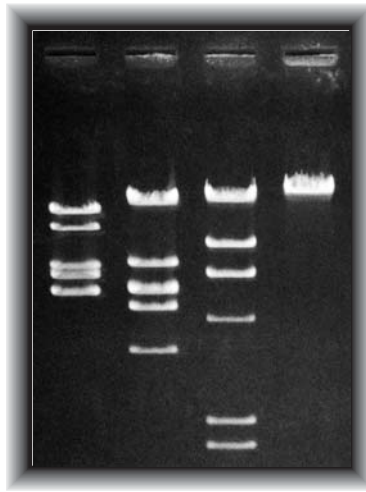


DNA Goes to the Races

Dry Lab Student Activity

An accompaniment to Restriction Enzymes and DNA Kit,
catalog #21-1185 and 21-1187.



Teacher Information

This is a reading and paper activity that introduces electrophoresis. Students should already be familiar with the activity of restriction enzymes through *DNA Scissors*. This lesson is an excellent introduction if you plan to conduct electrophoresis in your classroom.

Class periods required: 1

Objectives

At the end of this activity students should be able to

- If shown a simple restriction map, predict the number and arrangement of bands in a gel after digestion and electrophoresis.
- Explain why electrophoresis causes DNA fragments of different sizes to separate.

Materials

Scissors

Copies of student activity instructions, restriction maps, and gel outline

Preparation

Photocopy the appropriate pages for each student in your class.

Checking Answers

See figure. If possible, check the students' work as they do the exercise. Make sure they line the fragments up correctly between lanes as well as within an individual gel lane [for example, that the 2,500-base pair (bp) *Hind*III fragment is lined up between the 2,000-bp and 3,000-bp *Bam*HI fragments and even with the 2,500-bp *Eco*RI fragment]. Use their own fragment size labels to assist you.

Adapted by Carolina Biological Supply Company, from *Recombinant DNA and Biotechnology: A Guide for Teachers*, by Helen Kreuzer and Adrienne Massey, 1996, American Society for Microbiology Press.

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CB270339711

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Student Activity

You have already learned about restriction enzymes and how they cut DNA into fragments. You may have even looked at some DNA restriction maps and figured out how many pieces a particular enzyme would produce from that DNA. But when you actually perform a restriction digest, you put the DNA and the enzyme into a small tube and let the enzyme do its work. Before the reaction starts, the mixture in the tube looks like a clear fluid. Guess what! After the reaction is finished, it still looks like a clear fluid! Just by looking at it, you can't tell that anything happened.

In order for restriction digestion to mean much, you have to be able somehow to see the different DNA fragments that are produced. There are chemical dyes that stain DNA, but obviously it doesn't do much good to add them to the mixture in the test tube. In the laboratory, scientists separate DNA fragments so that they can look at the results of restriction digests (and other procedures) by a process called *gel electrophoresis*.

Gel electrophoresis takes advantage of the chemistry of DNA to separate fragments. Under normal circumstances, the phosphate groups in the backbone of DNA are negatively charged. In electrical society, opposites do attract, so DNA molecules are very much attracted to anything that is positively charged. In gel electrophoresis, DNA molecules are placed in an electric field (which has a positive and a negative pole) so that they will migrate towards the positive pole.

The electric field makes the DNA molecules move, but to cause them to separate and be easy to look at later on, the whole process is carried out in a gel (obviously the source of the name *gel* electrophoresis). If you have ever eaten Jell-O®, you have had experience with a gel. The gel material in Jell-O® is gelatin; different gel materials are used to separate DNA. One gel material often used for electrophoresis of DNA is called *agarose*, and it behaves much like Jell-O® but lacks the sugar and color. To make a gel for DNA (called *pouring* or *casting* a gel), you dissolve agarose powder in boiling water, pour it into the desired dish, and let it cool. As it cools, it hardens (sound familiar?).

Since the plan for agarose gels is usually to add DNA to them, scientists place a device called a *comb* in the liquid agarose after it has been poured into the desired dish, and let the agarose harden around the comb. Imagine what would happen if you stuck the teeth of a comb into liquid Jell-O® and let it harden. Afterwards, when you pulled the comb out, you would have a row of tiny holes in the solid Jell-O® where the teeth had been. This is exactly what happens with laboratory combs. When the comb is removed from the hardened agarose gel, a row of holes in the gel. The holes are called *sample wells*. DNA samples are placed into the wells before electrophoresis is begun.

For electrophoresis, the entire gel is placed in a tank of salt water (not table salt). An electric current is applied across the tank, so that it flows through the salt water and the gel. When the current is applied, the DNA molecules begin to migrate through the gel towards the positive pole of the electric field.

At this point the gel does its most important work. All of the DNA in the gel migrates through the gel towards the positive pole, but the gel material makes it more difficult for larger DNA molecules to move than smaller ones. So in the same amount of time, a small DNA fragment can migrate much further than a large one. You can therefore think of gel electrophoresis like a DNA footrace, where the "runners" (the molecules being separated) separate just like runners in a real race. The smaller the molecule, the faster it runs. Two molecules the same size run exactly together.

After a time, the electric current is turned off and the entire gel is placed into a DNA staining solution. After staining, the DNA can be seen. The resulting pattern looks like a series of stripes in the gel called *bands*, where each separate band is composed of one size of DNA molecule. There are millions of actual molecules in the band, but they are all the same size (or very close to it). At any rate, after a restriction digest, there should be one band in the gel for each different size fragment produced in the digest. The smallest fragment will be the one that has migrated furthest from the sample well, and the largest will be closest to the well.

Activity

You have three representations of a DNA molecule and an outline of an electrophoresis gel. The representations show the cut sites of three different restriction enzymes on the same DNA molecule. You will simulate the digestion of this DNA with each of the three enzymes, then simulate agarose gel electrophoresis of the restriction fragments.

1. Cut out the three pictures of the DNA molecule.
2. Simulate the activity of the restriction enzyme *EcoRI* on the DNA molecule that shows the *EcoRI* sites by cutting across the strip at the vertical lines representing *EcoRI* sites. You have now digested the molecule with *EcoRI*. Put your "restriction fragments" in a pile apart from the other two DNA strips.
3. "Digest" the second DNA strip with *BamHI*. Put the *BamHI* fragments in a separate pile.
4. Now digest the remaining DNA molecule with *HindIII*. Put these fragments in a third pile.
5. In our imaginary gel electrophoresis, you will separate the *EcoRI*, *BamHI*, and *HindIII* fragments as if you loaded the three sets of fragments into separate but adjacent sample wells. Arrange your fragments as they would be separated by agarose gel electrophoresis. Designate an area on your desk as the end of the gel with the sample wells. Starting with the *EcoRI* fragments, arrange them from longest to shortest, with the longest one closest to the well.
6. Next, separate the *BamHI* fragments adjacent to the *EcoRI* fragments. Be sure to order the fragments correctly by size with respect to the other *BamHI* fragments and to the *EcoRI* fragments you have already laid out.
7. Repeat the same procedure for the *HindIII* fragments. You should now have all three of your sets of fragments arranged in order in front of you.
8. Look at the outline of the electrophoresis gel. Notice that it has a size scale in base pairs on the left-hand side, and that sample wells are drawn in. Use the outline and draw the pattern your restriction digest would make in the gel, using the size scale as a guide for where to draw your fragments. Use the *EcoRI* sample well for the *EcoRI* fragments, and so on.
9. After you draw the bands representing the restriction fragments, use the size information on the paper DNA strips to label the bands on the gel with the sizes of the fragments in base pairs.
10. Use the actual fragment sizes as a check for your work.

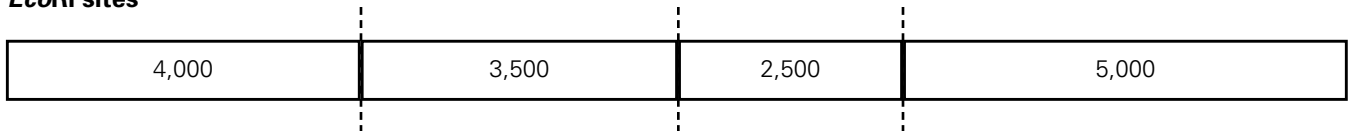
Are all the smaller fragments across all the gel "lanes" in front of all the larger fragments?

Did you notice that the size scale doesn't seem to have regular intervals? The size scale looks the way it does because agarose gels separate fragments that way.

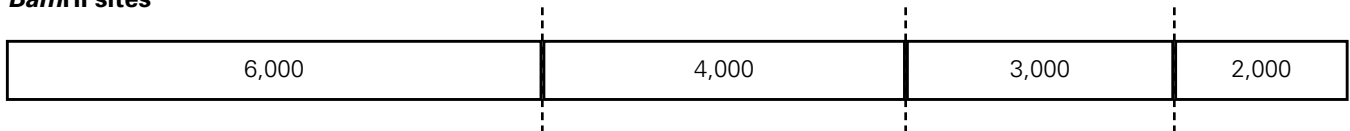
Restriction Maps for *DNA Goes to the Races*

Below are three representations of a 15,000-base-pair DNA molecule. Each representation shows the locations of different types of restriction site, with vertical lines representing the cut sites. The numbers between the cut sites show the sizes (in base pairs) of the fragments that would be generated by digesting the DNA with that enzyme.

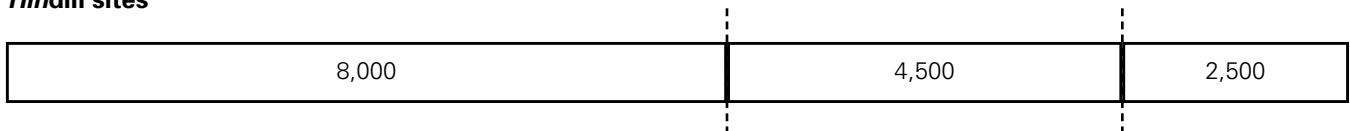
***EcoRI* sites**



***BamHI* sites**



***HindIII* sites**



Gel Outline for *DNA Goes to the Races*

