

Introduction to Gel Electrophoresis

The term '*electrophoresis*' literally means "to carry with electricity". It is a technique for separating and analyzing mixtures of charged molecules. Agarose, a linear polymer extracted from seaweed, is used to make the gel. As a powder, it is melted in the presence of a buffer until a clear, transparent solution is achieved. The melted solution is poured into a mold and allowed to solidify—much like jello. The density of the agarose matrix depends on the concentration of the agarose solution. Different sized molecules can be separated effectively by choosing the appropriate concentration of agarose. When an electric field is applied across the gel, negatively charged molecules move towards the anode. The rate of migration depends on the size of the molecules.

In this exercise we will be separating a mixture of dyes by size in order for you to see the movement of the molecules through the gel. In biomedical research, scientists can use this technique to separate pieces of DNA according to their sizes.

Part I. Cast an agarose gel

1. Prepare your casting tray by carefully placing a clean glass plate into bottom of tray.
2. Weigh out 0.8 grams of agarose. Put into a 250 ml Erlenmeyer flask. Add 100 ml of 1X TEA buffer (dilute 10X TEA buffer to make 1X- that is, add 10 ml of 10X TEA to 90 ml of distilled water in graduated cylinder). Place agarose and buffer in microwave and heat until agarose is dissolved and boiling (Instructor will demonstrate). Use caution the agarose solution is very HOT. When the flask is *just* cool enough to hold, pour the agarose *evenly* into the floor of the casting tray, making sure that it is completely in place and even. Place comb in the apparatus to ensure well formation.

3. DO NOT JAR or MOVE the casting tray as the gel solidifies. This ensures a smooth, even gel. As the agarose hardens (about 10 min), it changes from clear to slightly opaque.
4. Fill the plastic electrophoresis box with about 500 ml of 1X TEA—electrophoresis-running buffer. TEA is a salt solution made of Tris, pH 8.0, which keeps the pH constant, EDTA, which pulls out low levels of extraneous divalent ions and sodium acetate, a salt.
5. When the gel has solidified, remove the comb in a straight up motion and transfer the glass plate and gel from the casting tray to the gel box and submerge the gel and plate onto the platform in the gel box. You'll notice that removing the comb left behind five little empty "slots" or *wells* in the gel. The wells should be located at the cathode end (black lead; (-)). Remember- black-top; red-bottom. The level of the buffer should be slightly covering the surface of the gel.

Part II. Load the gel.

1. Is your gel ready to load? It should be in the gel box, under buffer solution; the comb should have been removed and the five empty wells in the gel should be at the cathode end of the box.
2. Load 10 μ l of the dye mixture into a separate well in the gel.
 - Lower the pipet tip under the surface of the buffer, but don't puncture the bottom of the gel.
 - Gently depress pipet plunger and slowly expel a sample into a well. Keep plunger depressed until the pipet is out of the gel box.
 - Change tips between samples.

Part III. Gel electrophoresis

The term '*electrophoresis*' literally means "to carry with electricity." It is a technique for separating and analyzing mixtures of charged molecules. A negatively charged molecule when placed in an electric field will migrate toward the anode (+). The speed of migration of DNA in an agarose gel depends on the size of the piece; small pieces experience

less resistance and move faster (farther) than the larger pieces.

CAUTIONS

Remember, it is good practice to turn the power supply OFF before touching or opening a gel box.

If two teams are connecting their boxes to one power supply, be sure to communicate with each other whenever the power supply is turned ON or OFF.

1. WITH THE POWER OFF, secure the lid of the gel box and connect the leads to the same channel of the power supply (red-red, black-black).
2. Set the power supply at about 100 Volts and 40 milliAmps current (80 mAmps will automatically result if a second box is connected to one power supply).
3. Turn the power supply ON. Notice there is a switch to direct the LED display to read either volts or milliamps. Use it to verify that current is flowing through the gel.
4. Shortly after the current is applied, you should notice something happening at each electrode...what is it? You may also notice that the loading dye “behaves” in an unexpected way. Why?
5. Continue to electrophorese until the fastest-moving dye front had advanced at least $\frac{3}{4}$ of the way across the gel (about 45 minutes)
6. Then, turn the power supply OFF and disconnect the leads.
7. Remove the casting tray from the gel box. CAREFULLY slide you gel off the casting tray and into its labeled plastic tray or “boat” and analyze the results from gel electrophoresis.