gel and tray out of the chamber. Carefully slide the gel into a shallow dish.



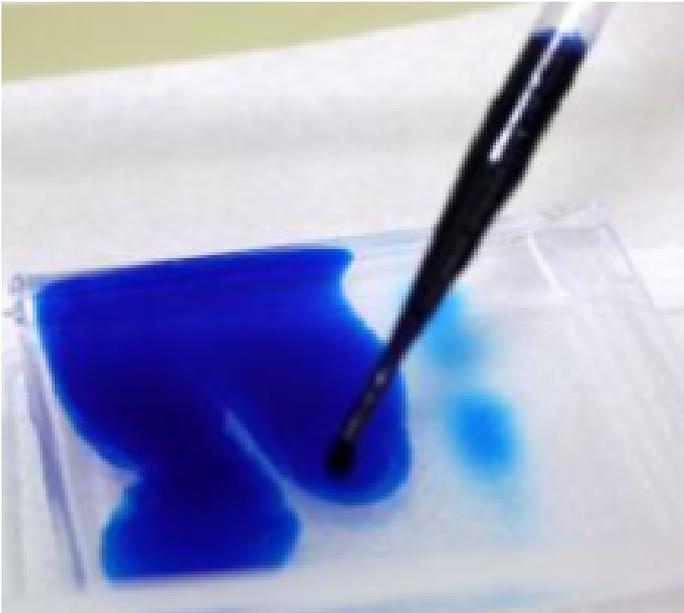
Transferring the gel to a tray for staining

TIPS:

- *The gel will be quite slippery at this point, so take care to keep the tray horizontal until you are ready to remove it.*
- *The buffer can be retained for reuse. It is suitable to use a buffer solution three times before it is discarded.*

SCIENCE:

- *Bromophenol blue will run at approximately the same rate as a DNA fragment of 400 base pairs, and xylene cyanol will run at approximately the same rate as a DNA fragment of 4000 base pairs.*



Covering the surface of the gel with concentrated stain



Before any de-staining

Staining the Gel

Methylene blue stain will bind to DNA molecules, and if allowed to diffuse through a completed gel, it will render the bands of DNA visible to the naked eye.

- 1 Use a plastic pipette to cover the upper surface of the gel with a small volume of concentrated stain solution.
- 2 Allow the stain to soak into the gel for 5 minutes, then rinse off excess stain with tap water.
- 3 Seal the dish and gel in cling wrap for several hours to allow the stain to develop.

To heighten the visual contrast between the DNA bands and the rest of the gel, you can perform a de-staining step to remove excess stain from the gel. To do this, immerse the gel in water and occasionally agitate to leach out unbound stain.

Storing the Gel

Place the gel in a press-seal bag with a few drops of dilute stain solution. Store in the refrigerator and handle with care. For long term storage, use 70% ethanol.

TIPS:

- *It is often convenient to allow this step to proceed overnight.*
- *A light box can help make the stained bands more visible. You can make a permanent record by capturing an image of the gel with a camera or a scanner.*



After three hours of de-staining



After overnight de-staining