a WOW Lab BLUEPRINT

Gel Electrophoresis

Activity Instructions

All of the following steps can be performed by the students.

Part I - Building the Chamber

The following items will be needed for this part of the activity:

per group:

- stainless steel wire
- wire cutters
- 500 mL square plastic container with lid
- four 9 V batteries

Step 1

Using the wire cutters, cut two pieces (25 cm in length) of the stainless steel wire.

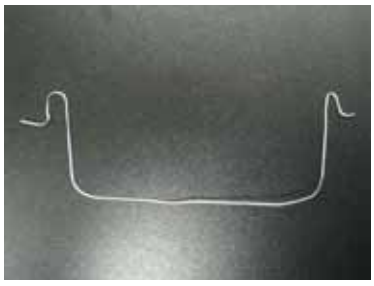

Figure 1

Step 2

Bend both wires so that they hook over the sides of the square plastic container and run along the width of the container just above the base. See **figure 1** for an example of the bent wire. Take the wires out of the plastic container and set them aside.

Step 3

Connect all four 9 V batteries together in series by snapping the positive terminal of one battery into the negative terminal of another (**Figure 2)**. There should be a positive and a negative terminal exposed. Set the batteries aside until later.

Figure 2

Part II - Setting up the Agar Gel

The following items will be needed for this part of the activity:

per group:

- styrofoam comb (see *Prep Instructions*)
- square plastic container
- gloves
- 500 mL beaker
- bottled water
- baking soda
- graduated cylinder (100 mL)
- 250 mL beaker
- agar powder
- stir stick
- craft knife

per class:

- microwave
- balance
- masking tape

Figure 3

Step 1

Place the styrofoam comb vertically into the plastic container. Make sure that there is a small gap between the bottom of the teeth and the bottom of the container (approximately 0.3 cm). A piece of paper should easily slide under the teeth. **Figure 3** is shown as a guide. Tape the styrofoam comb in place. Put on gloves before continuing.

Step 2

In a 500 mL beaker, mix 200 mL of bottled water with 2 g of baking soda. Stir well to create a 1% buffer solution.

Figure 4

Step 3

Using a graduated cylinder, measure 100 mL of the buffer solution and pour it into a 250 mL beaker. Add 0.5 g of agar powder to the buffer solution and mix well. Save the other 100 mL of buffer solution for later (Part III Step 6).

Step 4

Using a microwave, heat the agar solution for 15 seconds at a time for about 1 1/2 minutes (time may vary depending on the microwave). Stir after each 15 second period. When the agar solution begins to bubble (**Figure 4**), stop the microwave and let the agar cool for a minute. Carefully remove the beaker from the microwave. The agar solution should be transparent.

Step 5

Pour the agar into the corner of the plastic container until the level of the agar solution reaches the line on the middle tooth of the comb (**Figure 5**). Not all of the agar solution will be used. If the gel is too thick, it will be difficult to achieve good separation of the coloured samples. Adjust the styrofoam comb if it was moved when the agar was poured. Ensure that the comb is oriented vertically and is not tilted (**Figure 6**).

Figure 5

Figure 6

Step 6

It is important not to touch the gel while it solidifies. Wait approximately 15 minutes before touching the container again. After 15 minutes, carefully tap the side of the container to make sure the gel is firm. Slowly and carefully wiggle the comb out of the gel.

Step 7

Using a craft knife, estimate and carefully cut 0.5 cm above the line of the sample wells (**figure 7**) and 5 cm from the bottom of the edge of the container (**Figure 8**). Remove and dispose of the end pieces of the agar. It is possible to remove more of the gel from the bottom if deemed necessary. The final gel should resemble **figure 9**.

Figure 7

Figure 8

Figure 9

Part III - Running the Gel Electrophoresis

The following items will be needed for this part of the activity:

per group:

- coffee filter
- six prepared vials (see *Prep Instructions*)
- lid of plastic container
- scissors
- tweezers
- prepared agar gel (see Part II)
- 2 bent wires (see Part I)
- masking tape
- alligator clip leads
- connected batteries (see Part I)
- 100 mL of buffer solution (see Part II Step 3)

Step 1

Cut six 4 cm by 0.5 cm strips from the coffee filter. Label the strips "C" for control, "CS" for crime scene and 1 to 4 for the suspects. Match the labelled coffee filter strips with their vials.

Figure 10

Step 2

Touch the tip of each coffee filter strip to the liquid inside the corresponding vial for three seconds. **Figure 10** shows approximately how much the coffee filter will absorb. Place the strips on the lid of the plastic container and let them dry for one minute.

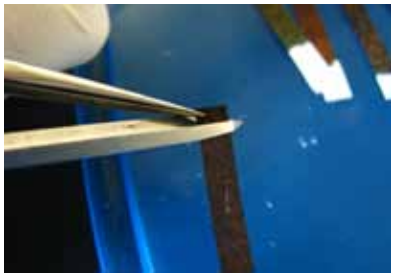

Figure 11

Figure 12

Step 3

Use the scissors and the tweezers to cut off a piece of filter paper approximately 0.3 cm wide (**Figure 11**). **Figure 12** shows the small sample pieces in line with the strips that they were cut from.

Step 4

Use tweezers to place each small piece of filter paper into the corresponding sample wells in the agar (**Figure 13**). Make sure that they are pressed against the front of the sample wells. Write down the order that the strips were placed in the agar gel.

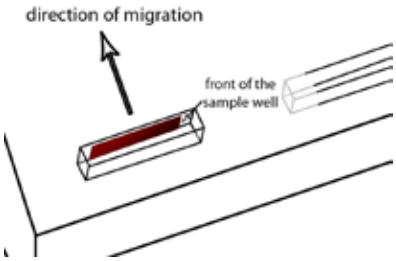

Figure 13

Step 5

Place the two wires in the plastic container, one at the top of the gel and one at the bottom (**Figure 14**). Tape the wires to the container.

Step 6

Attach the alligator clip leads to the stainless steel wires. Connect the positive terminal of the battery pack to the wire that the food coloring will migrate toward (the bottom wire). Connect the other wire to the negative terminal of the battery pack. Do not touch the opposite terminals of the alligator clips together—this will complete the circuit and cause a shock. If the circuit is set up correctly, there should be larger bubbles produced along the positive terminal wire (bottom wire) and smaller bubbles produced around the negative terminal wire (top wire). **Figure 15** shows the larger bubbles on the positive terminal wire.

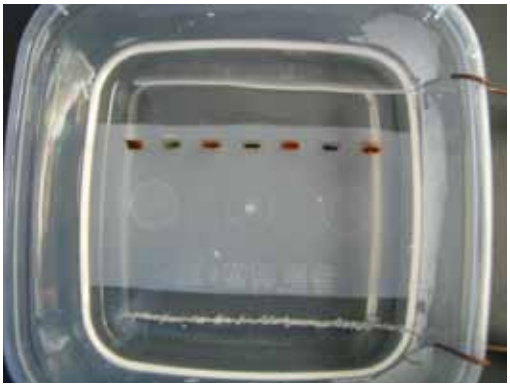

Figure 14

Figure 15

Step 7

Pour the 100 mL of buffer solution (from Part II Step 3) in a corner of the container, as this will reduce the chance of damaging the gel set-up. Do not knock the container or touch the gel once the buffer is added since any movement will disrupt the activity.

Step 8

Carefully place the lid of the container on top of the gel set-up so that most of the system is covered.

Step 9

Run the gel for 20 minutes, checking its progress every five minutes. The dyes should migrate towards the bottom and separate into different colours. It may look like the food colouring is travelling backwards in the buffer solution, but it is the migration of the food colouring in the agar gel that should be the focus.

Part IV - Analyzing the Results

The following items will be needed for this part of the activity:

per group:

- gel set-up (see Part III)
- rulers
- pencil crayons (red, yellow and blue)

Step 1

Disconnect the alligator clip leads from the stainless steel wires. Remove the wires from the plastic container and set them aside.

Step 2

Carefully discard the buffer solution while gently holding the agar gel in place against the container. Be careful as the agar gel is slippery. Place the agar gel on the lid of the plastic container.

Step 3

Line up the zero line of a ruler with the front edge of the sample wells on either side of the gel. Measure the distance from the front of the sample wells to the middle of each coloured band. Note the intensity of the colours using a five star system where 1 = barely visible and 5 = extremely intense.

Step 4

The gel cannot be stored as the food colouring will disperse through the gel in the absence of current. Draw the gel using pencil crayons to denote the colours of the bands and their intensity.

Step 5

Determine which colours of food colouring were used in each sample and estimate the quantities used. Determine which suspect matches the crime scene sample and explain why.