

Restriction Enzyme Digestion

Restriction endonucleases, or **restriction enzymes**, are enzymes that make double-stranded cuts in DNA molecules. In nature, they are made by bacteria and used to defend against foreign DNA, such as a virus that tries to take over the bacterial cell. Biochemists have purified many restriction enzymes, and they are now an important part of the molecular biology “toolbox.” Digestion (cutting) of DNA with restriction enzymes can be used in making a physical map of the DNA, cloning a gene, making a deliberate mutation and in many other ways.

Introduction to restriction enzymes

Dozens of different restriction enzymes have been isolated from different kinds of bacteria, and each one cleaves DNA at a different, specific sequence. The DNA sequences recognized by the enzymes are usually 4-8 nucleotides long and are **palindromes**. A palindrome is a sequence which is the same on the two DNA strands, if you read from 5' toward 3'. Because the sequence occurs on both strands at nearly the same place, the enzyme can cut both strands of the DNA. Figure 44 shows the recognition sequences for two different enzymes and how they make a double-stranded cut in the DNA molecule.

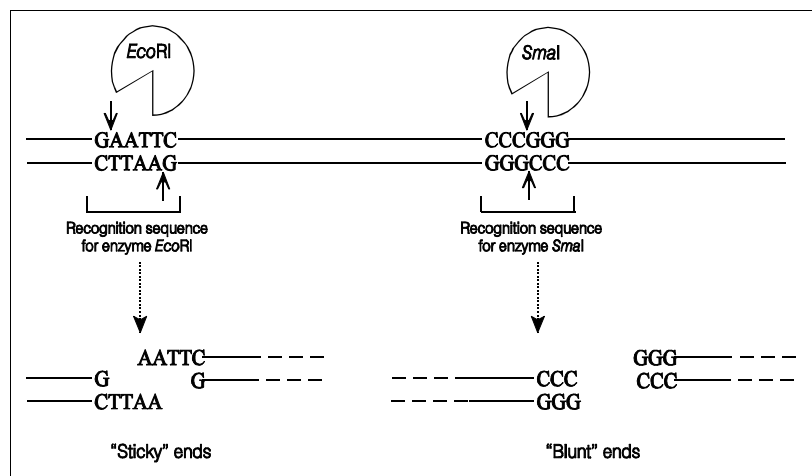


Figure 44. Cleavage of a DNA molecule by restriction enzymes *EcoRI* and *SmaI*.

The DNA molecule shown in the figure contains the sequence 5'-GAATTC-3', which is recognized by the enzyme *EcoRI* (an enzyme isolated from the bacterium *E. coli*). When *EcoRI* finds the sequence GAATTC in a DNA molecule, it cuts between the G and the first A. This is a palindrome, so each strand has a GAATTC here; the enzyme therefore cuts both. Notice that because it cuts off-center, the ends left behind each have a short, single-stranded region. We call these “**sticky ends**” because they can base-pair with a matching sticky end. A different enzyme, *SmaI* (from the bacterium *Serratia marcescens*) recognizes 5'-CCCGGG-3' and cuts between the last C and the first G. The cut site is in the center of the recognition sequence, so this enzyme cuts both strands in the same place, leaving **blunt ends** rather than sticky ones.

Because each enzyme recognizes one specific DNA sequence, each enzyme will cut a particular DNA molecule only at specific points. The number of cut sites and their locations depend on the nucleotide sequence. For example, Figure 45 shows a plasmid that has one GAATTC sequence. The enzyme *EcoRI* would cut the plasmid once, converting the circular DNA molecule to a linear piece of DNA of the same length.

The very same plasmid might happen to have two recognition sequences for another enzyme, such as *HindIII*. The very same plasmid

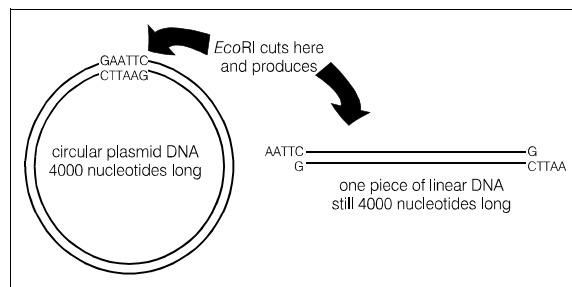


Figure 45. *EcoRI* cuts a circular plasmid once, producing a single, linear DNA fragment.

might happen to have two recognition sequences for another enzyme, such as *Hind*III (which recognizes 5'-AAGCTT-3'). This means that *Hind*III would cut the plasmid twice, producing two linear DNA fragments (Figure 46). In this example, the two recognition sites are 1700 base-pairs (bp) apart, so the circle is cut into two pieces: one is 1700 bp long, and the other is 2300—together, they add up to the original size of the plasmid, 4000 bp. Figure 46 also shows what would happen if we decided to cut the plasmid with both *Eco*RI and *Hind*III at the same time. Now we'd get three cuts, so three fragments.

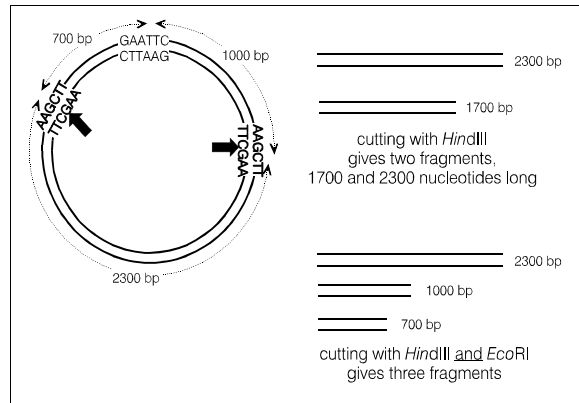


Figure 46. *Hind*III cuts the plasmid twice (two fragments)

Digesting DNA with a restriction enzyme

A restriction digest requires DNA, the enzyme and a buffer. The buffer contains a pH buffer, Mg^{2+} (a needed cofactor) and salts. It is supplied at 10× concentration, so you always add a volume equal to 1/10 of the total volume of your digest (final concentration is then 1×).

We currently use two different kinds of restriction enzymes. **FastDigest** enzymes are purchased from Fermentas and digest DNA sufficiently well for most purposes in as little as 5 min. All FastDigest enzymes use the same buffer. Enzymes not available in FastDigest format are usually purchased from New England Biolabs (NEB), and different NEB enzymes need different buffers, so consult their catalog or Web site (www.neb.com) to find out what buffer your enzyme needs as well as the optimal incubation temperature (usually 37°C).

A typical volume for a restriction digest is 20 μ l, but you can increase this volume if you have a large amount of DNA. Restriction enzymes are measured in “units,” and one unit is the amount of enzyme needed to digest 1 μ g of DNA in one hour under optimal conditions. In order to ensure complete digestion, it's common to add 5-10 units of enzyme per μ g of DNA.

- ▶ Determine the volume of DNA you want to digest.
- ▶ Determine the number of units of enzyme you need and what volume this would be.

◇ You can digest the DNA with two (or more) enzymes at the same time, if they both work well in the same buffer. If the enzymes are not compatible, you will have to digest with one, purify the DNA or adjust the buffer, and then digest with the other.

- ▶ Determine what buffer to use and what volume (1/10 of total volume; e.g., 2 μ l for a 20- μ l digest).
- ▶ Add up the volumes of DNA, enzyme and buffer. Calculate the amount of water needed to bring the volume up to the desired total.
- ▶ Add the water (be sure it's *sterile* dH₂O) to a microcentrifuge tube, then add the buffer with the same pipette tip. Add the DNA and mix by pipetting up and down a couple of times.

◇ If you are using *FastDigest* enzymes, use a 0.5-ml thin-wall microcentrifuge tube so that the contents of the tube will warm up faster.

- ▶ Add the enzyme last, mix, centrifuge for a few seconds to bring down all the liquid and incubate in a water bath at the appropriate temperature for 1 hour (10 min. for *FastDigest* enzymes).
- ▶ Add a volume of loading buffer equal to 1/5 of the total volume of your digest and mix well.

◇ **IMPORTANT! Restriction enzymes are very sensitive to heat!** Keep them in their blue cooler racks anytime you take them out of the freezer, and keep their time outside the freezer as short as possible. Never touch the part of the tube where the enzyme is with your warm fingers.
