Restriction enzymes & DNA ligase

Key points:

- **Restriction enzymes** are DNA-cutting enzymes. Each enzyme recognizes one or a few target sequences and cuts DNA at or near those sequences.
- Many restriction enzymes make staggered cuts, producing ends with single-stranded DNA overhangs. However, some produce blunt ends.
- **DNA ligase** is a DNA-joining enzyme. If two pieces of DNA have matching ends, ligase can link them to form a single, unbroken molecule of DNA.
- In DNA cloning, restriction enzymes and DNA ligase are used to insert genes and other pieces of DNA into plasmids.

How do you cut and paste DNA?

In DNA cloning, researchers make many copies of a piece of DNA, such as a gene. In many cases, cloning involves inserting the gene into a piece of circular DNA called a **plasmid**, which can be copied in bacteria.

How can pieces of DNA from different sources (such as a human gene and a bacterial plasmid) be joined together to make a single DNA molecule? One common method is based on restriction enzymes and DNA ligase.

- A **restriction enzyme** is a DNA-cutting enzyme that recognizes specific sites in DNA. Many restriction enzymes make staggered cuts at or near their recognition sites, producing ends with a single-stranded overhang.
- If two DNA molecules have matching ends, they can be joined by the enzyme **DNA ligase**. DNA ligase seals the gap between the molecules, forming a single piece of DNA.

  Restriction enzymes and DNA ligase are often used to insert genes and other pieces of DNA into plasmids during DNA cloning.

Restriction enzymes

Restriction enzymes are found in bacteria (and other prokaryotes). They recognize and bind to specific sequences of DNA, called **restriction sites**. Each restriction enzyme recognizes just one or a few restriction sites. When it finds its target sequence, a restriction enzyme will make a

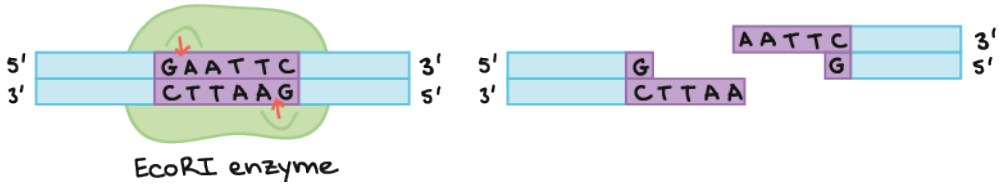
double-stranded cut in the DNA molecule. Typically, the cut is at or near the restriction site and occurs in a tidy, predictable pattern.
[Why do bacteria have restriction enzymes?]

As an example of how a restriction enzyme recognizes and cuts at a DNA sequence, let's consider *Eco*RI, a common restriction enzyme used in labs. *Eco*RI cuts at the following site:

5'-...GAATTC...-3' 3'-...CTTAAG...-5'

EcoRIsite

When EcoRI recognizes and cuts this site, it always does so in a very specific pattern that produces ends with single-stranded DNA “overhangs”:



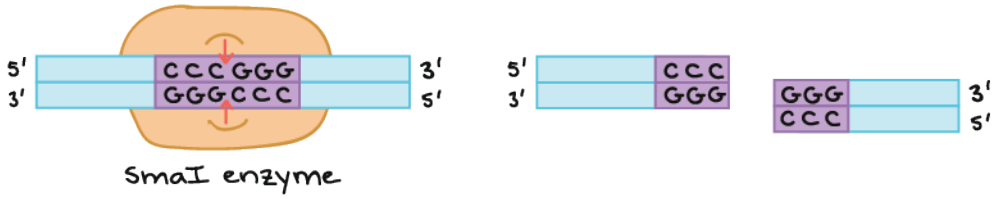
An *Eco*RI enzyme binds to an *Eco*RI site in a piece of DNA and makes a cut on both strands of the DNA. The pattern of the cut is:

5'-...G|AATTC...-3' 3'-...CTTAA|G...-5'

Thus, it produces an overhang of 5'-AATT-3' on each end of the cut DNA.

If another piece of DNA has matching overhangs (for instance, because it has also been cut by EcoRI), the overhangs can stick together by complementary base pairing. For this reason, enzymes that leave single-stranded overhangs are said to produce **sticky ends**. Sticky ends are helpful in cloning because they hold two pieces of DNA together so they can be linked by DNA ligase.

Not all restriction enzymes produce sticky ends. Some are “blunt cutters,” which cut straight down the middle of a target sequence and leave no overhang. The restriction enzyme *Sma*I is an example of a blunt cutter:



A *Sma*I enzyme binds to the *Sma*I restriction site, which is:

5'-...CCCGGG...-3' 3'-...GGGCCC...5'

It makes a cut right in the middle of this sequence on both strands, producing blunt ends. The cut sites are:

5'-...CCC|GGG...-3' 3'-...GGG|CCC...5'

Blunt-ended fragments can be joined to each other by DNA ligase. However, blunt-ended fragments are harder to ligate together (the ligation reaction is less efficient and more likely to fail) because there are no single-stranded overhangs to hold the DNA molecules in position.

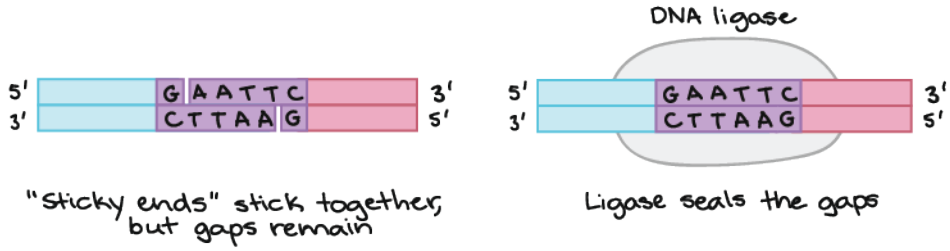
[Where do restriction enzymes get these weird names?]

3,3, comma000000

5′–GAATTC–3′3′–CTTAAG–3′	5′–G↓AATTC–3′3′–CTTAA↑G–5′
5′–GGATCC–3′3′–CCTAGG–3′	5′–G↓GATCC–3′3′–CCTAG↑G–5′
5′–AAGCTT–33′–TTCGAA–5′	5′–A↓AGCTT–33′–TTCGA↑A–5′
5′–CCCGGG–33′–GGGCCC–5′	5′–CCC↓GGG–33′–GGG↑CCC–5′
5′–GAGCTC–33′–CTCGAG–5′	5′–GAGCT↓C–33′–C↑TCGAG–5′

DNA ligase

If you've learned about DNA replication, you may already have met DNA ligase. In DNA replication, ligase's job is to join together fragments of newly synthesized DNA to form a seamless strand. The ligases used in DNA cloning do basically the same thing. If two pieces of DNA have matching ends, DNA ligase can join them together to make an unbroken molecule.



Fragment 1 of DNA:

5'-...G 3'-...CTTAA

Fragment 2 of DNA:

AATTC...-3' G...-5'

The single-stranded regions of the two molecules can stick together by hydrogen bonding, but there are still gaps in the backbone:

5'-...G|AATTC...-3' 3'-...CTTAA|G...-5'

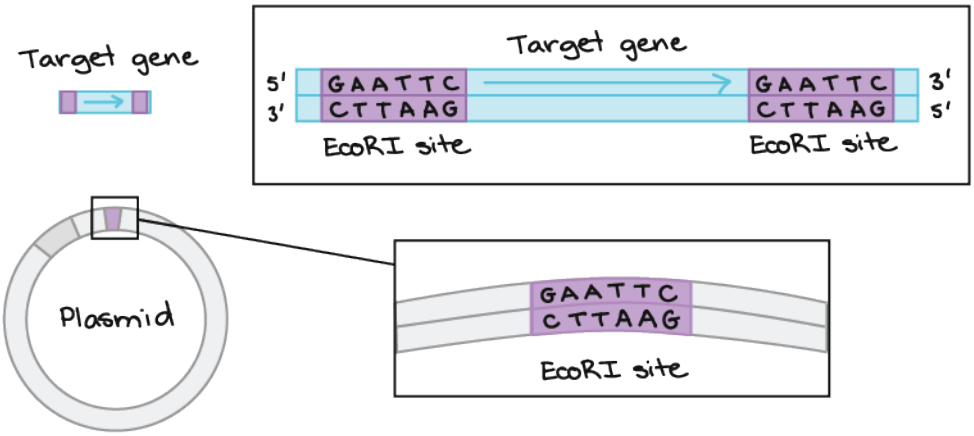
DNA ligase seals the gaps to make an unbroken molecule of DNA:

5'-...GAATTC...-3' 3'-...CTTAAG...-5'

How does DNA ligase do this? Using ATP as an energy source, ligase catalyzes a reaction in which the phosphate group sticking off the 5’ end of one DNA strand is linked to the hydroxyl group sticking off the 3’ end of the other. This reaction produces an intact sugar-phosphate backbone.

Example: Building a recombinant plasmid

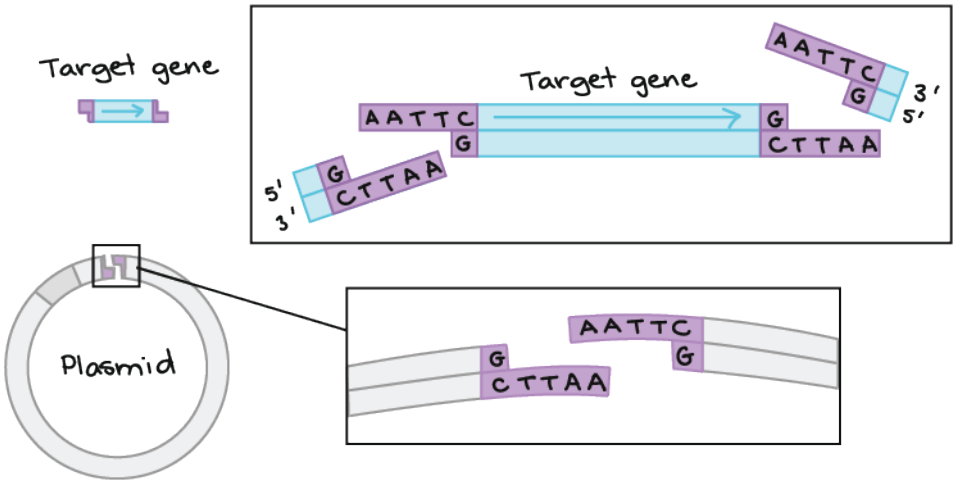
Let's see how restriction digestion and ligation can be used to insert a gene into a plasmid. Suppose we have a target gene, flanked with *Eco*RI recognition sites, and a plasmid, containing a single *Eco*RI site:



We start off with a target gene and a circular plasmid. The target gene has two *Eco*RI restriction sites near its ends. The plasmid has one *Eco*RI site in it, lying just after a promoter that drives expression in bacteria. The sequence of the *Eco*RI sites is:

5'-GAATTC-3' 3'-CTTAAG-5'

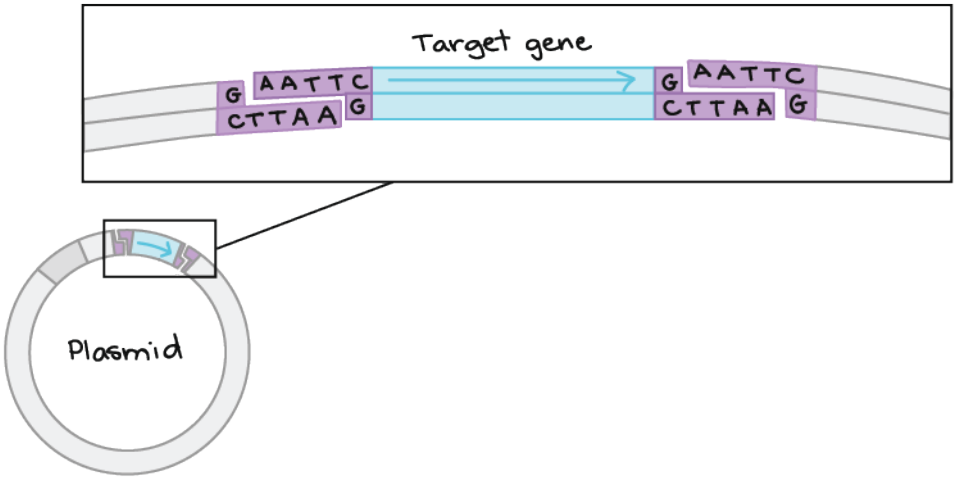
Our goal is to use the enzyme *Eco*RI to insert the gene into the plasmid. First, we separately digest (cut) the gene fragment and the plasmid with *Eco*RI. This step produces fragments with sticky ends:



We separately digest (cut) the gene fragment and the plasmid with *Eco*RI. This step produces fragments with sticky ends. All of the ends have an overhang of four nucleotides, with the sequence 5'-AATT-3'. That's because *Eco*RI's cut pattern is:

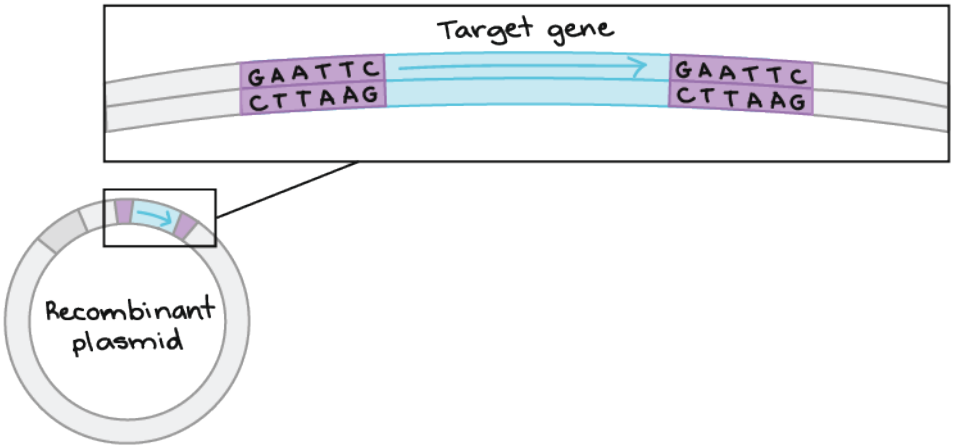
5'-G|AATTC-3' 3'-CTTAA|G-5'

Next, we take the gene fragment and the linearized (opened-up) plasmid and combine them along with DNA ligase. The sticky ends of the two fragments stick together by complementary base pairing:



Next, we take the gene fragment and the linearized (opened-up) plasmid and combine them along with DNA ligase. The sticky ends of the two fragments stick together by complementary base pairing. However, there are still gaps in the sugar-phosphate backbones of the DNA double helix at the junction sites where the gene and plasmid DNA meet.

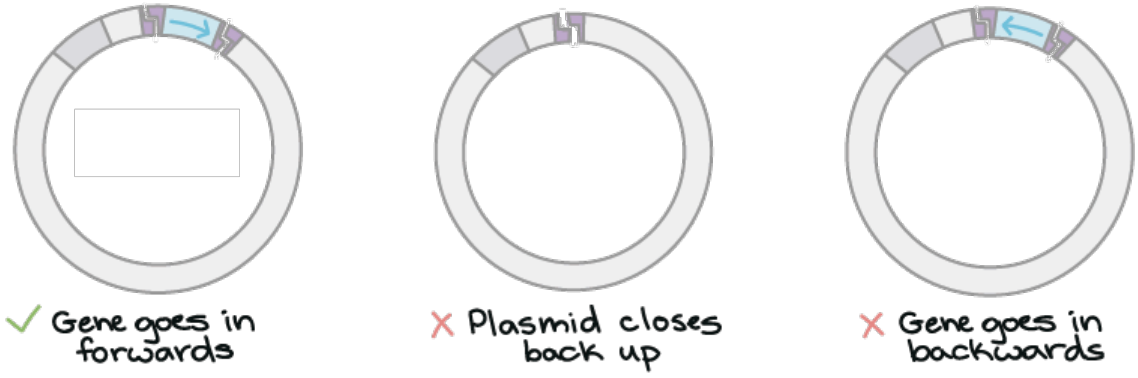
Once they are joined by ligase, the fragments become a single piece of unbroken DNA. The target gene has now been inserted into the plasmid, making a recombinant plasmid.



Once they are joined by ligase, the fragments become a single piece of unbroken DNA. The target gene has now been inserted into the plasmid, making a recombinant plasmid. In the plasmid, the gene is now flanked by two *Eco*RI sites that were generated when the cut ends were ligated together.

Restriction digests and ligations involve many molecules of DNA

In the example above, we saw one outcome of a ligation between a gene and plasmid cut with *Eco*RI. However, other outcomes could happen in this exact same ligation. For instance, the cut plasmid could recircularize (close back up) without taking in the gene. Similarly, the gene could go into the plasmid, but flipped backwards (since its two *Eco*RI sticky ends are identical).



Left: recombinant plasmid produced when gene goes in forwards ("pointing" away from the promoter that is already in the plasmid).

Middle: non-recombinant plasmid produced when the cut plasmid simply closes back up (its ends ligate with each other).

Right: recombinant plasmid produced when gene goes in backwards ("pointing" back towards the promoter that is already in the plasmid).

Restriction digests and ligations like this one are performed using many copies of plasmid and gene DNA. In fact, billions of molecules of DNA are used in a single ligation! These molecules are all bumping into one another, and into DNA ligase, at random in different ways. So, if multiple products can be made, all of them *will* be made at some frequency – including ones we don't want.

How can we avoid the "bad" plasmids? When we transform bacteria with DNA from a ligation, each one takes up a different piece of DNA. We can check the bacteria after transformation and use only the ones with the correct plasmid. In many cases, plasmid from transformed bacteria is analyzed using another restriction digest to see if it contains the right insert in the right orientation.