

# DNA Goes to the Races



## Classroom Activities

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 has 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 these dyes to the mixture in the test tube. In the laboratory, scientists use a process called **gel electrophoresis** to separate DNA fragments so that they can look at the results of restriction digests (and other procedures).

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 toward the positive pole.

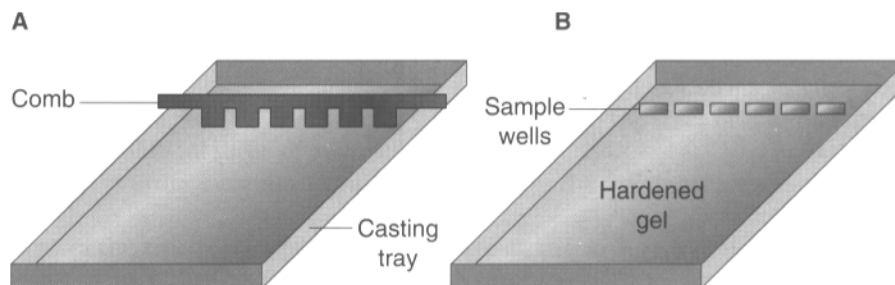
The electric field makes the DNA molecules move, but to cause the molecules to separate and be easy to look at later on, the whole process is carried out in a gel (obviously the source of the name *gel* electrophoresis). If you have ever eaten Jell-O, you have had experience with a gel. The gel material 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 without the sugar and color. To make a gel for DNA (called *pouring* or *casting* a gel), you dis-

solve agarose powder in boiling buffer, 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 remains (look at Fig. 11.1). 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) called buffer. 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 toward the positive pole of the electric field (Fig. 11.2). Figure 11.3 shows a scientist loading a DNA sample into an agarose gel sitting in an electrophoresis tank.

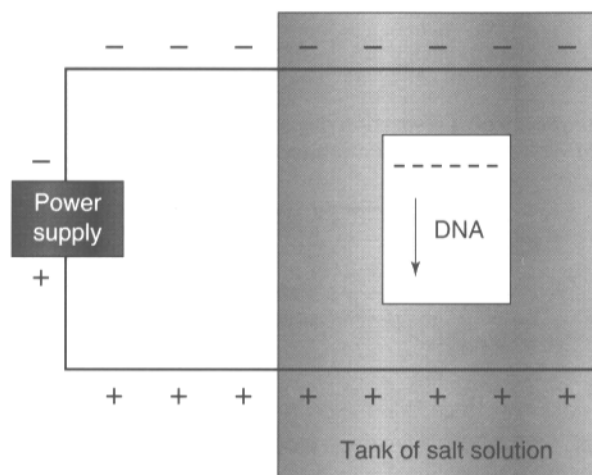
At this point, the gel does its most important work. All of the DNA in the gel migrates through the gel toward the positive pole, but the gel material makes it more difficult for larger DNA molecules to move than smaller ones. So in the same amount of time, a small DNA fragment can migrate much farther than a large one. You can therefore think of gel electrophoresis as a DNA footrace, where the "runners" (the molecules being separated) separate just like runners in a real race (Fig. 11.4). The smaller the molecule, the faster it runs. Two molecules the same size run exactly together.



**Figure 11.1** Casting an agarose gel. (A) To make a gel, hot liquid agarose solution is poured into a casting tray (any shallow container), and the comb is put in place. (B) After the agarose cools and hardens, the comb is removed, leaving behind pits in the gel called sample wells. Samples are loaded into the wells prior to electrophoresis.

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 pattern looks like a series of stripes (bands) in the gel; 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, as shown in Fig. 11.5.

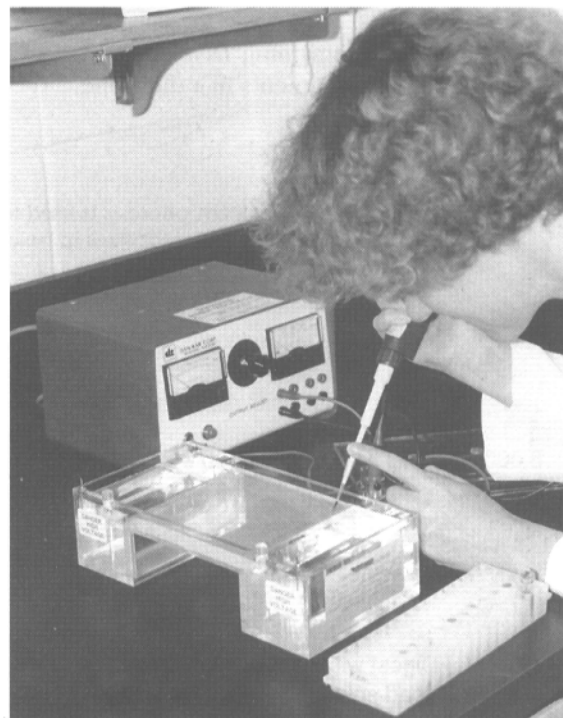
**Figure 11.2** In electrophoresis, the gel is placed in a tank of salt solution, and an electric current is applied. The DNA migrates toward the positive pole.

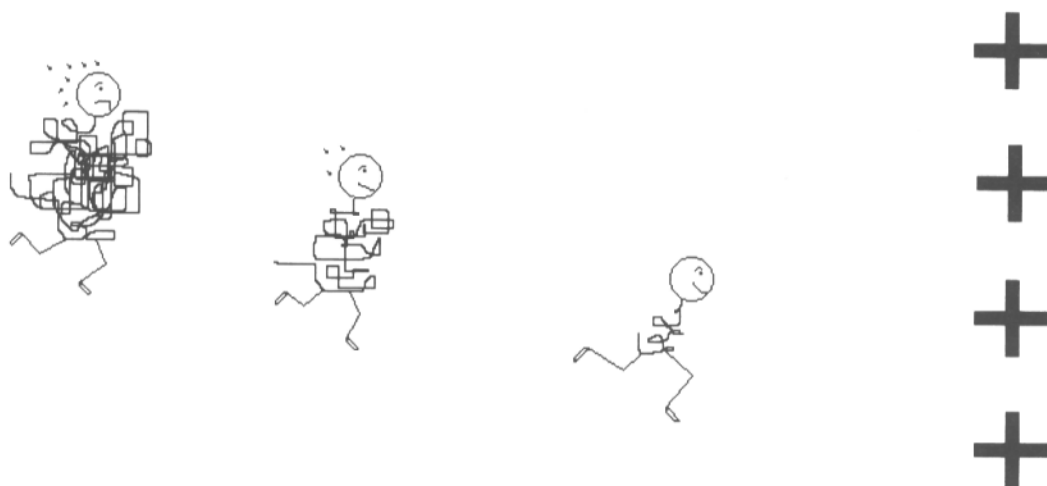


## Activity

In Appendix A, you have three representations of a DNA molecule and the 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 and then simulate agarose gel electrophoresis of the restriction fragments.

**Figure 11.3** A scientist is using a micropipette to load a DNA sample into an agarose gel. The gel is in an electrophoresis chamber full of buffer. The power supply for the chamber is on the laboratory bench behind the chamber.

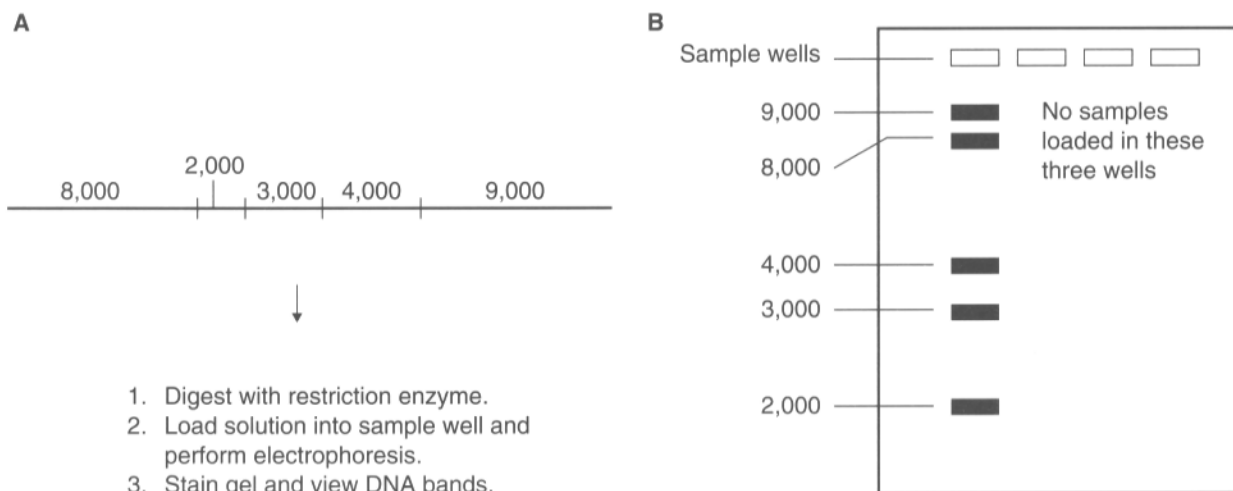




**Figure 11.4** In electrophoresis races, the small DNA always wins!

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 had 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, and place them adjacent to the *EcoRI* fragments. Be sure to order each fragment correctly by size with re-

**Figure 11.5** Gel electrophoresis is used to separate products of restriction digestion. (A) Restriction map, with fragment sizes in base pairs; (B) view of gel after electrophoresis.



spect to other *Bam*HI fragments and to the *Eco*RI fragments you have already laid out.

7. Repeat the same procedure for the *Hind*III 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 provided in Appendix A. Notice that it has a size scale in base pairs on the left-hand side and that sample wells are drawn in. Using the outline and the size scale as a guide for where to draw your fragments, draw the pattern your restriction digest would make in the gel. Use the *Eco*RI sample well for the *Eco*RI 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 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 site. 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

4,000	3,500	2,500	5,000
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### *BamHI* sites

6,000	4,000	3,000	2,000
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### *HindIII* sites

8,000	4,500	2,500
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Gel outline for *DNA Goes to the Races*

