LAB 9: GEL ELECTROPHORESIS

The term *electrophoresis* literally means, "to carry with electricity." It is a technique for separating and analyzing mixtures of charged molecules. Because of its sugar-phosphate backbones, DNA is a negatively-charged molecule. When DNA is placed in an electric field, it will migrate toward the positive electrode (anode). The speed of migration of DNA in an agarose gel depends on the size of the piece. Small pieces experience less resistance when moving through the mesh created by the agarose gel and move faster than the larger pieces. Thus, small pieces of DNA migrate farther in the gel than large pieces and are found closer to the bottom of the gel.

Cast an agarose gel

1. Prepare the casting tray and insert a 6-wel comb into the end slots.
2. Get a beaker of 25-30 ml of agarose that has been prepared and kept liquid in a water bath at 65-70 °C. Slowly and evenly pour the agarose into the casting tray.
3. DO NOT MOVE the casting gel until the gel solidifies, which takes about 10-15 minutes. It will change from clear to slightly opaque.
4. After the gel is solidified, wrap it carefully in plastic wrap to make sure it doesn't dry out. Store the gel overnight in the refrigerator.

Preparing the gel for electrophoresis

1. Get the gel out of the refrigerator, let it warm up to room temperature and remove the plastic wrap.
2. Put your gel in the casting tray in the electrophoresis chamber on the gel platform with the side with the comb nearest the negative electrode (cathode or black lead).
3. Fill the electrophoresis chamber with approximately 300 ml of 1X TAE or TBE electrophoresis running buffer. The buffer should just cover the surface of the gel by a few mm. Add more buffer if needed.
4. Carefully and slowly remove the comb from the gel by pulling it straight up. There will be six wells or slots in the well where you can put your DNA samples.

Loading the agarose gel

1. To each of your six samples, add 2 µl of loading dye. Finger vortex to mix and then microcentrifuge for 1-2 minutes. (Remember to balance the microfuge tubes in the rotor.)
2. By convention, DNA gels are read from left to right, with the wells located at the top of the gel. Your gel apparatus may be set up so that your gel is lined up in its box with the wells to your left. Thus, the contents of Tube "1" should be loaded in the well closest to you. Thus, when the gel is turned so that the wells are at the top, "1" will be in the left-hand corner. See diagram below.
3. Load 12 µl of each sample into wells or lanes 2-6 (if you used lane 1 for practice)
    a. Fill your pipet tip slowly and carefully to 12 µl (the teacher will instruct you on how to correctly use a micropipettor)
    b. Position your arm and pipet over the well or lane where you want to put your sample. Steady your pipet arm with your other arm.
    c. Lower the pipet tip to the top edge of the well opening. The loading dye contains a substance that is denser than the buffer so your solution will sink to the bottom of the well. DON'T PUNCTURE THE BOTTOM OF THE WELL.
    d. Gently expel the sample into the well by depressing the pipet plunger. Don't release the plunger until all the sample is out of the pipet tip and into the well. Then remove the pipet tip from the buffer before releasing the plunger.
    e. Change tips between EVERY sample.

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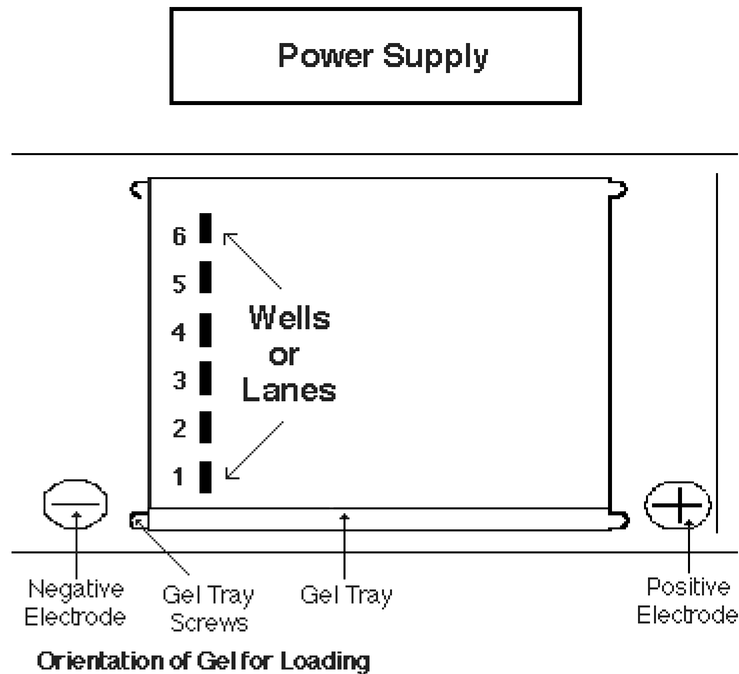

Running the Gel

PRECAUTIONS

- Always make sure the power supply is turned OFF before touching or opening the gel electrophoresis box.
- If two groups are connecting their gel electrophoresis boxes to the SAME power supply, always tell each other when the power supply is turned ON or OFF.

1. WITH THE POWER SUPPLY OFF, place the lid onto the gel electrophoresis box and connect the electrical leads cathode to cathode (black to black) and anode to anode (red to red). Both electrodes should be connected to one power supply channel.
2. Make sure the voltage knob is at ZERO. Then turn ON the power supply. Set the power supply to 100 Volts.
3. There is a switch that allows one to change the LED display reading either to Volts or milliAmps. Use it to check how much current is flowing through the gel. It should read about 40 milliAmps with one gel box hooked up or 80 milliAmps with two boxes hooked up when using 1X TAE buffer.
4. You should notice bubbles forming near each electrode if the system is working properly. Allow the electrophoresis to continue until the dye front is at least 2/3 to ¾ of the way down the gel, which is approximately 45 minutes.
5. Turn OFF the power supply and disconnect the leads.
6. Remove the casting tray from the gel electrophoresis box and carefully slide your gel off the casting tray a piece of plastic wrap in a plastic tray labeled with your group name Carefully wrap the gel and place it in the refrigerator overnight.

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