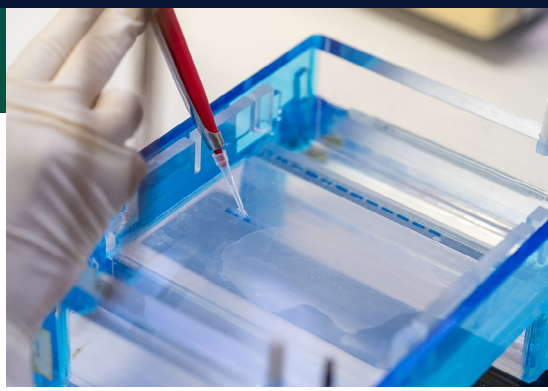
BIO LAB:
GEL ELECTROPHORESIS OF DYES

TEACHER

LEVEL: Year 11&12 | TOPIC: Biotechnology | TIME REQUIREMENT: 45 mins



CURRICULUM ALIGNMENT

- *Identify, research and construct questions for investigation; propose hypotheses; and predict possible outcomes (ACSBL061)*
- *Conduct investigations, including the use of probabilities to predict inheritance patterns, real or virtual gel electrophoresis, and population simulations to predict population changes, safely, competently and methodically for the collection of valid and reliable data (ACSBL063)*

BACKGROUND

Gel electrophoresis is a commonly used technique that separates samples of molecules by size and charge. A gel electrophoresis system consists of a platform that holds the gel, a chamber for buffer, electrodes positioned on opposite sides of the chamber. It also includes a power supply that connects to the negative and positive electrodes, and applies an electric charge to the electrodes. The procedure is frequently used to analyse DNA, RNA, and proteins. The process of gel electrophoresis begins with pouring agar gel into a platform that lies within the chamber. The platform and gel are then submerged in the buffer; which conducts electrical current through the gel. The gel contains small wells, whereby, DNA or other molecules may be inserted. When the electric field is activated, the molecules are triggered to migrate towards the oppositely charged electrode. Hence, the electrical field causes the molecules to move through the small pores of the gel. This will reveal the size of the molecules, as smaller molecules move more easily through the pores of the gel, compared to larger molecules of the same charge. When using electrophoresis to separate nucleic acids, the wells of the gel should be placed closer to the end with the negative electrode, as the negatively charged DNA and RNA will be attracted to the positive pole when the electric field is applied. DNA or RNA will both move through the gel towards the positive poll, however, when using dyes rather than DNA, this is not the case. When using dyes, some will move towards the positive end while others will travel in the opposite direction. This is due to the fact that some dyes are negatively charged and others are positively charged. As a result, it is best to cast the gels in the wells as close to the centre as possible to allow them room to move in both directions. Using dyes means conditions during electrophoresis do not have to be as carefully controlled as they would with DNA. The dyes also make it much easier to visualise the process.

In this practical, students use water soluble dyes in gel electrophoresis. This method is economical and allows for clear visualisation. Using dyes before the more complex procedure of DNA electrophoresis allows students and teachers to practice techniques in a simpler experiment with less variables. Students are tasked with preparing the gel electrophoresis chamber, loading the dyes, predicting their migration patterns and observing the running of the gel. Students will load 6 dyes into the wells; 4 known dyes and 2 'Unknowns'. Southern Biological stocks 4 different 'Unknown' mixtures (known as: Mixtures A, B, C and D) that are comprised of different combinations of the four known dyes; however, you will receive a selection of 2 of the 4 mixtures with your kit. After gel running, students observe the gel migration and identify the composition of the 'Unknown' dyes. Students then determine whether their predictions regarding how the dyes would behave were correct. Through this practical, students gain a deeper understanding of the molecules and molecular properties that are relevant to DNA and proteins. Students explore how the size and charge of a molecule influences its migration through an agar gel.

MATERIALS

Each Dyes for Electrophoresis Kit is comprised of 4 known dyes and two dye mixtures ('Unknowns'). You will recieve:

- 1 Xylene Cyanol Dye
- 1 Orange G Dye
- 1 Methyl Green Dye
- 1 Bromophenol Blue Dye
- 2 'Unknowns' (labelled Mixture A, B, C or D)
- Plain Agar Powder to make 1% agar gel (0.5 g per group)
- Horizontal gel electrophoresis apparatus: e.g. blueGel™
- Sodium bicarbonate buffer (100mL per group)
- Micropipettes 10uL capacity
- Disposable micropipette tips
- Plastic tubes
- Microcentrifuge (optional)
- Scale for weighing agar
- Glass beaker, 2000mL
- Glass beaker, 500mL to dissolve agar
- Microwave or hot plate
- Plastic ruler
- Piece of white paper: (if using blueGelTM)

SOUTHERN Biological

SAFETY PRECAUTIONS

- Wear appropriate personal protective equipment (PPE).
- Know and follow all regulatory guidelines for the disposal of laboratory wastes.
- The dyes used in this practical are non-toxic. We recommend wearing gloves to ensure the dye does not stain your skin.
- Do not pour the agar before it has cooled to 55-60°C.
- Never remove the lid from the gel tank while the unit is still connected to the power supply.

PREPARATION - BY LAB TECHNICIAN

Preparing Sodium Bicarbonate Buffer Solution

1. Dissolve 1g of $NaHCO_3$ (Sodium Bicarbonate) in 1000mL of distilled water to prepare a 0.1% w/v stock solution.

Preparing Plain Agar Gel

1. To make a 1% w/v agar gel, dissolve 3g of plain agar in 250ml of boiling buffer solution (see previous step)
2. Once dissolved, make up to 300ml.
3. This volume should make approximately 6 gels (allowing 50ml per gel)
4. If using blueGel apparatus, you should be able to make approximately 15 gels from 300ml.(approximately 20ml per gel).

Note: You may wish students to practice the process of pouring gels themselves, as part of this practical. If so, allow for 15-20 minutes to complete the procedure or instruct students to prepare the gel the day before and stored in a sealed container in the refrigerator overnight. To make a 1% w/v agar gel, add 50mL of your working buffer solution (0.1% w/v NaHCO3) to a conical flask and sprinkle in 0.5g of plain agar powder.) Heat the solution to boiling using a microwave or hot plate. If using a microwave, use short bursts to avoid boiling over. The powder should be fully dissolved and the gel clear. Note: if you are using blueGelTM equipment, you will only need 20mL of agar (0.2g of agar powder in 20ml of buffer solution).
Allow it to cool to about 60°C.

- Supply each student group with 15µL of each dye/dye mix.

METHOD - STUDENT PRACTICAL

Pouring the Agar Gel

1. Seal each end of the casting tray with the dams if your chamber has them. If you are using a chamber without dams, seal the ends with masking tape. Note that this does not apply if you are using blueGelTM.
2. Place the comb into the position at the middle of the tray. Put the tray on a flat and level surface. There are negatively and positively charged dyes represented in this kit, so you should expect to see the dyes traveling in opposite directions based on their charge, to the anode and cathode respectively.
3. Carefully and slowly pour the cooled agar solution into the tray with the comb in the middle.
4. Fill according to the requirements of the specific chamber you are using (consult the chamber's documentation).
5. 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.
6. Allow 20 to 60 minutes for the agar solution to solidify.
7. Remove the comb from the gel by carefully wiggling the comb slowly to dislodge it. Complete this step just prior to use.

Running the Agar Gel

Note: If you are using blueGelTM, place a piece of white paper on the blue surface and then place the buffer chamber on top.

1. Remove the dams or tape as applicable from the ends of the casting tray and position the tray in the buffer chamber. Keep the tray level during this step to ensure the gel does not slip off.
2. Place the chamber, with the casting tray inside, on a level surface then carefully pour your dilute 0.1% working strength buffer solution into the chamber until it just covers the surface to submerge the gel. Ensure there are no bubbles trapped in the wells. You can shake the gel gently to remove air bubbles.
3. Load 10 µL samples of each dye into the wells in the following sequence:

- Lane 1: Leave empty
- Lane 2: Orange G
- Lane 3: Xylene cyanol
- Lane 4: Unknown 1
- Lane 5: Unknown 2
- Lane 6: Methyl Green
- Lane 7: Bromophenol Blue
- Lane 8: Leave empty

4. Carefully fit the lid of the chamber onto the tank. Never force the lid to close!
5. Plug the leads into the power supply if applicable, choose a suitable voltage setting and turn on the power. It is usual to start at a setting of 100V. This causes the samples to move quickly into the gel where they cannot be disturbed. At this voltage, the entire run will take approximately 30-40 minutes. If you are using blueGel™ you need only plug it in and turn it on.
6. Once the dyes have moved into the gel, you can reduce the voltage to slow the process if required, if your equipment allows adjustment.
7. To ensure that the unit is receiving power, be sure to check for the presence of small bubbles forming on both electrodes. This occurs due to electrolysis. Never remove the lid from the gel tank while the unit is still connected to the power supply! During the run, some heat will be liberated and the tank will warm up slightly. This is normal and will cause some condensation to form on the inside of the tank lid.
8. Monitor the progress by observing the position of the dyes. Switch off the power when the dyes have been sufficiently separated.

After Gel running

1. 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.
2. Carefully slide the gel into a shallow dish. The gel will be quite slippery at this point, so take care to keep the tray horizontal until you are ready to remove it.
3. Place a piece of white paper under the entire chamber to view the dyes more clearly.
4. Observe location of dyes in the gel. If needed, use a flashlight or other light source to better see the dyes.
5. If you do not see clear separation between dyes, increase running time.
6. 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. Within a few hours, the bands of dye will not be clearly defined in the gel.

Predictions

1. While the dye is running, predict how the dyes will behave during the electrophoresis procedure. Predict the intensity and colour of the dyes.
2. Predict the migration patterns of the dyes and expected results based on the molecular weights of the dyes below:
    - Orange g approx 450 g/mol
    - Xylene Cyanol approx 540 g/mol
    - Methyl Green approx 650 g/mol
    - Bromophenol Blue approx 670 g/mol
3. Predict the relative distance of the dyes to one another. Provide evidence to support your answer.
4. Predict which dye will run the farthest. Provide evidence to support your answer.
5. Predict which dyes are positive and which are negative. Provide evidence to support your answer.

OBSERVATION AND RESULTS

The migration of the dyes can be measured using a ruler positioned on the surface of the gel. The distance migrated is the distance between the leading edge of the dye band and the front edge of the well. The intensity of the bands, their colour as well as their migration will depend upon the gel-loading, the length of electrophoresis and the voltage. Positive dyes travel towards the negative electrode, whereas negative dyes travel towards the positive electrode. The dyes we supply are recognised commercial compounds; hence there may be some coloured by-products in the dye that have carried over from manufacture. When you run the Methyl Green you will see two colour streaks (purple and blue) extending from the well.

Dye migration

- Negatively charged, Orange G and Bromophenol Blue dyes migrate relatively fast towards the positive electrode.
- Positively charged, Methyl Green (double positive charged) also migrates quickly; however, it migrates towards the negative electrode.
- Negatively charged Xylene Cyanol migrates very slowly towards the positive electrode.
- The migration of the unknown dyes will vary depending on the mixture. The distances and direction will depend upon charge and molecular weight.

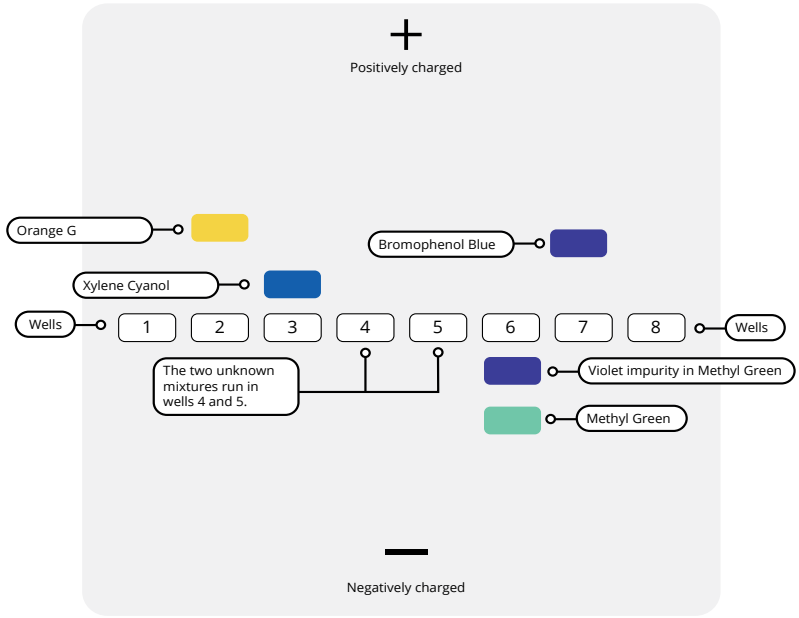
Below is an example of an idealised result if you followed the above sequence of loading the gels into the wells. Also included are examples of the results for the 'Unknown' mixtures. The results of gel electrophoresis will reveal the compositions of the 'Unknowns', as follows:

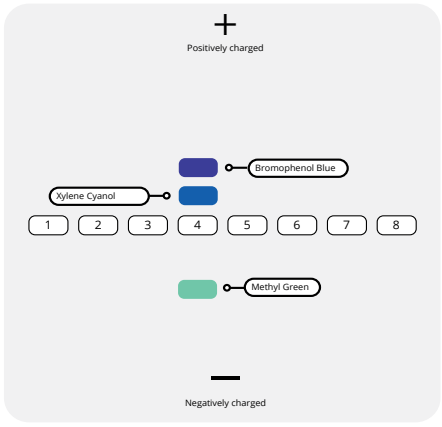
Mixture A:
- Xylene Cyanol
- Bromophenol Blue
- Methyl Green

Mixture B:
- Orange G
- Bromophenol Blue
- Methyl Green

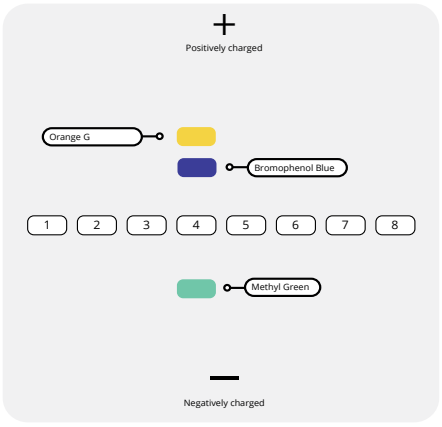
Mixture C:
- Xylene Cyanol
- Orange G
- Bromophenol Blue

Mixture D:
- Xylene cyanol
- Orange G
- Methyl green

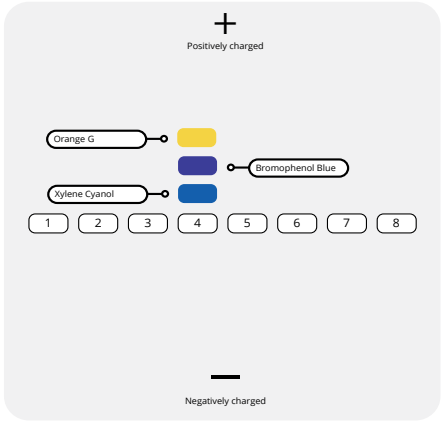




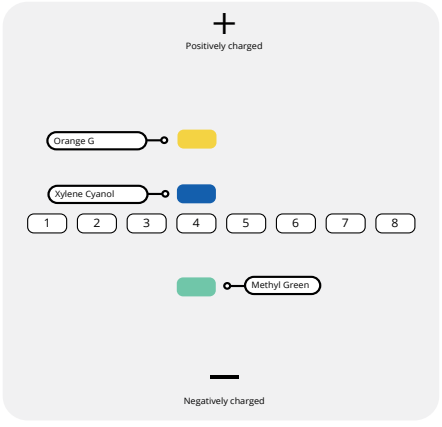
Mixture A



Mixture B



Mixture C



Mixture D

INVESTIGATION

Analysis of results:

1. Ask students to describe the gel running results. Question whether the colour and intensity of the dyes met their predictions. Students should provide potential reasons for the dyes not meeting the expected results.
2. Ask students if their predictions for the migration patterns of the dye were accurate. Students should identify which dyes met their predictions and which did not. Students should also include potential reasons for the dyes not meeting their predictions. One potential reason for the dyes not meeting expected predictions is the double positive charge of Methyl Green. This double positive charge gives the negative electrode a stronger pull on it, causing the dye to run more quickly than expected for its size. You may prefer to tell students this up front or let them work it out based on its behaviour in the gel. The speed the molecules migrate during gel running is dependent on the size, charge, and shape of the molecule.
3. Ask students whether predicted relative distances of the dyes to one another were correct. Ask students to provide potential reasons for the dyes not meeting the expected results.
4. Ask students which dye ran the farthest. Students should include information on the dye's weight and charge.
5. Ask students whether their predictions of which dyes are positive and which are negative were proven correct.

Class discussion

1. Ask students what force that makes the molecules move in gel electrophoresis. Students should identify electrical pull of the negative and positive electrodes as the force that causes the positively and negatively charged dye molecules to travel within the gel.
2. Ask students to describe the function of the buffer solution in the process of electrophoresis. Student answers should explain that the buffer contains the ions necessary to provide conduction, and keeps the pH at a constant level. Some students may be able to identify that constant pH is particularly important for DNA separation, as a change in acidity may change the shape of the molecule, affecting its run.
3. Ask students why the comb is placed in the middle of the gel in this procedure. In this case, the comb is placed in the middle to allow room for the negatively and positively charged dyes to move in opposite directions.
4. Ask students to consider why two dyes that are the same size travel different distances after the gel has run. Dyes with a double charge, will have an electrical pull that is much stronger than a single charge.
5. Ask students to provide reasons why the same dye molecule might move at different distances when run in two different gel electrophoresis experiments. This variation may be the result of variations in buffer concentration or even volume; variations in gel composition; different power settings; different running time.

EXTENSION EXERCISE

Calculating individual molecules

A great extension exercise for this practical, is to ask students to calculate the number of individual molecules in the 10 µL of volumes of dyes in the wells. We recommend tasking students with calculating only 1-2 dyes, as it can be a time-consuming activity. Instruct students to use the molecular weight and weight per volume concentration of the dye to calculate the approximate number of total molecules placed in the well for Orange G. Students will use Avogadro's number, 6.022 x 1023 (the number of molecules in a mole) to calculate the answer.

Dye = 0.2% w/v => mass (m) = 0.02 µg in 10 µL

For Orange G, molecular mass (M) ~ 450 g/mol (approximate mass will be enough for this exercise)
Number of moles (n) = m/M
n = (0.02 x 10-6) / 450 = 4.44 x 10-11 mol
Number of molecules = 4.44 x 10-11 mol x 6.022 x 1023 = 2.7 x 1013 molecules

For Methyl Green, molecular mass (M) ~ 650 g/mol
Number of moles (n) = m/M
n = (0.02 x 10-6) / 650 = 3.07 x 10-11 mol
Number of molecules = 3.07 x 10-11 mol x 6.022 x 1023 = 1.8 x 1013 molecules

For Xylene Cyanol, molecular mass (M) ~ 540 g/mol
Number of moles (n) = m/M
n = (0.02 x 10-6) / 540 = 3.7 x 10-11 mol
Number of molecules = 3.7 x 10-11 mol x 6.022 x 1023 = 2.2 x 1013 molecules

For Bromophenol Blue, molecular mass (M) ~ 670 g/mol
Number of moles (n) = m/M
n = (0.02 x 10-6) / 670 = 2.98 x 10-11 mol
Number of molecules = 2.98 x 10-11 mol x 6.022 x 1023 = 1.8 x 1013 molecules