



University of
Massachusetts
Medical School

Regional Science Resource Center

SUPPORTING MATHEMATICS, SCIENCE AND TECHNOLOGY EDUCATION



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RESTRICTION ENZYME ANALYSIS OF DNA

Background

Many of the revolutionary changes that have occurred in biology over the past fifteen years can be attributed directly to the ability to manipulate DNA in defined ways. The principal tools for this recombinant DNA technology are *enzymes* that can cut, mend, wind, unwind, transcribed, repress, and replicate DNA.

Restriction enzymes are the “chemical scissors” of the molecular biologist; these enzymes cut DNA at specific nucleotides sequence. A sample of someone’s DNA, incubated with restriction enzymes, is reduced to millions of DNA fragments of varying sizes. A DNA sample from a different person would have a different nucleotide sequence and would thus be enzymatically “chopped up” into a very different collection of fragments. The DNA fragments are separated by electricity – agarose gel electrophoresis – and tagged or stained in some fashion so that they can be visualized and studied. The resulting patterns of restriction fragment resembles the pricing bar code used on supermarket products – small bars lined up in a column, the largest closest to the beginning of the gel, the smallest at the end of the gel.

Purpose

In this laboratory, we will:

- Cut DNA sample by incubating it with a restriction enzyme,
- Load an agarose gel with the restriction digest,
- Conduct gel electrophoresis to spread out the mixture of DNA fragments in the digest,

- Photograph the gel to visualize the DNA,
- Analyze the resulting banding pattern of DNA

Materials, per team

Gel electrophoresis box

Power supply

Casting tray and comb

Agarose and glass flask

P-20 micropipette and tips

Electrophoresis buffer, 500 mL (1X TEA)

DNA sample, on ice

6x loading dye

Class use: Water bath, crushed ice containers, microcentrifuges, documentation stations (camera, film, filter, hood, UV transilluminator)

Part I Cast an agarose gel

1. Prepare your casting tray by carefully placing a clean glass plate into bottom of tray.
2. Weight 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 – 10ml of 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.

Place the comb into the slot on the casting tray; making sure that it is completely in place and even.

3. DO NOT JAR or MOVE the casting tray as the gel solidifies. This ensures a smooth, even gel. As the agarose hardens (about 10min), it changes from clear to slightly opaque.
4. Fill the plastic electrophoresis box with about 500ml of 1X TEA – electrophoresis running buffer. TEA is a salt solution, made of Tris, pH 8.0, which keeps the pH constant, which pulls out low levels of extraneous divalent ions and sodium acetate, a salt.
5. While the gel is solidifying, begin part II.

Part II Receive DNA Sample

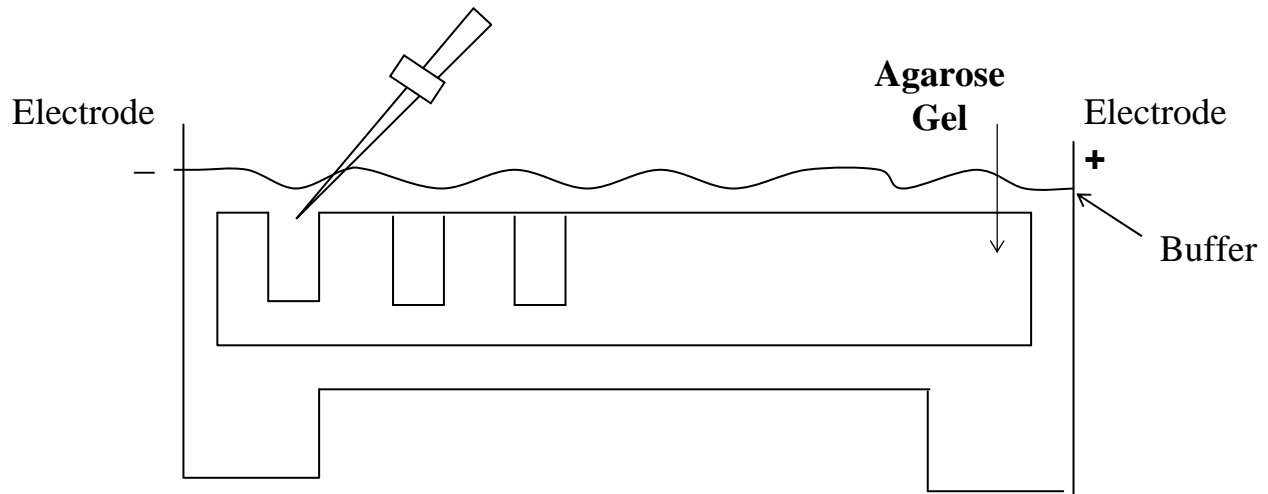
1. Obtain DNA already digested with the restriction enzyme. Keep on ICE.

Part III Load the gel

1. To each of your tubes, add 2 μ l loading dye. Then give them all quick spin in the microcentrifuge to mix the dye. Temporarily set them aside.
2. 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.
3. Load 10 μ l of each sample into a separate well in the gel.

Lower the pipette tip under the surface of the buffer, but don't puncture the bottom of the gel.

Gently depress pipette plunger and slowly expel a sample into a well. Keep plunger depressed until the pipette is out of the gel box. Change tips between samples.



Part IV Gel electrophoresis

The term *electrophoresis* literally means, “to carry with electricity”. It is a technique for separating and analyzing mixtures of charged molecules. Due to its sugar-phosphate backbone, DNA is a negatively charged molecule. When placed in an electric field, it will migrate toward the anode (+). The speed of migration of DNA in an agarose gel depends on the size of the piece; small pieces experiences less resistance and move faster (farther) than the large 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 gel 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 SUPPLY 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 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 in 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 it is? You may also notice that the loading dye “behaves” in an unexpected way. Why?
5. Continue to electrophorese until the fastest-moving dye front has advanced at least $\frac{2}{3}$ to $\frac{3}{4}$ of the way across the gel (about 45 minutes times).
6. Then, turn the power supply **OFF** and disconnect the leads.
7. Remove the casting tray from the gel box. Wear gloves; remember there is ethidium bromide in the gel and also the buffer. **CAREFULLY** slide your gel off the casting tray and into its labeled plastic tray or “boat”.

Part V Photograph the gel

There are many stain and tagged that allow us to visualize DNA. In our lab, we will stain the DNA with a fluorescent dye double helix. When excited by ultraviolet (UV) light, the EtBr absorbs some of the energy and emits orange (visible) light.

CAUTIONS

- Do not allow your skin, eyes, mouth, etc. to come into contact with ethidium bromide solution. Always wear goggles and gloves if working with this chemical.
 - If you accidentally spill ethidium bromide staining solution, notify the instructor. She/he will deactivate it using powdered activated charcoal.
 - UV light can damage unprotected eyes and skin. Never look directly into an unshielded UV light source. Our transilluminators are safe, since a plastic safety shield is lowered over the gel.
 - An operator at the Documentation Station will put your gel on the surface of the UV transilluminator. When the safety-lid is closed, this “box” emits ultraviolet light. The ultraviolet light makes EtBr-coated DNA fragments glow.
 - The operator takes Polaroid 67 photographs of your gel. After about 45 seconds of developing time, peel the backing away to separate the print from the negative. The developing fluid is caustic – don’t let it touch you.
1. Steady the camera and squeeze the cable release to take the picture.
 2. Grasp and pull out the white tab. Then pull out the yellow tab, which is actually one end of the photograph, right out of the camera. This starts development. After 45 seconds, separate picture from backing (negative).
 - 3.

Upon completion of this lab

- Dispose of designated materials in the appropriate places.
- Leave equipment as you found it.
- Check your workstation is in order.
- Wash your hands.