RESTRICTION ENZYME BACKGROUND

In order to make new combinations of DNA (for example, if you wanted to stick your favorite gene into a plasmid), you need a tool to help you cut and paste DNA together. Luckily, there is a large class of enzymes called **restriction endonucleases**, or **restriction enzymes**. Each restriction enzyme recognizes a specific, short (typically 4-8 bp) sequence of DNA and makes a cut wherever that sequence is found. The resulting pieces of DNA are typically called "fragments." What makes these enzymes particularly useful is that the cut they make is often staggered, rather than blunt; on each side of the cut, it will leave one strand of the DNA a little longer than the other (the difference is typically just a handful of base pairs). Think about what this means--the resulting ends of DNA are "sticky" because there are unpaired bases (Figure 1).

Another very cool feature of restriction enzymes is that they cut at palindromic sequences. In English, a palindrome refers to a word or sentence that reads the same forwards and backwards: "dud" is a very short palindrome, and "A man, a plan, a canal, Panama!" is one of the most famous palindromes. (You can find huge archives with a search on the web.) An example of a palindromic DNA sequence (for the restriction enzyme Xho I) is read "CTCGAG" in the 5'-3' direction. Note that this strand does not read the same backwards (CTCGAG ≠ GAGCTC). The palindrome here lies in the fact that the *other,* complementary (3'-5') strand reads backwards: GAGCTC.

In other words, **both strands are identical in the 5'-3' direction**. So why is this cool? Beyond being interesting, it means that any Xho I sticky end can bind with any other Xho I sticky end. Therefore, if you cut on either side of your favorite gene with the same restriction enzyme you cut your plasmid with, the sticky ends will sometimes pair up correctly to splice your gene into the plasmid. Cool, no?

Because the sequence of bases that Xho I recognizes is not the same as that for a different restriction enzyme like EcoR I, the bases in their sticky ends will not pair with each other. In other words, the stickiness of the cut ends is very specific.

A note on restriction enzyme names: most are named after the scientific name of the species they were isolated from; Xho I is from *Xanthomonas holcicola*, and EcoR I is from *E. coli.* Pronunciation is a little funny and sometimes varies by lab; for example, in some labs, EcoR I is pronounced "eek oh are one." In other places, you may hear "eck oh are one."

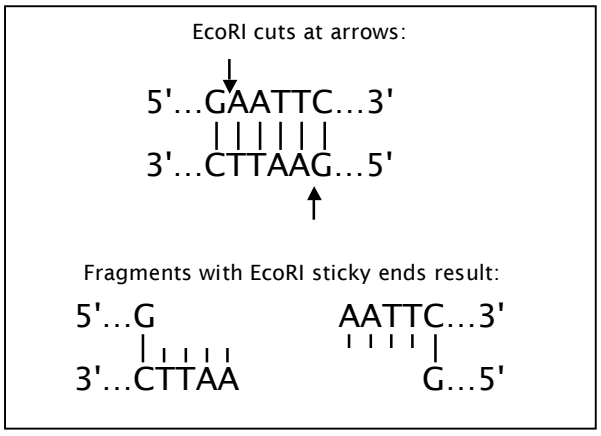

Figure 1. Example of restriction enzyme digestion.