USING GEL ELECTROPHORESIS (AGAROSE GELS) TO SOLVE A CRIME

A high school cheerleader, Caroline, was missing after the basketball game and later found dead in her boyfriend's car. Although Caroline's boyfriend, Brian, claimed that he was innocent, police immediately arrested him. The crime scene investigator (CSI) found a small drop of blood on the passenger seat of Brian's car. From the camera footage taken in the school's parking lot, Caroline was last seen with Brian in his car. However, police also discovered that a vehicle was following them after they left the parking lot. Based on what they saw on camera, police interviewed the drivers of both vehicles: Brian and the driver of the vehicle who was following them, Caroline's long-time friend Lauren. The time gap between them leaving the school and the discovery of the cheerleader's body made them the last people with her and therefore possible suspects. The CSI determined that Brian's car was not the first crime scene, meaning that Caroline was killed in another place then transferred to his car. To make matters worse, both of the suspects had motives.

The only way to find out who actually did it would be by comparing the DNA sample from the blood drop left in the car to the DNA of each of the suspects and Caroline. The CSI collected DNA samples from each person for testing. In this scenario, you are the investigator who will conduct an experiment using a technique called gel electrophoresis. Your task is to identify the suspect who committed the crime. The sample that matches best with the unknown sample will be the primary suspect, and this person will be charged with the crime.

Part I: Preparing the Samples for Gel Electrophoresis

1. Pick up solutions CS, V, S1, and S2 from your teacher.
2. Label 4 tubes 1, 2, 3 and 4 and add 3µL DI water to each tube
3. Mix solution CS by shaking the tube a couple of times. Then, using a clean tip, transfer 12µL of the solution to tube 1.
4. Mix solution V by shaking the tube a couple of times. Then, using a clean tip, transfer 12µL of the solution to tube 2.
5. Mix solution S1 by shaking the tube a couple of times. Then, using a clean tip, transfer 12µL of the solution to tube 3.
6. Mix solution S2 by shaking the tube a couple of times. Then, using a clean tip, transfer 12µL of the solution to tube 4.
7. The following table summarizes the contents of each tube

Data Table

Tube	Sample name	Amount of solution CS, V, S1 or S2	DI water	Total
1	Crime Scene (CS)	12µL	3µL	15µL
2	Victim (V)(Caroline)	12µL	3µL	15µL
3	Suspect 1 (S1)(Brian)	12µL	3µL	15µL
4	Suspect 2 (S2)(Lauren)	12µL	3µL	15µL

Part II: Standard Operating Procedure to Perform Gel Electrophoresis

A. Preparing the Gel Tray to Pour the Gel

1. Lower the ends on the gel tray and place it into the electrophoresis chamber. Make sure the wells of the gel are located toward the negative (black) electrode. This will ensure that the samples move toward the positive (red) electrode when they are charged.
2. Fill the chamber with 1X SB buffer solution to a level that just covers the entire surface of the gel to a depth of 1-2 mm. The gel should be completely submerged under the buffer and wells should be filled with buffer as well.

B. Loading the Samples

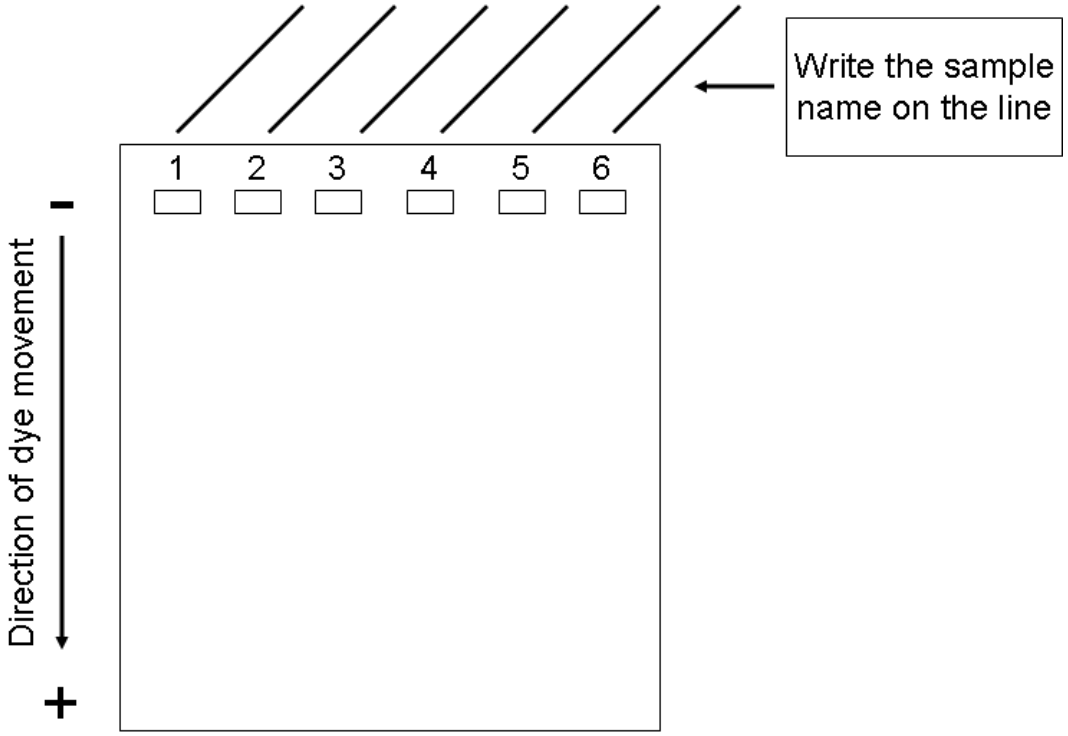
3. If necessary, spin tubes 1, 2, 3 and 4 in the micro centrifuge to pull all the solution to the bottom of the tubes.
4. Set the micropipette to 15µL and load each sample into separate wells. Use a fresh tip for each sample to avoid contamination. When loading each sample, center the pipette tip over the well and slowly expel the sample. Use your other hand to support your pipette hand to avoid shaking. The samples will sink into the bottom of the wells because they have a greater density than SB buffer.
5. Remember to record which sample you load into each well

C. Performing Gel Electrophoresis

6. Tightly close the cover over the electrophoresis chamber. Connect the electrical leads to the power supply.
7. Turn on the power supply and set the voltage to 150~155V.
8. You may observe the movements of the dyes to make certain that they are moving toward the positive electrode. You should be able to see the separation of the dye. Do not open the cover of the chamber while the power supply is on.
9. After 20~30 minutes you should be able to distinguish all the dyes. If not you may let it run longer to get a better separation. Turn off the power supply and unplug the electrodes from the power supply. Carefully remove the cover from the chamber so that you can observe the dyes in the gel. Leave the gel in the chamber while observing the dyes and answer the questions in the conclusions section.
10. Keep your gel for the second part of the lab, using gel electrophoresis to make a standard curve.

Part III: Analyzing Results

1. On the diagram below, record the banding or color pattern in each of the lanes containing your samples or paste a picture of you gel here.



2. According to the results from your gel, who killed the cheerleader? How did you reach this conclusion?

3. After loading your gel, did any solution remain in tubes 1, 2, 3, and/or 4? What could account for solution remaining in these tubes?

4. What electrical charge did the dyes that you loaded on your gel carry? How do you know?

5. Just for fun: Why do you think the murderer killed the cheerleader?

Part IV: Using Gel Electrophoresis to make a Standard Curve (Performance Assessment)

6. Observe the colored bands on your gel or gel picture.
7. Locate the lane that Suspect 2's sample was loaded in. There should be 3 distinct bands in this lane.
8. These bands correspond to the dyes xylene cyanol (blue), bromophenol blue (purple) and orange G (orange).
9. Each band traveled a certain distance in the 1/2 % agarose gel based on the base pair size equivalent of each dye.
10. By measuring the distance each band travelled and knowing the base pair equivalent (see data table on the next page) you can use this information to construct a standard curve.

    *A **standard curve** is a plot of known x and y values. By generating a "best fit" line from the known values you can determine the value of an Unknown.*

11. To measure the distance each band in the S2 lane, obtain a ruler that has metric measurements (mm and cm).
12. Place the ruler on the gel or the picture of the gel so that the zero mark is at the bottom of the well in the S2 lane.
13. **For the S2 lane only**, record the distance that each band traveled from the bottom of the well to the bottom of each band.
14. Record the measurement for each dye in the "distance from origin" column in the appropriate row of the data table on the next page.
15. You are now ready to construct your standard curve.
16. Using your data table and the graph on the next page, find the base pair equivalent on the y-axis and the distance migrated on the x-axis for xylene cyanol (blue).
17. Place a dot on the graph where these two values intersect.
18. Do the same for the bromophenol blue (purple) and the orange G (orange).
19. You should have three distinct dots on your graph.
20. Use your ruler to draw a "best fit" line thorough the dots. Continue the line through the X and Y axis.
21. This line is your standard curve.

22. You can now use your standard curve to determine the size (base pair equivalent) of the unknown band.
23. Measure the distance that the unknown band traveled. This band is in the S1 lane.
24. Repeat steps 12 and 13 above to measure the distance that the unknown band traveled.
25. Record this measurement in the "distance from origin" column in the data table below.
26. Look at your data table and identify the distance that the unknown band traveled.
27. On the x-axis of your graph, find this number.
28. Draw a dashed line from this number on the x-axis until you touch the line of your standard curve.
29. Once you touch the line continue the dashed line horizontally in the direction of the y-axis until you touch the y-axis.
30. The place where the dashed line touches the y-axis is your answer, which is the measurement that corresponds to the base pair equivalent of the unknown.
31. Identify this value on your graph, label it "unknown" on the graph and record the number in your data table.

Data Table

Dye	Lane	Color	Base pair equivalent	Distance from origin
Xylene Cyanol	S2	Blue	2800	
Bromophenol Blue	S2	Purple	250	
Orange G	S2	Orange	70	
Unknown	S1	Yellow		

Use the table to construct a standard curve and determine the unknown on the log graph below