RESTRICTION ENZYMES

Synthesis of nucleic acids is catalyzed by specific enzymes. DNA polymerase synthesizes DNA and RNA polymerase synthesize RNA. Degradation of nucleic acids also occurs under the influence of specific enzymes. There are exonucleases and endonucleases. Exonucleases remove residues one at a time from the end of the molecule, while endonucleases cut individual bonds within the molecule. Several endonucleases, derived from bacteria, are used by the molecular biologist to fragment nucleic acids at defined points. These are frequently referred to as restriction enzymes.

Carefully isolated DNA is often too large in length for us to easily work with it; consequently, we need to cut it up into pieces of a manageable size. This is typically done using **restriction enzymes** which bind to DNA at specific recognition sites (specific sequences of bases). The recognition site is typically 4 to 8 bases long and in the form of an **inverted repeat**.

5' -- G - G - A - T - C -C --3'

3' -- C - C - T - A - G - G --5'

Recognition site for BamH1

5' -- G - A - A - T - T - C --3'

3' -- C - T - T - A - A - G -- 5'

Recognition site for EcoR1

5' -- G - T - T - A - A - C -- 3'

3' -- C - A - A - T - T - G --5'

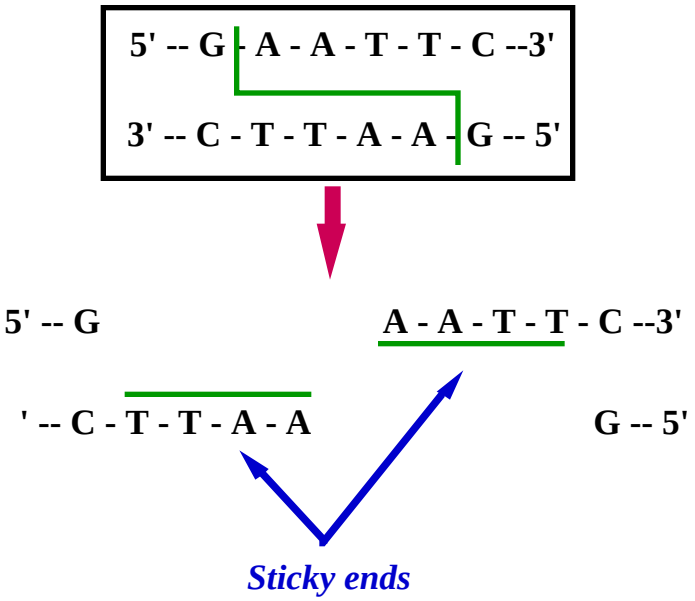
Recognition site for Hpa1

Restriction enzymes are bacterial endonucleases and they are widely used in the molecular biology laboratory. Like many other situations in molecular biology, the nomenclature of restriction endonucleases is historical and may not have apparent logic.
Restriction enzymes get their name from the bacteria from which they were isolated in combination with some input from the molecular biologist. The

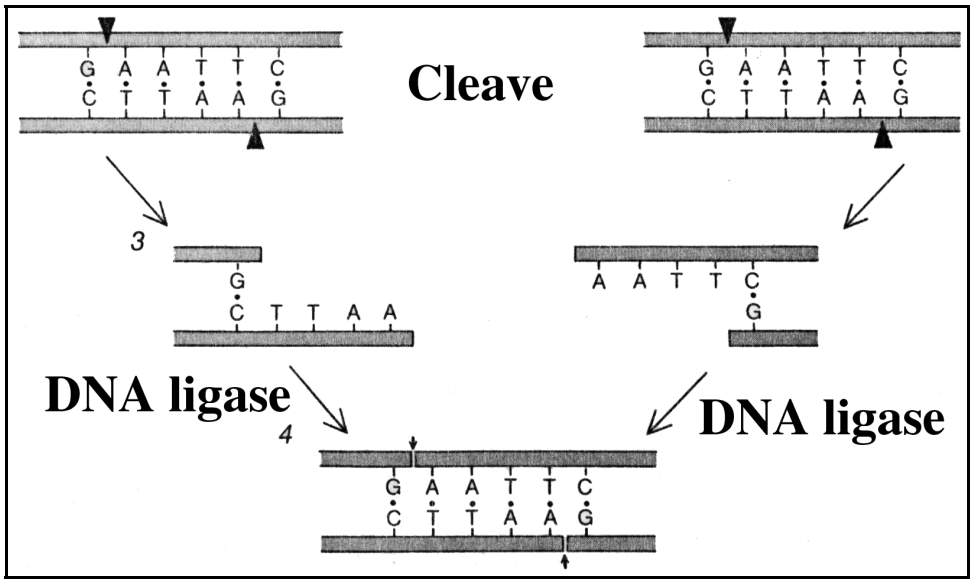
restriction enzyme **EcoRI** originated from **E**schericia **co**li strain **R**Y13 and the "**I**" derives from the fact that this was the first restriction enzyme found in this species of bacteria.

Type II restriction enzymes cut the DNA at the **recognition site** in an known position and yield either blunt ends or sticky ends.

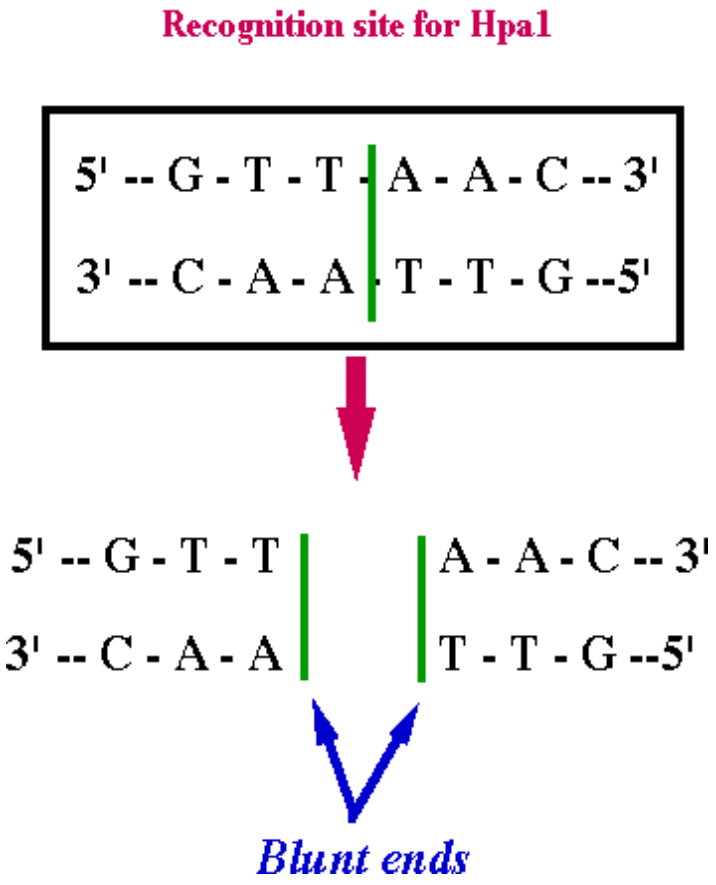
Recognition site for EcoR1



In the presence of **DNA ligase**, the sticky ends can hybridize with complementary nucleotide sequences.



Ligation of blunt ends is inefficient and requires large amounts of DNA ligase while sticky ends are more readily susceptible to ligation.



Restriction Fragment Length Polymorphisms

Restriction fragment length polymorphisms **(RFLPs)** are found as a consequence of fragmenting the DNA with restriction endonucleases. Since each restriction enzyme recognizes a specific sequence, then a single base change will prevent the site-specific cutting of that DNA.

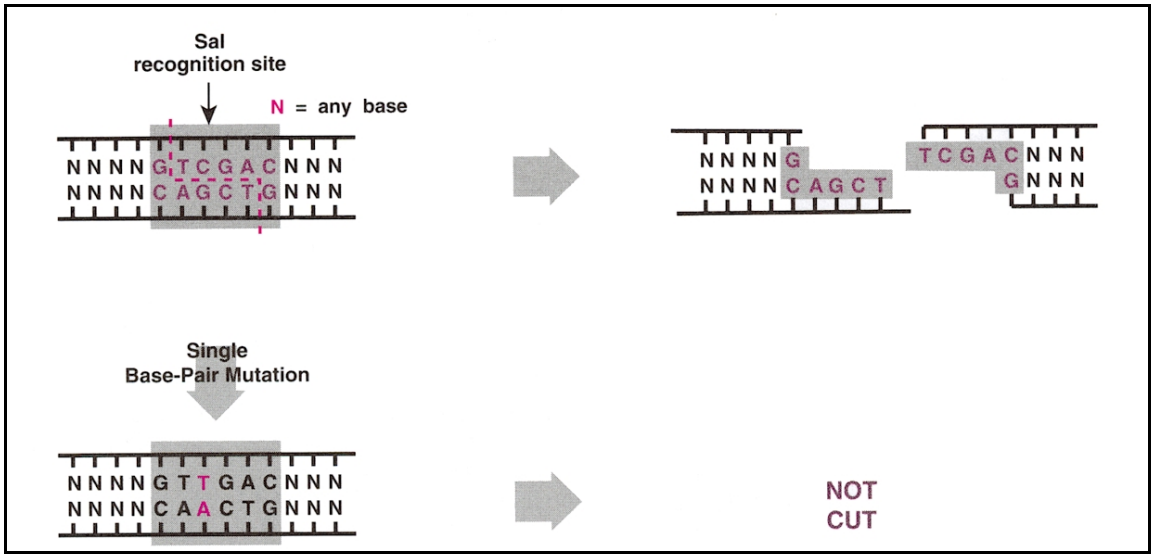


Figure from: D.P. Clark and L.D. Russell (1997) *Molecular Biology Made Fun and Simple* with permission from Cache River Press, Vienna, Illinois, USA.

Small differences in DNA sequences frequently exist within the genome usually as a result of differences in inherited maternal and paternal alleles. In some instances the difference resides within the recognition site for a specific restriction enzyme. If we cut two related, but different, DNA molecules with the appropriate restriction endonuclease, we may get segments of DNA that differ in length. These can be detected based upon their different size using an electrophoretic gel. The resulting difference between the two fragmented DNA molecules is referred to as an RFLP. Even though we may not appreciate the function of the DNA segments in question, their identification allows use to identify organisms, establish relationships, and detect differences. The RFLP does not need to originate from a coding region of a gene to be a useful marker.