Restriction Enzymes

Restriction Enzymes

- Enzyme that cuts DNA at specific nucleotide sequences known as restriction sites.
- Found in bacteria and have evolved to provide a defense mechanism against invading viruses.
- In bacteria they selectively cut up *foreign* DNA in a process called *restriction*
- To cut the DNA, restriction enzyme makes two incisions, each strand of the DNA double helix.

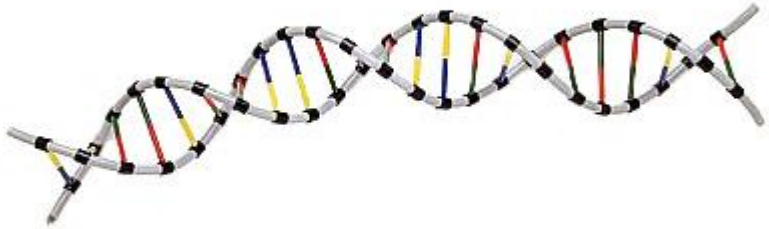
Picking a palindrome

Words that read the same forwards as backwards

Hannah	**hannaH**
Level	**leveL**
Madam	**madaM**

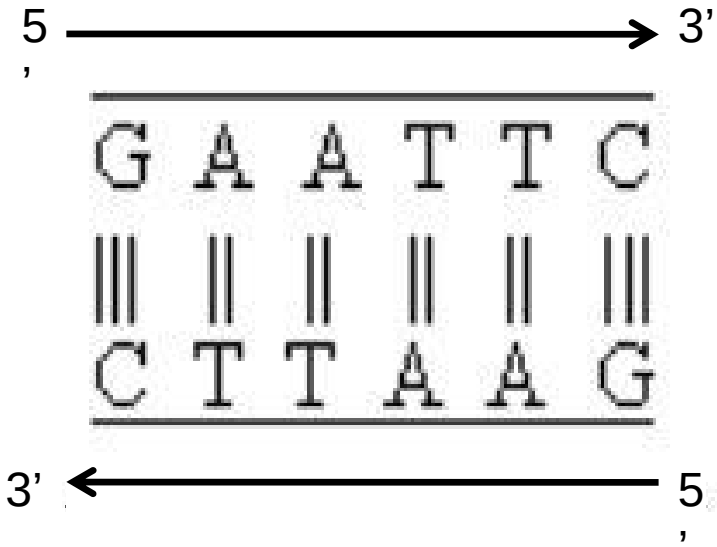
Restriction Enzymes

- Over 3000 have been identified
- More than 600 available commercially
- Routinely used for DNA modification and manipulation in laboratories.
- http://en.wikipedia.org/wiki/Restriction_enzyme



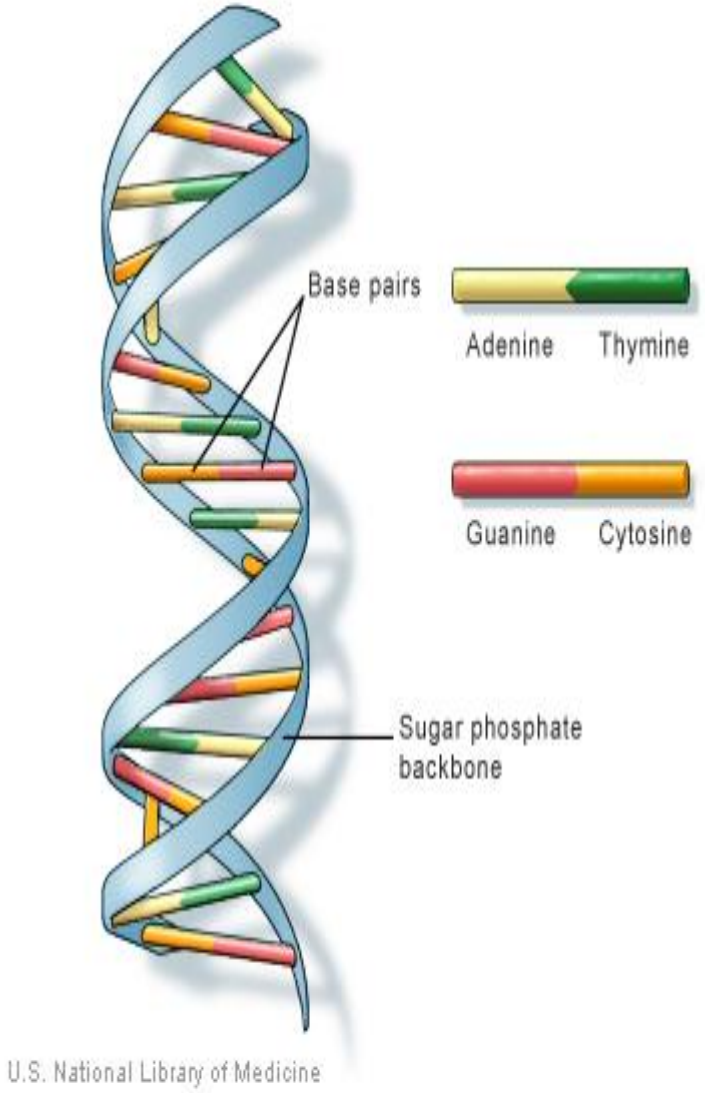
- Restriction Enzymes scan the DNA sequence
- Find a very specific set of nucleotides
- Make a specific cut

Palindromes in DNA sequences



Genetic palindromes are similar to verbal palindromes. A palindromic sequence in DNA is one in which the 5’ to 3’ base pair sequence is identical on both strands.

Restriction enzymes recognize and make a cut within specific palindromic sequences, known as ***restriction sites***, in the DNA. This is usually a 4- or 6 base pair sequence.



Each of the double strands of the DNA molecule is complimentary to the other; thus adenine pairs with thymine, and guanine with cytosine.

Restriction Endonuclease Types

Type I- multi-subunit, both endonuclease and methylase activities, cleave at random up to 1000 bp from recognition sequence

Type II- most single subunit, cleave DNA within recognition sequence

Type III- multi-subunit, endonuclease and methylase about 25 bp from recognition sequence

Restriction enzymes recognize and make a cut within specific palindromic sequences, known as *restriction sites*, in the genetic code. This is usually a 4- or 6 base pair sequence.

Example?

Hae III

HaeIII is a restriction enzyme that searches the DNA molecule until it finds this sequence of four nitrogen bases.

5’ TGACGGGTTCGA**GGCC**AG 3’

3’ ACTGCCCAAGGT**CCGG**TC 5’

Once the recognition site was found HaeIII could go to work cutting (cleaving) the DNA

5’ TGACGGGTTCGA**GGCC**AG 3’
3’ ACTGCCCAAGGT**CCGG**TC 5’

These cuts produce what scientists call
"blunt ends"

5' TGACGGGTTCGA**GG** **CC**AG 3'
3' ACTGCCCAAGGT**CC** **GG**TC 5'

The names for restriction enzymes come from:

- the type of bacteria in which the enzyme is found
- the order in which the restriction enzyme was identified and isolated.

***Eco*RI** for example

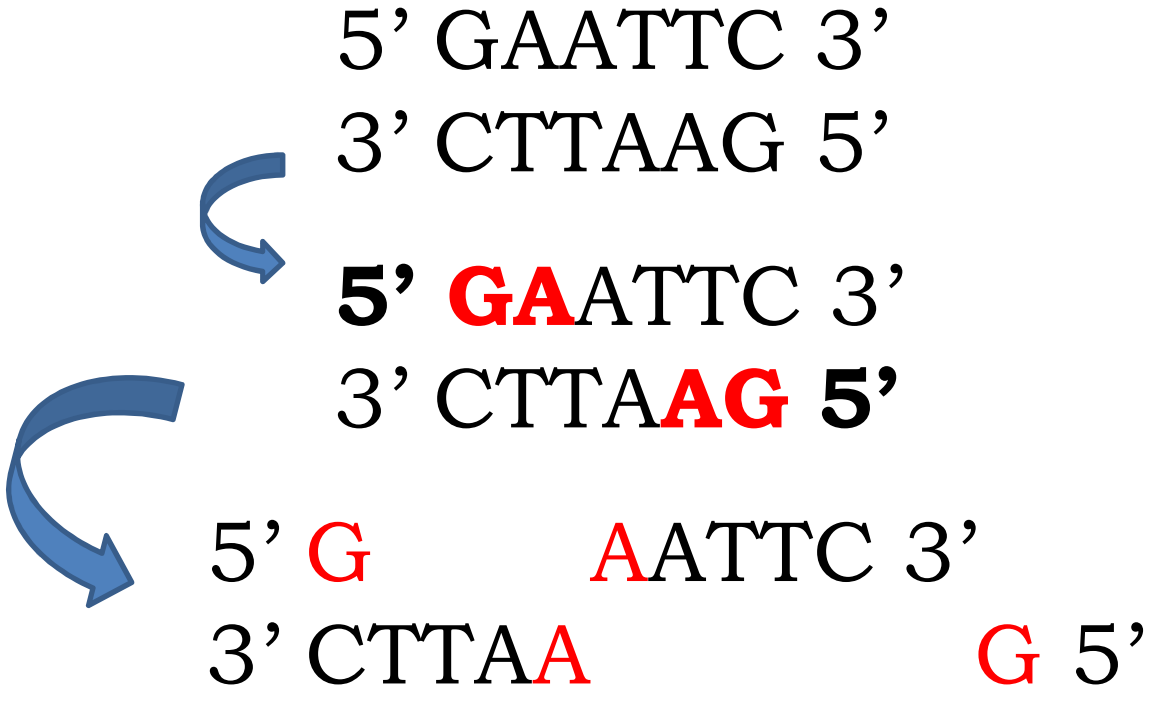
R strain of ***E.coli*** bacteria

I as it is was the first *E. coli* restriction enzyme to be discovered.

“blunt ends” and “sticky ends”

Remember how *Hae*III produced a “blunt end”?

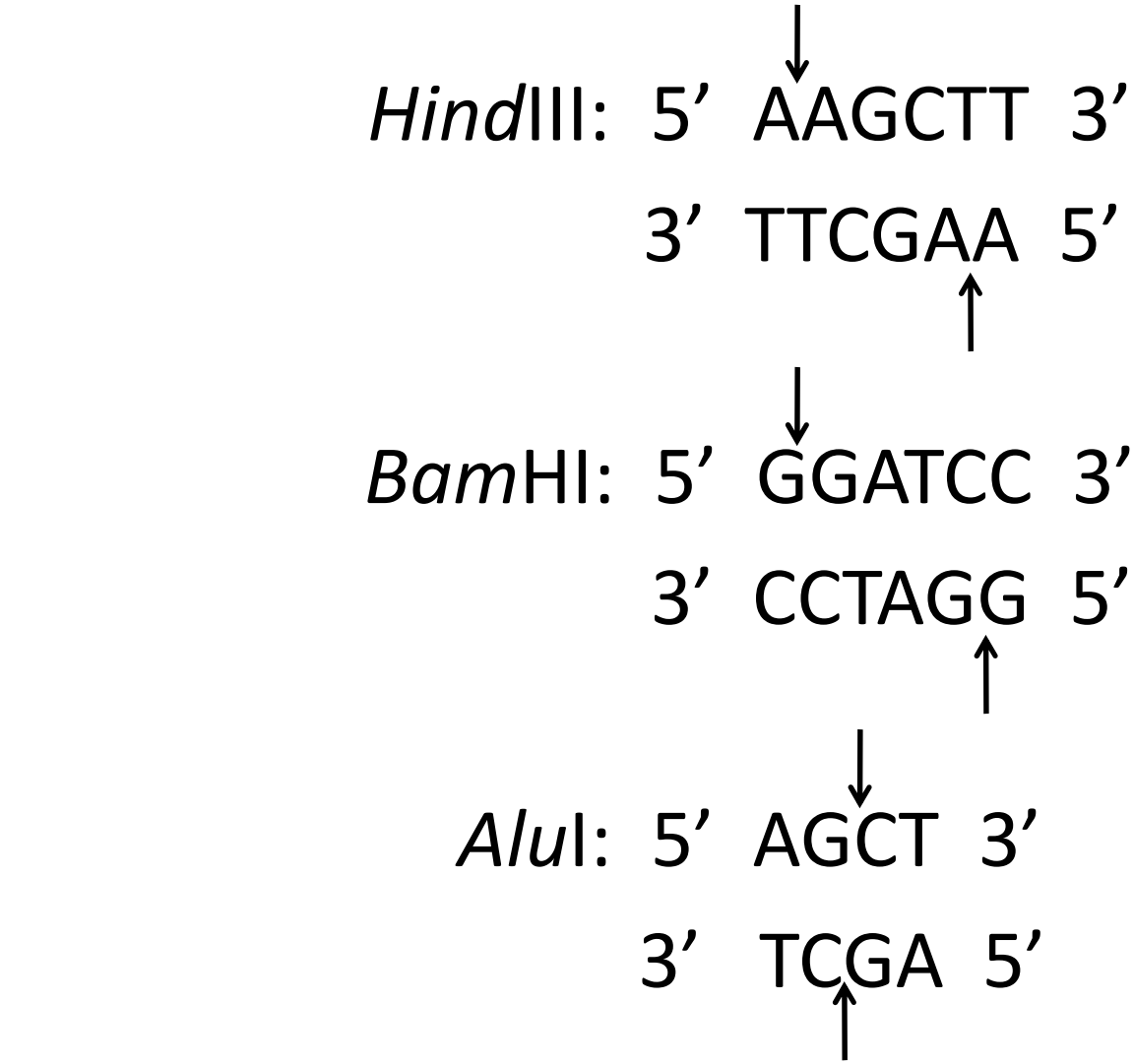
*Eco*RI, for instance, makes a staggered cut and produces a “sticky end”



blunt end

sticky end

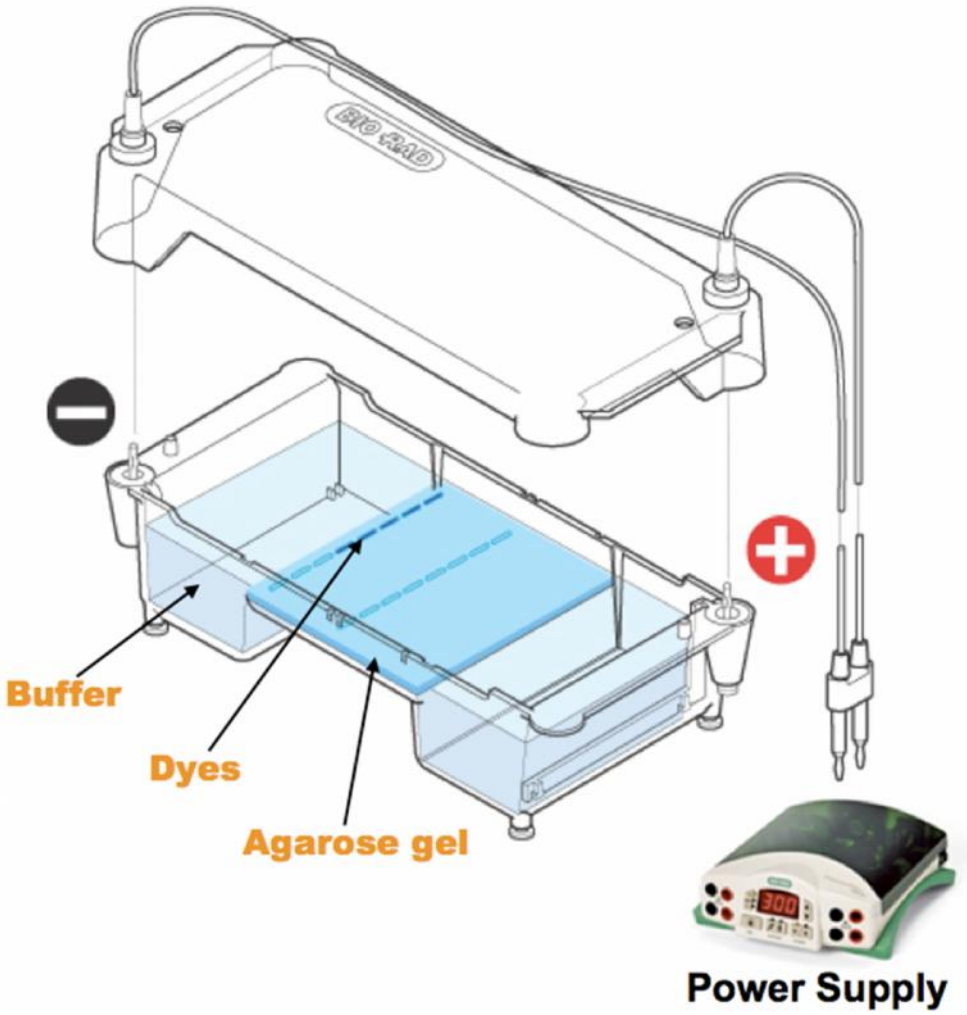
Some more examples of restriction sites of restriction enzymes with their cut sites:



Separating Restriction Fragments, I

Agarose Electrophoresis Loading

- **Electrical current** carries negatively-charged DNA through gel towards positive (red) electrode



Separating Restriction Fragments, II

Agarose Electrophoresis Running

- **Agarose gel sieves DNA fragments according to size**
  - **Small fragments move farther than large fragments**