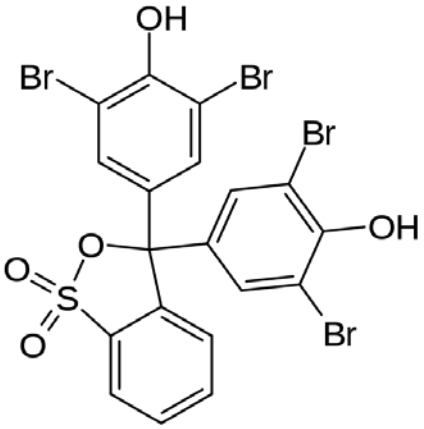
Gel Electrophoresis Run Prediction with Dyes

1) Intro

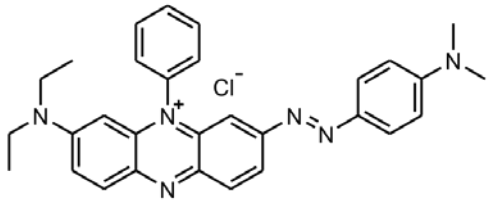
This experiment will introduce students to gel electrophoresis and have students make prediction based on what they will learn. Electrophoresis is an analyzing technique that separates particles based on both size and charge. Negative particles will move towards the anode (negatively charged), or the black end. Positive particles will travel towards the cathode, or the red end. The smaller the particle, the farther it will travel across the gel. Even if two particles are mixed, their own individual travel paths will not be affected. This makes gel electrophoresis a great tool for identifying mystery DNA—it is the technique often used for forensic data analysis like DNA fingerprinting.

Students will be shown the structure of the 4 particles they will be electrophoresing, and asked to make predictions about its course of travel—namely, will it travel towards the cathode or the anode, and what will be the relative distances traveled?

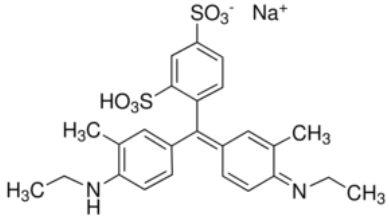
Bromophenol Blue



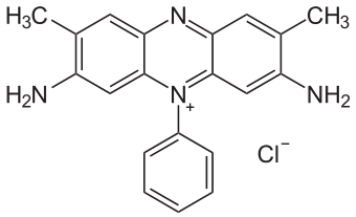
Janus Green



Safranin O



Xylene Cyanol



The molecular weights are:

1) Bromophenol Blue: 669.96 g/mol
2) Janus Green: 511.06 g/mol
3) Safranin O: 350.84 g/mol
4) Xylene Cyanol: 538.61 g/mol

2) Method

Making the Gel

1) Measure out 0.7 grams of agarose and place into a 250ml beaker. This will be enough for about 3 gels.
2) Add 100 ml of 1X TBE buffer.
3) Heat in the microwave for 30-second intervals. Take it out and swirl the beaker every 30 seconds. Do this until the agarose is completely dissolved.
4) Allow the agarose to cool for about 3-5 minutes, or until the beaker is still very warm but not scalding.
5) Pour the agarose mixture into the gel block, until it is about ½ of the way up the teeth of the comb. Make sure the comb does not move. Allow this to sit and solidify, typically around 30 minutes.
6) After the gel is solid, gently remove the comb by pulling straight up gently out of the gel, and then remove the black rubber stoppers.

Making the dyes

1) All the solid dyes must be mixed, but Xylene Cyanol can just be used directly in the experiment.
2) For the other dyes (Bromophenol Blue, Janus Green, and Safranin O), do the following:
    a. Take 25 mg of dye
    b. Add 4 grams of sugar (I used 98% D-Fructose)
    c. Make up to 10 ml with distilled water
    d. Disperse volumes up to .5 ml of each dye into separate centrifuge tubes
    e. Make several mixtures of dyes in a 1:1 mixture

Procedure for gel electrophoresis

1) Once the gel has solidified and the comb and black stoppers have been removed, pour about 150 mL of TBE buffer into the unit. The gel should be completely covered.
2) Use the 10 μL pipettes to add 10 μL of each of the dyes/dye mixtures into the wells.
3) Once all 8 wells are filled, place the lid on top of the gel box.
4) Plug in the two electrodes into the power supply.
5) Switch the power supply on, press the green start button, and then ensure that the voltage goes to 100V.
6) Allow to run for about 30 minutes, keeping an eye on the apparatus to be sure that the dyes do not run off the gel and that the voltage remains at 100V. The dyes should be about 1 cm from the edge of the gel at the end of the run.
7) After the run is complete, turn off the power supply.
8) Remove lid
9) Remove gel, and look at each of the lanes.
10) Using the given information and by looking at the gel run, try to deduce which mystery dyes were in which lanes.

Results

1) Safarin O travels the farthest, and travels towards the anode
2) Janus Green travels towards the anode, but only moves a little bit
3) Bomophenol Blue moves towards the cathode and travels slightly less than Xylene Cyanol.
4) Xylene Cyanol travels towards the cathode and moves far down the gel

These results are the expected outcomes.

Helpful Hints

The agarose gel should be made ahead of time, because it is a hazardous material and also takes a while to prepare. You can make one mixture of it, keeping with the 0.7% ratio, and it will keep indefinitely if kept at around 4 °C. If kept at room temp, it should be used in two weeks.

The dyes will keep for a long time, so make a master mix and store it in the dark and hand out the small centrifuge tubes full of dye to the students.

When running the gel, ensure that bubbles form—this means that electricity is being supplied.

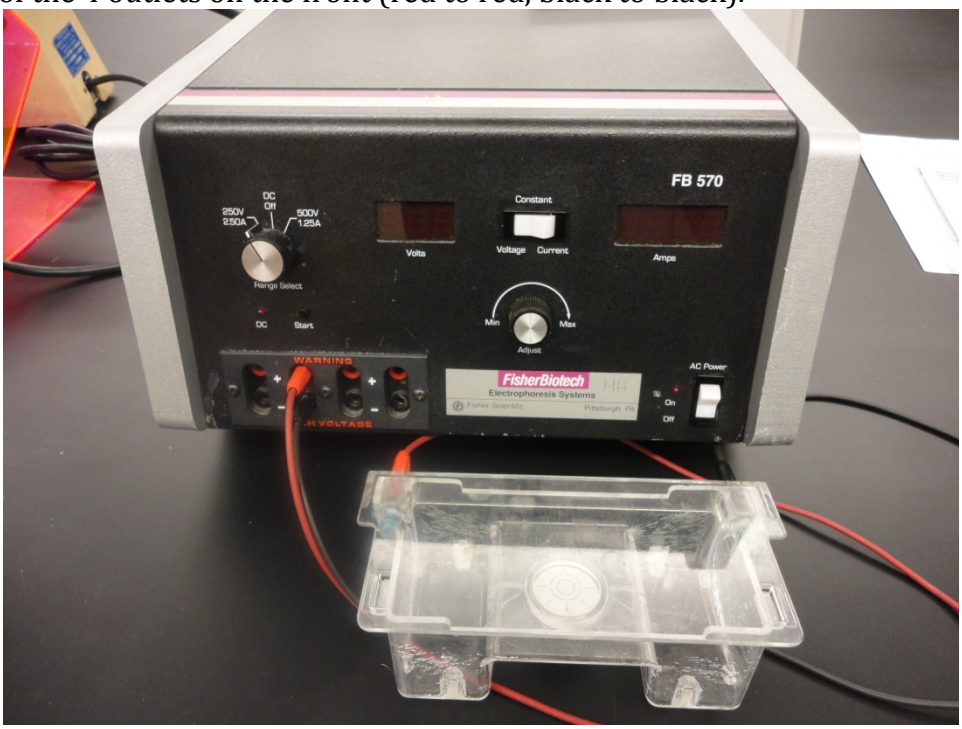
Safety tips:

1) Xylene Cyanol is a skin and eye irritant
2) Janus Green is slightly flammable, and ingestion can cause health effects
3) Bromophenol blue slightly flammable, and ingestion can cause health effects
4) Safranin O can cause some serious health effects if it is ingested
5) Agarose is slightly flammable, can cause effects if ingested, and is an irritant
6) The power supply and the gel apparatus uses high voltage and should be handled with extreme caution. Nothing besides the proper electrodes should be placed into the apparatus, and it should be turned off at all times when not in use
7) Gloves and eye protection should be worn at all times during the procedure

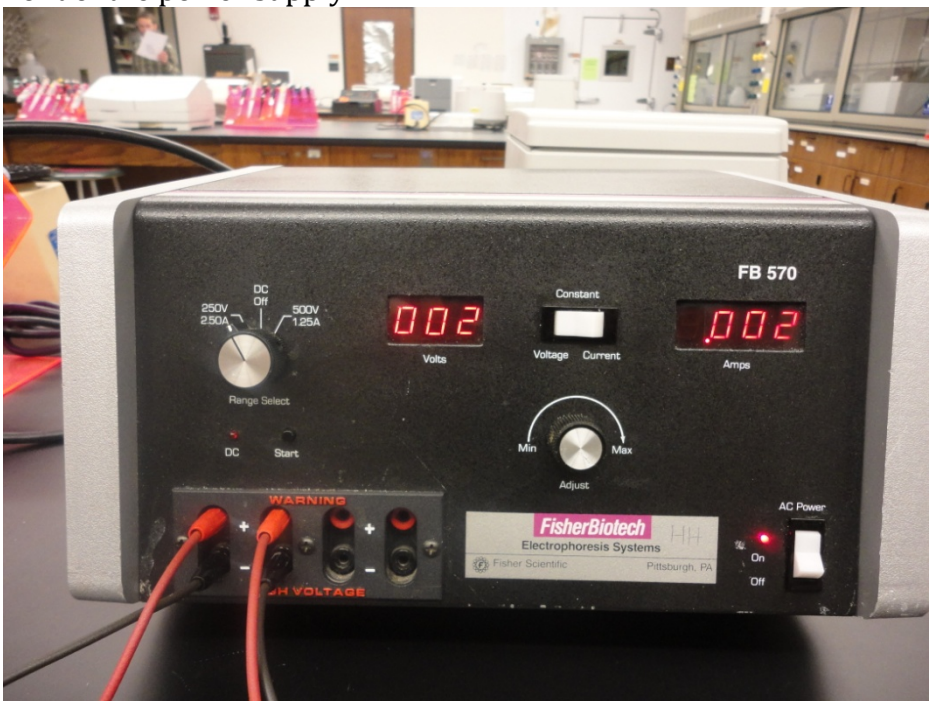
GEL ELECTROPHORESIS RUN PREDICTION WITH DYES

Gel Electrophoresis power supply overview

1) Before making the gel, you will need to set the power supply to the correct voltage, in the case for this experiment it will need to be at 100
2) Take an empty gel tray, put the lid on it, and then plug in the two electrodes into one of the 4 outlets on the front (red to red, black to black).

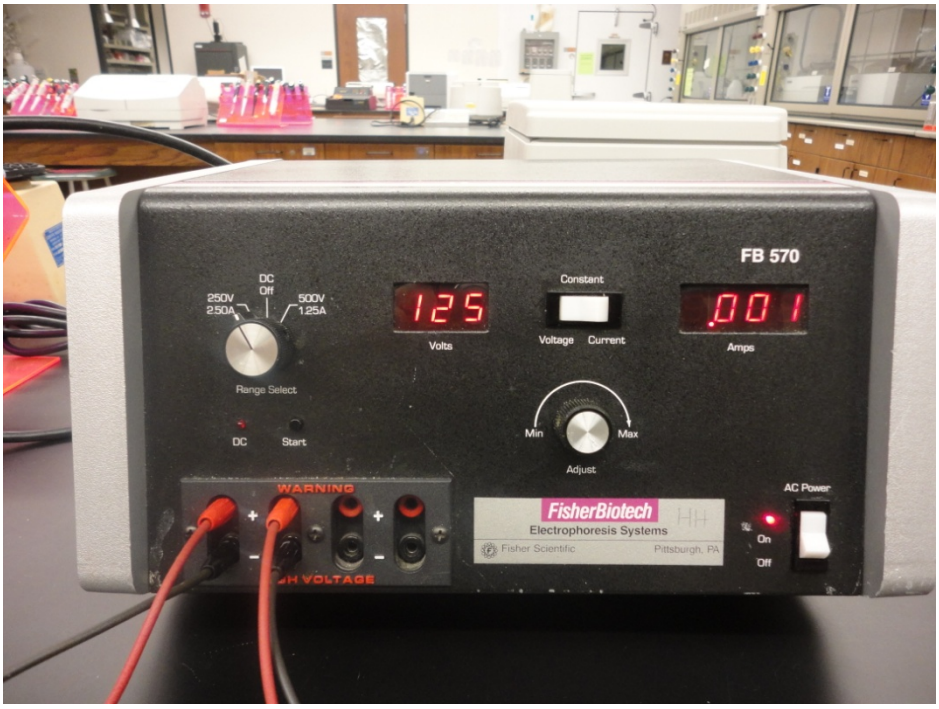


3) To turn the power supply on, flip the switch in the lower right hand corner on the front of the power supply.



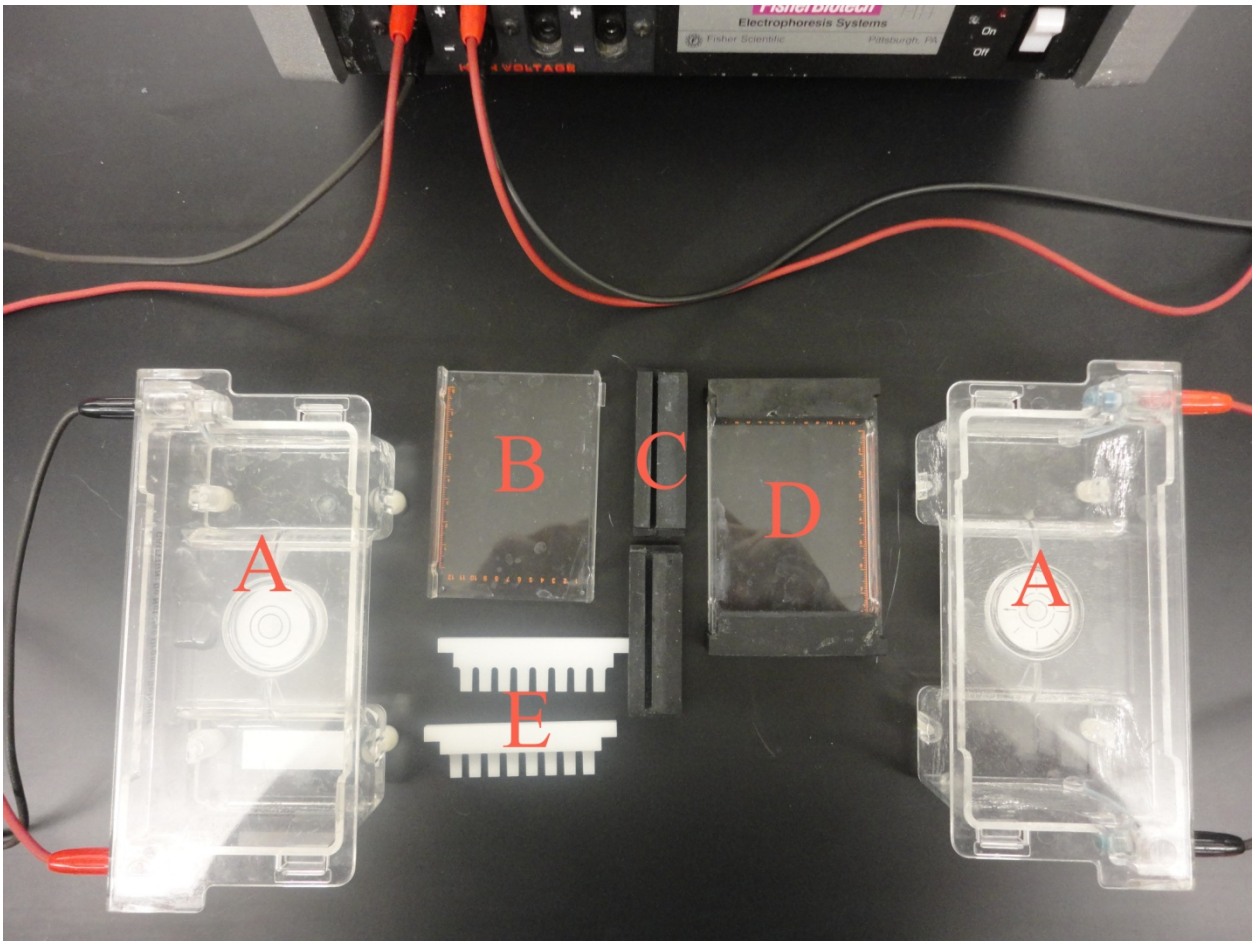
4) Next, push the small green button that says “Start”. Located in the middle left, just below the Voltage range select. Once you do this, after a few second, the voltage will jump up. Now, use the “Adjust” knob in the middle of the power supply to make sure that the voltage is set to 100V. Notice the horizontal white switch in the middle of the two displays that says “Constant”. Ensure that this is always set to Voltage and is

never set to Current.



5) Once you have the voltage set to 100V, turn of the power supply and disconnect the two electrodes. After doing this once, as long as the voltage is not changed by accident, it should be set to a constant 100V.

Supplies for gel electrophoresis run

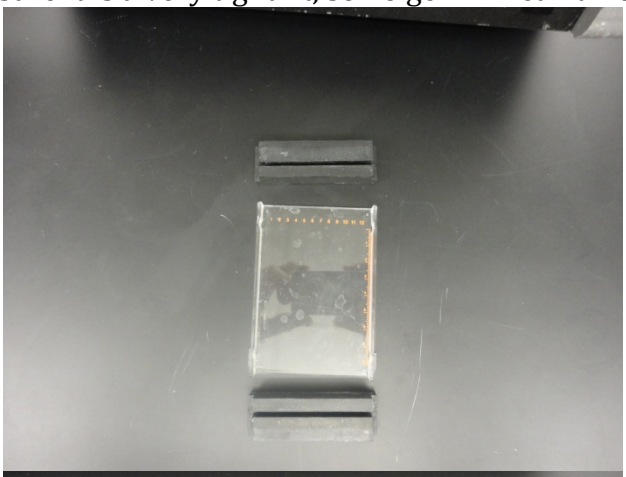


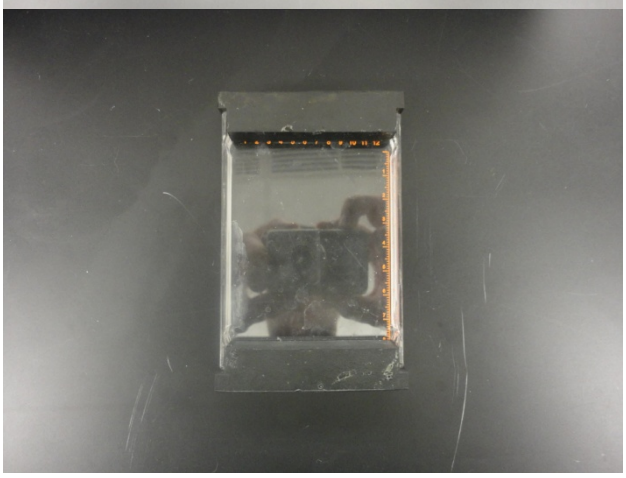
These are the other necessary supplies to run electrophoresis:

A. These are the two gel blocks, with their lids on and electrodes plugged into the power supply.
B. This is the gel block, as it will look when the electrophoresis is running.
C. These are the two black rubber stoppers, used when pouring and setting the gel.
D. This is the gel block and the rubber stoppers assembled and ready to have the gel poured.
E. These are two combs, placed in the gel block when pouring the gel. These are what cause the wells to form in the gel.

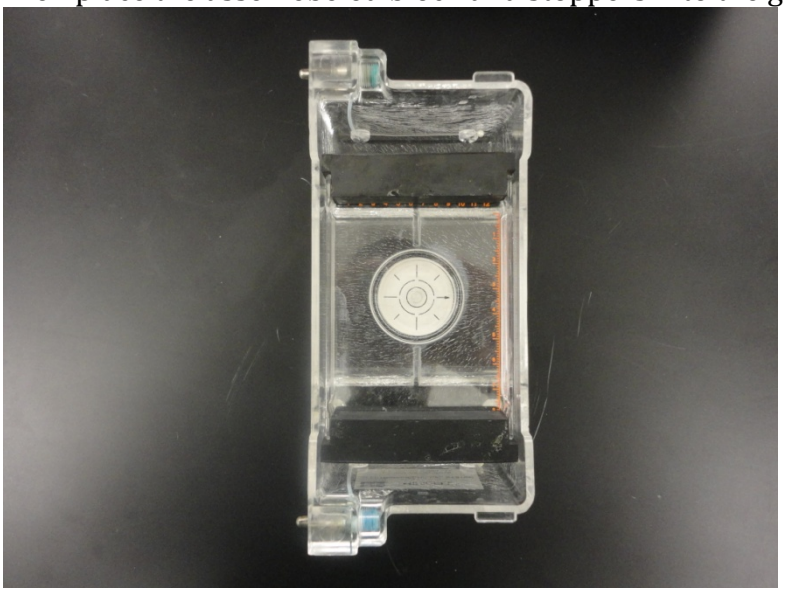
Pouring and making the agarose gel

7) Take the gel block and the two rubber stoppers, and assemble them together. Make sure it is a very tight fit, so no gel will leak through.

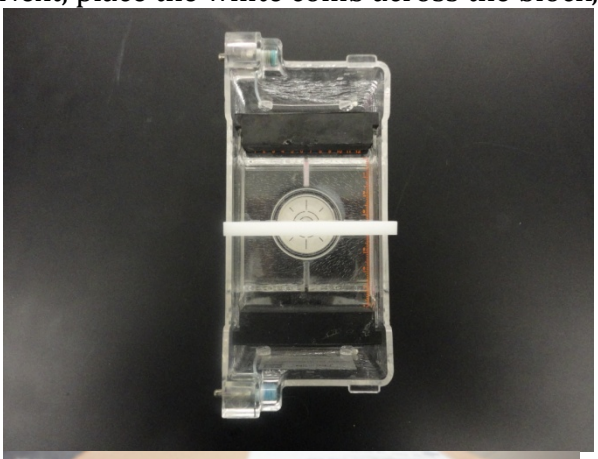


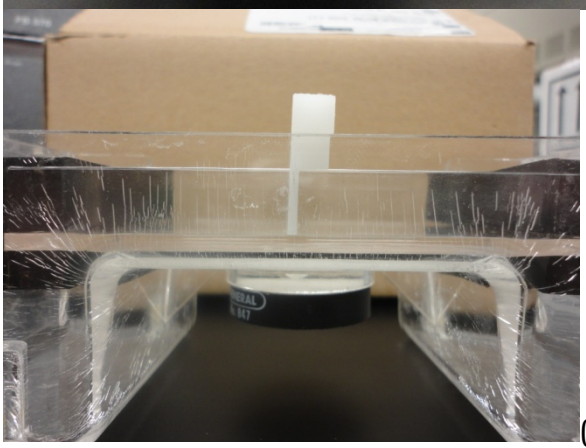


8) Then place the assemebeled block and stoppers into the gel electrophoresis box



9) Next, place the white comb across the block, as close to halfway down as possible





(Side view of comb)

10) Now make the 0.7% agarose gel.
11) Measure out 0.7 grams of agarose and place into a 250ml beaker. This will be enough for about 3 gels.
12) Add 100 ml of 1X TBE buffer.
13) Heat in the microwave for 30-second intervals. Take it out and swirl the beaker every 30 seconds. Do this until the agarose is completely dissolved.
14) Allow the agarose to cool for about 3-5 minutes, or until the beaker is still very warm but not scalding.
15) Pour the agarose mixture into the gel block, until it is about ½ of the way up the teeth of the comb. Make sure the comb does not move. Allow this to sit and solidify, typically around 30 minutes.
16) Cover any left over and keep it in the fridge. To use, simply melt this leftover in the microwave.
17) After the gel is solid, gently remove the comb by pulling straight up gently out of the gel, and then remove the black rubber stoppers.

Making the dyes

3) All the solid dyes must be mixed, but Xylene Cyanol can just be used directly in the experiment.
4) For the other dyes (Bromophenol Blue, Janus Green, and Safranin O), do the following:
    a. Take 25 mg of dye
    b. Add 4 grams of sugar (I used 98% D-Fructose)
    c. Make up to 10 ml with distilled water
    d. Disperse volumes up to .5 ml of each dye into separate centrifuge tubes
    e. Make several mixtures of dyes in a 1:1 mixture

Making the 1X TBE Buffer

1) The TBE buffer given is 10X, so it needs to be diluted
2) Add 900 ml of water for every 100 ml of buffer used

Procedure for gel electrophoresis

11) Once the gel has solidified and the comb and black stoppers have been removed, pour about 150 mL of TBE buffer into the unit. The gel should be completely covered.
12) Use the 10 μL pipettes to add 10 μL of each of the dyes/dye mixtures into the wells.
13) Once all 8 wells are filled, place the lid on top of the gel box.
14) Plug in the two electrodes into the power supply.
15) Switch the power supply on, press the green start button, and then ensure that the voltage goes to 100V.
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17) After the run is complete, turn off the power supply.
18) Remove lid
19) Remove gel, and look at each of the lanes.
20) Using the given information and by looking at the gel run, try to deduce which mystery dyes were in which lanes.

Supply Numbers for Reorder

1) 250 ml beakers: 89044-514
2) Pipette Tips: 53509-130
3) Bromephenol Blue: 97061-690
4) Janus Green: AAA17391-06
5) Safranin O: 97061-954
6) Xylene Cyanol: 97062-406
7) TBE Buffer: EM-8820
8) Microcentrifuge Tubes: 20170-650
9) Weighboats: 89106-764
10) Fructose: 95033-338