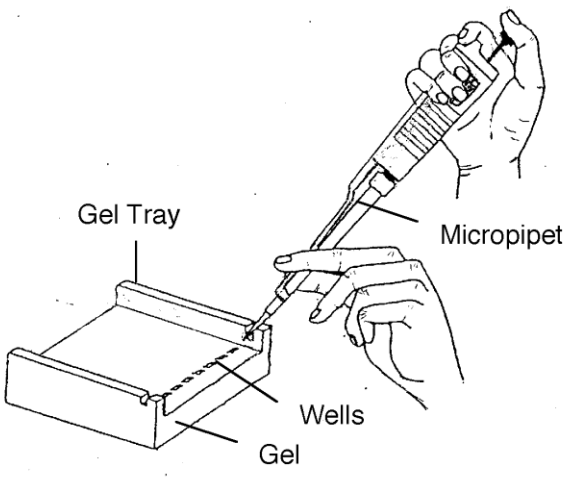
Gel Electrophoresis Lab; SB2f

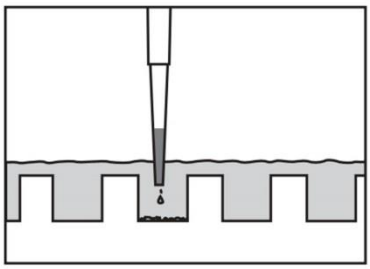
How can a mixture of molecules, too small to be seen with even a high-powered microscope, be separated from one another? This was the dilemma that faced scientists and biotechnologists until the development of a process that has now become standard in many laboratories worldwide- gel electrophoresis. Biotechnologists rely heavily on this proven and reliable technique for separating a wide variety of samples, from DNA used in forensics and paternity tests. Scientists use this process for mapping genes and the human genome. Proteins can even be separated and used to determine evolutionary relations, which are depicted in diagrams called cladograms. This separation technique uses special enzymes, called restriction enzymes that cut up the DNA into smaller pieces of varying sizes. Like all enzymes, these special catalysts are very specific, and thus, only bind and cut the DNA ii specific locations. Because DNA is negatively charged, biotechnologists can then use electricity and some of the basic fundamental laws of physics to separate the macromolecules through an agarose gel matrix, which results in a "genetic barcode" often referred to as a DNA fingerprint. The purpose of this lab is to demonstrate gel electrophoresis using protein samples that vary in their charge, molecular mass and shape and to understand how this process can be used in forensics, medicine and agriculture. Please understand that this is an expensive and complicated process that requires a great amount of time and preparation. Students in the Biotechnology pathway have graciously prepared 0.8 % agarose gels and a 1X tris-acetate buffer solution to make this lab possible to complete in one class period.

Procedure: Please read before you proceed and always ask questions, if you have any doubts about the methods. All of these materials and reagents are very sensitive and expensive. You will also be working in larger groups, as you will only have one electrophoresis chamber per lab bench. Everyone must have an opportunity to pipette samples into the electrophoresis holding wells.

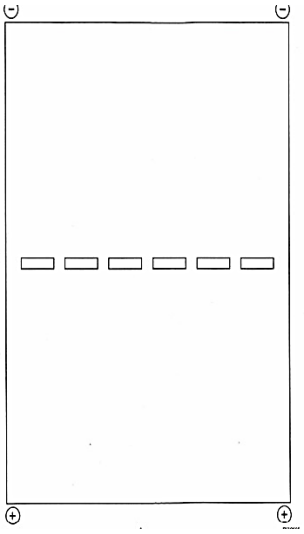
Dye Name	Dye Well Number	Migration Distance (mm)	Migration Direction (+/-)	Dye Molecule Speed Ranking

1). Assemble the gel electrophoresis chamber as modeled by your teacher. 2). Place the chamber in an area free of clutter and in a locations that is accessible for pipetting, but also will not be bumped or disturbed accidently. 3). Place a sheet of white paper on the counter (horizontally) next to or below the chamber. Draw a rectangle representing your gel on the sheet of paper. Number your wells in the rectangle, so you can organize your data. 4). Carefully remove an agarose gel out of the plastic wrap or bag. It will be very slippery. Slide the gel onto the chamber with the opening for the wells pointing upward. I will come around the room to assist your groups as needed. 5). Next slowly pour 1X tris-acetate buffer solution to fill the chamber and barely cover the gel by around 2 millimeters. 6). If you have not already done so, copy down the data table. 7). Label your piece of paper according to which dye sample you are going to put in each well. 7). Practice pipetting 10 µL of food coloring several times to ensure accurate pipetting skills. When your group is ready, proceed to the next step. Study the schematic diagram to the right.





8). Withdraw 10 µL of dye from the first available micro-centrifuge tube sample that is available and aliquot the fluid into the corresponding well of the gel as depicted in the above schematic diagram. Do not puncture the gel and while you pipette use both hands (see diagram to right). 9). When everyone has had a chance to load a well, place the lid on the chamber ensuring that the polarity for the electricity is aligned properly. Please double check that the cathodes (black) and anodes (red) match. 10).Connect the chamber to a power supply, again assuring the proper alignment of the electrodes. 11). Ask your teacher for the proper voltage setting for this particular lab, as it will vary with other electrophoresis applications. 12). You will run the gel for about 15-20 minutes, during which time you will need to carefully observe the movement of the dyes and the appearance of the buffer. You can also start working on Q2/Q3 of the post lab questions during this time. 13). When the power is turned off, you will need to very carefully remove the gel from the chamber. It will be even more slippery this time. If the gel breaks, your group will need to repeat this lab before or after school, so please be careful. 14). Place the gel on a white sheet of paper or a light box. 15). Measure the distance that each dye migrated in millimeters (mm). This distance should be measured from the side of the well closest to the direction the dye traveled to the leading edge of each colored band (see board). 16). Record this data in your data table. 17). Create a gel diagram to map out the bands (see sample diagram to right; copies might be provided). You will need to make this diagram at least half the size of a piece of paper, but it should also be accurate to scale and representative of the actual well. I also recommend that you take a picture of the gel to include in your lab write up for reference. 18). Complete the remaining data sections of your data table. 19). Please save all pipette tips and leave the buffer in the gel chamber. You will discard of the agarose gel in the trash.



Post Lab Questions:

Q1. Explain in detail why the various dyes traveled different distances, speeds and directions.

Q2. Explain how this process could be used to determine paternity.

Q3. Explain how this process could be used in forensics at a crime scene.

Q4. Explain how transgenic biotechnology could be used in agriculture.

Q5. Define the term genetic engineering and then explain how this biotechnology could be used in medicine.

Q6. Explain the concept of recombinant DNA technology. Examine the schematic diagram to the right and create descriptions for #1-3.