Dye/Indicator Lab

**Separation of Molecules Using Agarose Gel Electrophoresis**

**Objectives:**

You will be able to:

* Make and load the wells of an agarose gell
* Electrophorese samples in the gel and interpret the results
* Identify and manipulate variables involved in separating biological dyes & pH indicators
* Design and carry out a simple electrophoresis experiment
* Explain the theory behind separation of molecules by electrophoresis

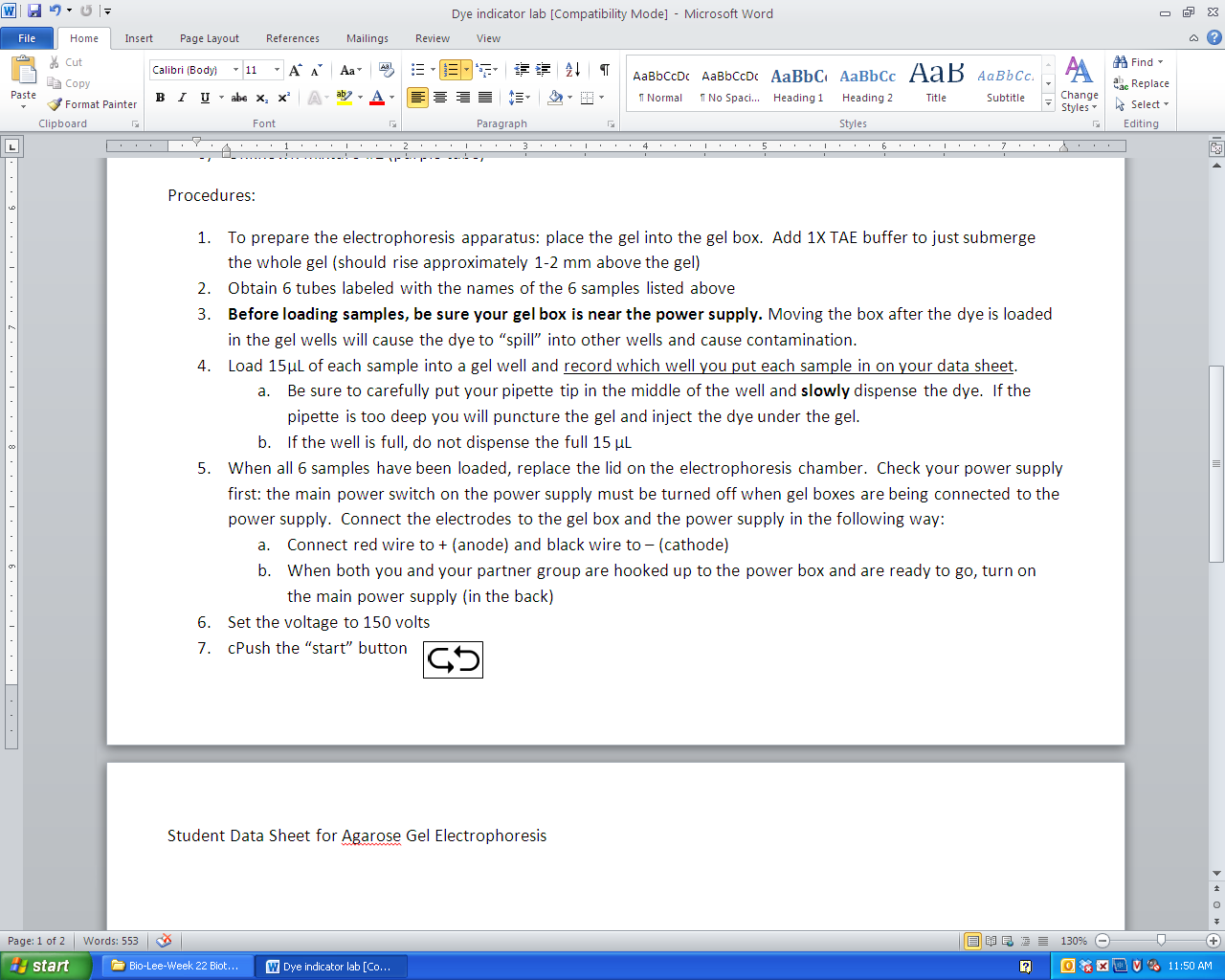
**Materials (for a lab group of 4):**

* 1 electrophoresis chamber
* 0.8% Agarose gel (prepared)
* Micropipette
* 1 Plastic overhead square
* Power supply (shared with other groups)
* 1X TAE buffer
* 1 Dye samples pack
* 1 ruler (mm)
* 1 beaker for used tips
* Micropipette tips
* Permanent marker
* Microtube rack

**Samples to run:**

|  |  |  |
| --- | --- | --- |
| **Well** | **Color of microcentrifuge tube** | **Sample** |
| Well 1 | Orange | Orange G (A) |
| Well 2 | Blue | Bromophenol blue (B) |
| Well 3 | Red | Xylene cyanol (C) |
| Well 4 | Green | Methyl green (D) |
| Well 5 | Yellow | Unknown mixture #1 |
| Well 6 | Purple | Unknown mixture #2 |

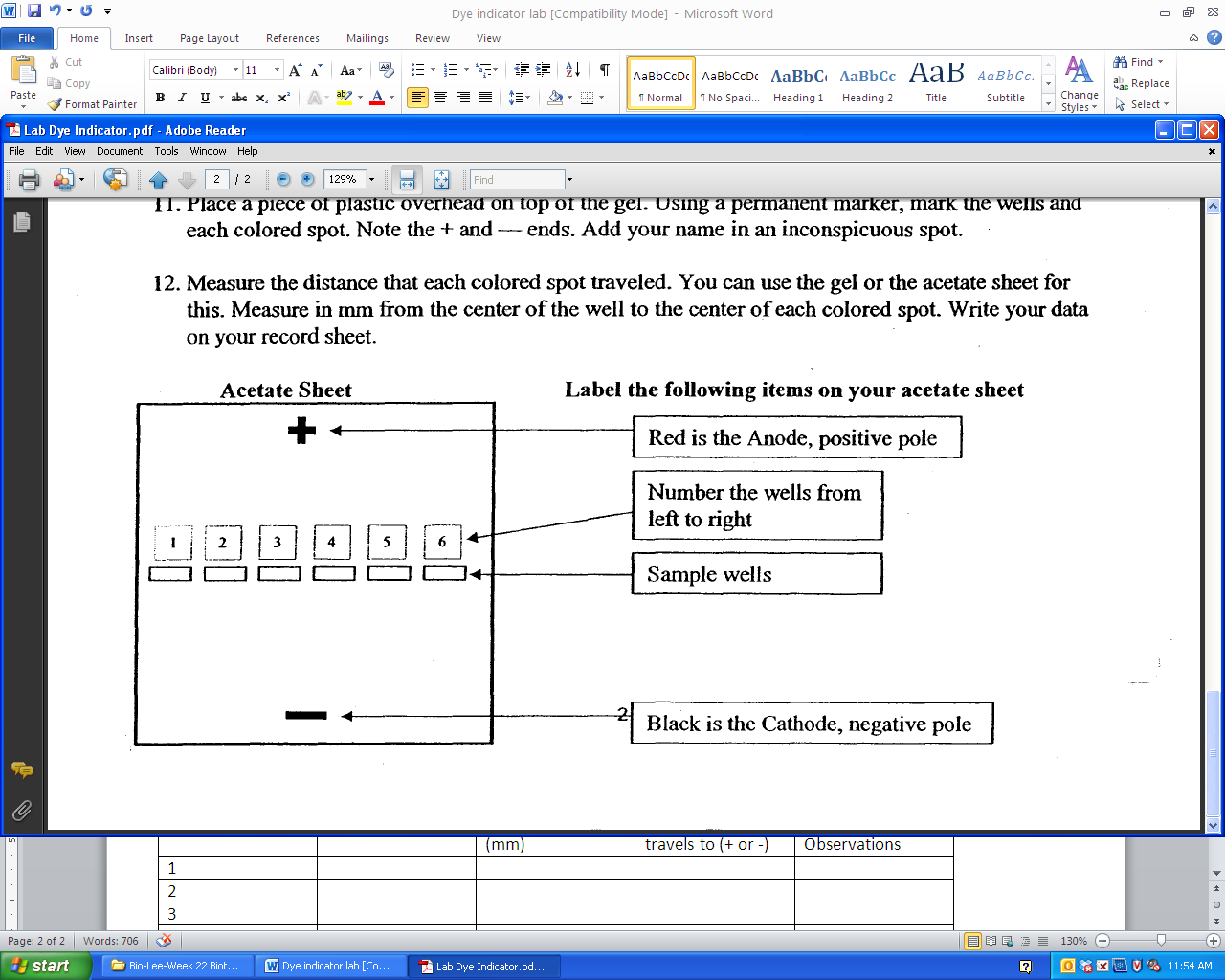
**Procedures:**

1. To prepare the electrophoresis apparatus: place the gel into the electrophoresis chamber. Add 1X TAE buffer to just submerge the whole gel (should rise approximately 1-2 mm above the gel)
2. Obtain 6 tubes color-coded for the 6 samples above
3. **Before loading samples, be sure your gel box is near the power supply.** Moving the box after the dye is loaded in the gel wells will cause the dye to “spill” into other wells and cause contamination.
4. Load 15µL of each sample into a gel well and record which well you put each sample in on your data sheet.
   1. Be sure to carefully put your pipette tip in the middle of the well and **slowly** dispense the dye. If the pipette is too deep you will puncture the gel and inject the dye under the gel.
   2. A new tip should be used for each sample
   3. If the well is full, do not dispense the full 15 µL
5. When all 6 samples have been loaded, replace the lid on the electrophoresis chamber. Check your power supply first: the main power switch on the power supply must be turned off when gel boxes are being connected to the power supply. Connect the electrodes to the gel box and the power supply in the following way:
   1. Connect red wire to + (anode) and black wire to – (cathode)
   2. When both you and your partner group are hooked up to the power box and are ready to go, turn on the main power supply (in the back)
6. Set the voltage to 150 volts
7. Push the “start” button and record the start time on your data sheet
8. Allow your gel to run for a total of 20 minutes and press the ‘start” button again to stop the power. Then turn off the main power switch on the back.
9. Unplug the electrodes and open the gel box. Lift out the gel deck. Slide the gel directly onto a piece of plastic wrap or paper towel and dab off the excess buffer.

(continued on back)

1. Obtain a piece of plastic overhead sheet from the front of the classroom. Place it on top of the gel. Using a permanent marker, mark the wells and each colored spot. Note the + and – ends. Add your name in an inconspicuous spot.

Ex:



1. Measure the distance that each sample mixture traveled. You can use the gel or the acetate sheet to do this. Measure in **mm** from the center of the well to the center of each colored spot. Record your data on your data sheet.
2. To clean up, dispose of your gel in the flask labeled “used gel”, make sure all materials are as you found them at the beginning of the lab (in their proper location on the place mat). Double check to make sure power source is off and that everything is unplugged.

\*\* Once your lab station is clean, please return to your seat and begin answering the analysis questions.

Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Period: \_\_\_\_\_\_ DUE Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Student Data Sheet for Dye/Indicator Lab**

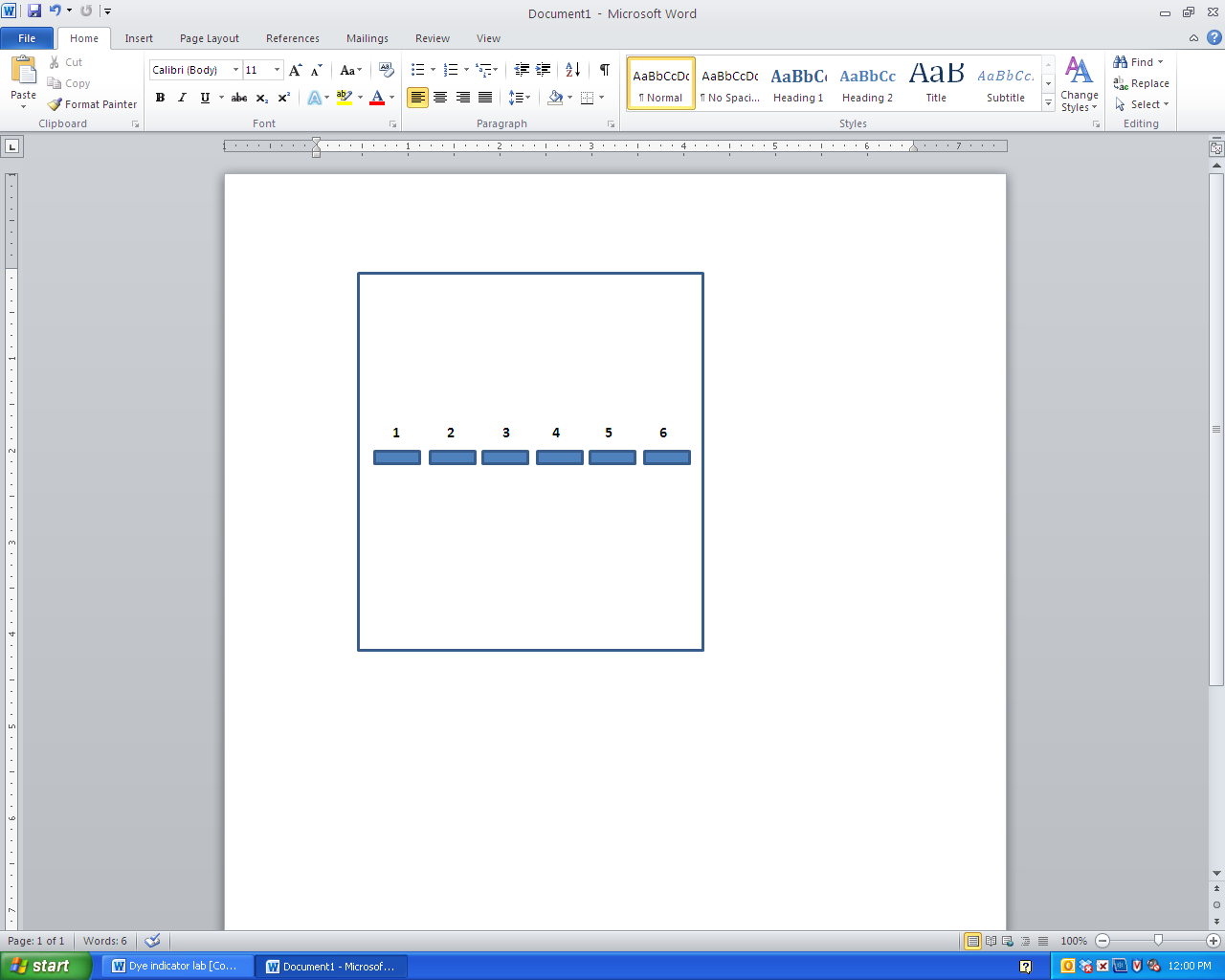
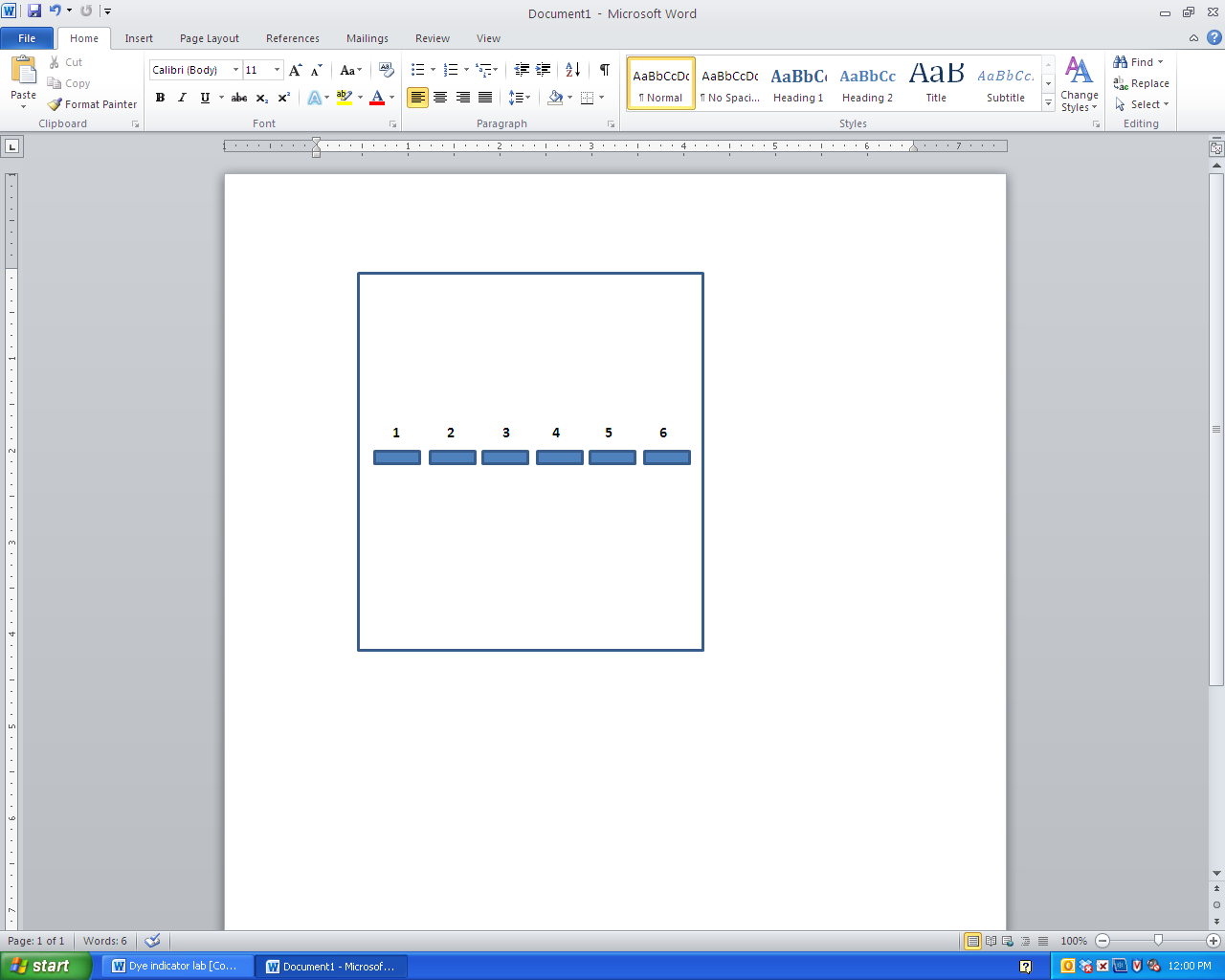
**Before Running Gel**

**After Running Gel**

**Diagrams:**

**+ (red)**

**+ (red)**



Start Time: \_\_\_\_\_\_\_\_\_\_

Stop Time: \_\_\_\_\_\_\_\_\_\_

**- (black)**

**- (black)**

**Data Collection:**

Observations of the gel after 20 minutes of electrophoresis:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Well # | Sample Name | Distance Traveled (mm) | Pole that DNA travels to (+ or -) | Comments & Observations |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |
| 4 |  |  |  |  |
| 5 |  |  |  |  |
| 6 |  |  |  |  |

**Data Analysis (please answer, using complete sentences, on a separate sheet of paper and staple to this handout):**

1. Unknown #1 and unknown #2 are mixtures of the different dyes.

a. What dye(s) are in unknown #1? What evidence do you have to support your answer?

b. What dye(s) are in unknown #2? What evidence do you have to support your answer?

1. By what properties does gel electrophoresis separate molecules?
2. Why do some dyes move faster/further than others?
3. If you were asked to improve the separation of these molecules, what are some of the variables you could modify in your own experiment?
4. Write two questions you have about this process and the results. Indicate how you might generate answers to these questions through experimentation.