University of Duhok

College of Sciences

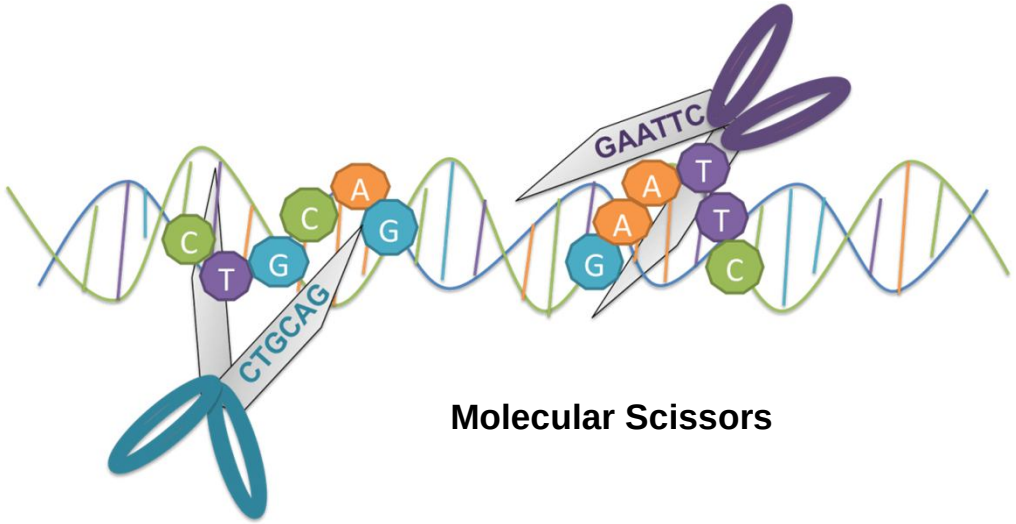
Department of Biology

4th Year Class

Lecture6. Restriction Enzymes

Lecture outlines:

- What are restriction enzymes?
- The discovery of restriction enzymes.
- Where these enzymes are found?
- Nomenclature and mode of action.
- Types of restriction enzymes.
- Applications and uses of restriction enzymes.



Molecular Scissors

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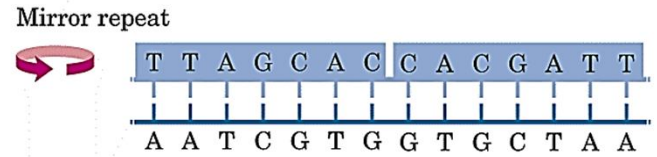
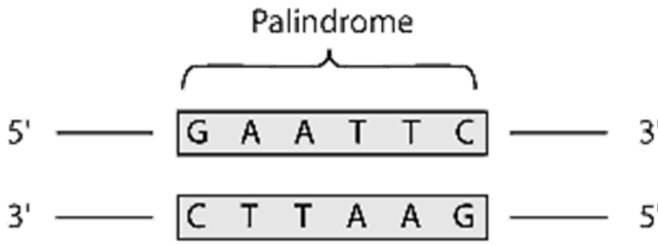
What are restriction enzymes?

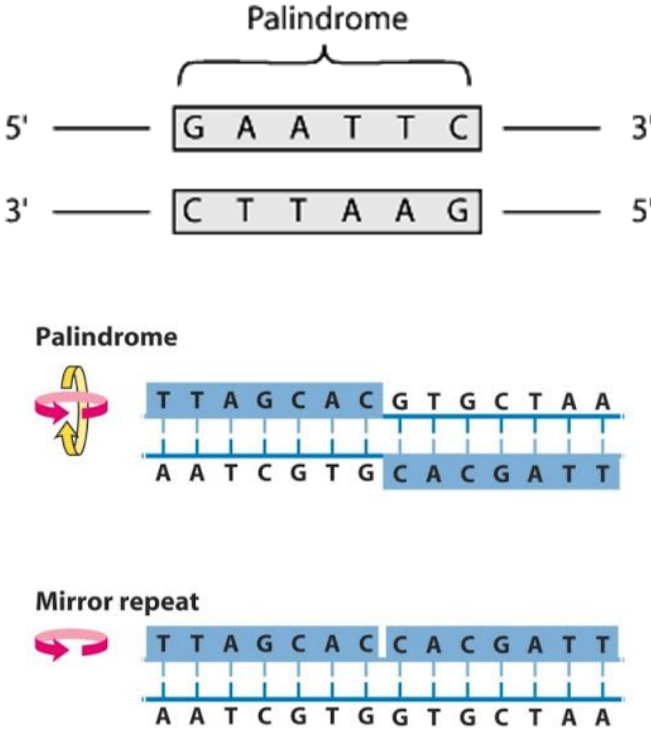
- Restriction enzymes or restriction endonucleases (molecular scissors) are enzymes that cut the sugar-phosphate backbone of double-stranded or single stranded DNA at specific recognition nucleotide sequences (mostly palindromic or symmetry) known as restriction sites (or cleavage site).
- A **palindromic sequence** is a nucleic acid **sequence** on double-stranded **DNA** or RNA wherein reading 5' to 3' forward on one strand matches the **sequence** reading 5' to 3' on the complementary strand with which it forms a double helix (**5'-GTATAC-3'** being complementary to **3'-CATATG- 5'**).
- They are found naturally in a wide variety of prokaryotes.



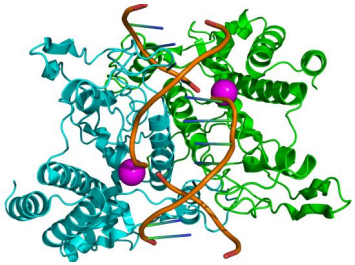


Palindrome Sequences

- The **mirror like palindrome** in which the same forward and backwards are on a single strand of DNA strand, as in …..**GTAATG**….
- The **Inverted repeat palindromes** is also a sequence that reads the same forward and backwards, but the forward and backward sequences are found in complementary DNA strands (GTATAC being complementary to CATATG).
- Inverted repeat palindromes are more common and have greater biological importance than mirror- like palindromes.



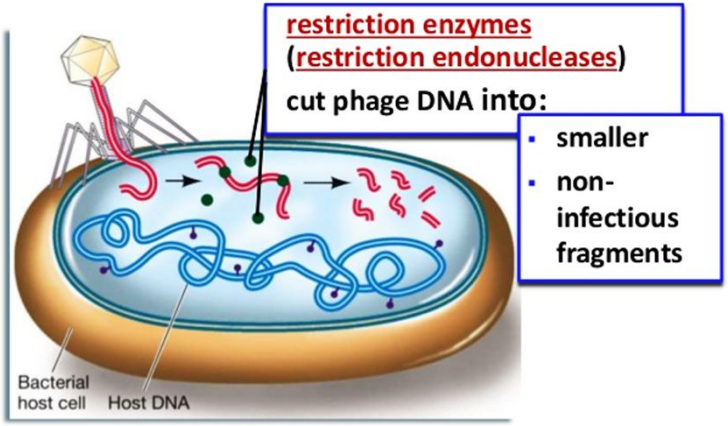
The discovery of restriction enzymes



- Restriction enzymes were first postulated by Arber and Dussoix in 1962, who discovered that certain bacteria contain Endonucleases which have the ability to cleave DNA.
- The first true restriction endonucleases was isolated in 1970 by Nathans and Smith, who purified and characterized the cleavage site of a Restriction Enzyme.
- All three scientists, Werner Arber, Hamilton Smith and Daniel Nathans, were awarded the Noble Prize in 1978 for Medicine and Physiology for their discovery of Restriction Enzymes.

Where these enzymes are found?

- Restriction enzymes are found in many different strains of bacteria where their biological role is to participate in cell defense. A bacterium uses a restriction enzyme to defend against bacterial viruses called bacteriophages, or phages.
- When a phage infects a bacterium, these enzymes restrict foreign (viral) DNA that enters the cells by cutting them into many pieces, destroying them, and prevent replication of the phage DNA.



- The host cells have a restriction-modification system that methylates their own DNA at sites specific for their respective restriction enzymes, thereby protecting them from cleavage.
- Restriction enzymes were named for their ability to restrict, or limit, the number of strains of bacteriophage that can infect a bacterium.
- Over 10,000 bacterial species have been screened for restriction enzymes. More than 3000 known enzymes have been discovered and studied in detail. More than 600 are available commercially and are routinely used for DNA modification and manipulation in laboratories.

Nomenclature of restriction enzymes

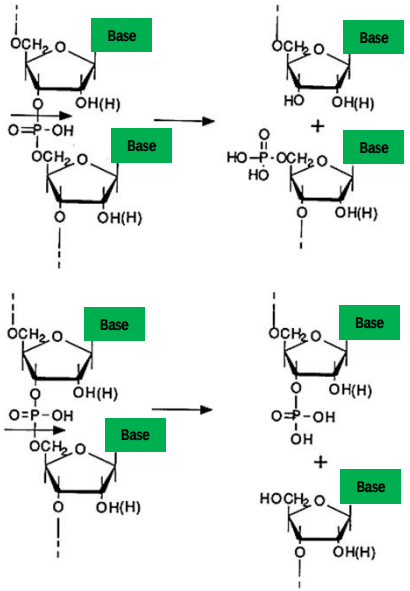
- Since their discovery in the 1970s, many restriction enzymes have been identified; for example, more than 3500 different Type II restriction enzymes have been characterized.
- Each enzyme is named after the bacterium from which it was isolated, using a naming system based on bacterial genus, species and strain.
- First letter from genus.
- Next two letters represent species.
- Additional letter or number represent the strain or serotypes.

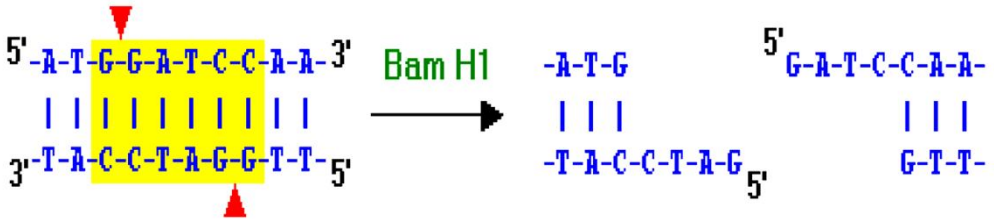
- **Example: *EcoR*1**
- **Genus: *Escherichia***
- **Species: *coli***
- **Strain: R**
- **Order discovered: 1**

- **Example: *Hind*II**
- **Genus: *Haemophilus***
  Species: *influenzae*
  serotype: d
- **Order discovered: II**

Modes of action (how restriction enzyme cuts DNA)

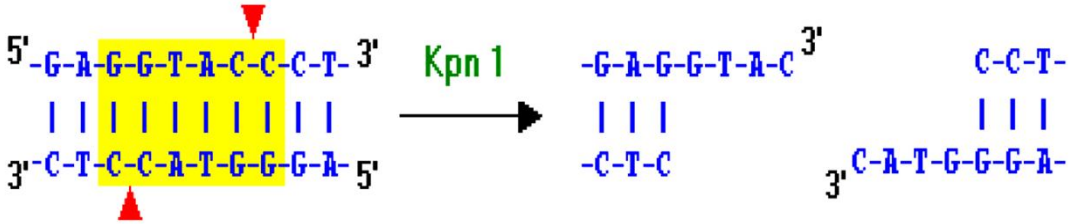
- The enzyme makes two incisions, one through each of the sugar-phosphate backbones (i.e., each strand) of the double helix without damaging the nitrogenous bases.
- Restriction enzymes hydrolyze the backbone of DNA between deoxyribose and phosphate groups. This leaves a phosphate group on the 5' ends and a hydroxyl on the 3' ends of both strands. A few restriction enzymes will cleave single stranded DNA, although usually at low efficiency.
- The restriction enzymes most used in molecular biology labs cut within their recognition sites and generate one of three different types of ends. In the diagrams below, the recognition site is boxed in yellow and the cut sites indicated by red triangles.
- **5' overhangs:** The enzyme cuts asymmetrically within the recognition site such that a short single-stranded segment extends from the 5' ends. *Bam*HI cuts in this manner.



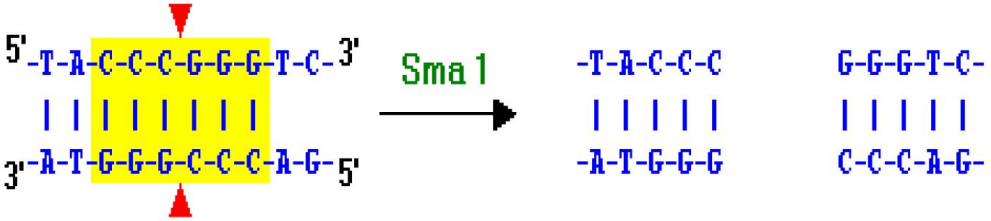


Modes of action..... continue

- **3' overhangs:** Again, we see asymmetrical cutting within the recognition site, but the result is a single-stranded overhang from the two **3'** ends. **Kpn1** cuts in this manner.



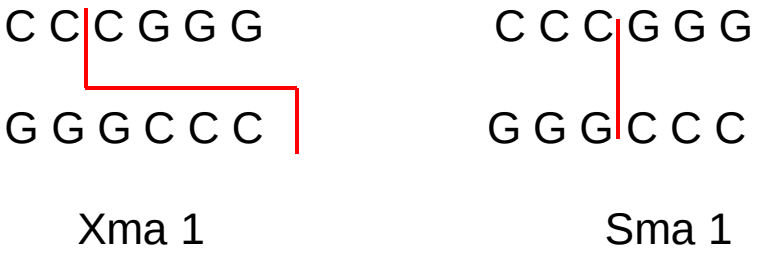
- **Blunts:** Enzymes that cut at precisely opposite sites in the two strands of DNA generate blunt ends without overhangs. **Sma1** is an example of an enzyme that generates blunt ends.



- The 5' or 3' overhangs generated by enzymes that cut asymmetrically are called sticky ends or cohesive ends, because they will readily stick or anneal with their partner by base pairing. The sticky end is also called a cohesive end or complementary end in some reference.

Isoschizomers and Neochischizomers

- Restriction enzymes that have the same recognition sequence as well as the same cleavage site are Isoschizomers. E.g: *Sph*I and *Bbu*I
- Restriction enzymes that have the same recognition sequence but cleave the DNA at a different site within that sequence are Neochizomers. E.g: Sma1 and Xma1



Types of restriction enzymes.

Restriction endonucleases are categorized into four general groups

- **Type I**
- **Type II**
- **Type III**
- **Type IV**

These types are categorized based on:

- Their composition.
- Enzyme co-factor requirement.
- The nature of their target sequence.
- Position of their DNA cleavage site relative to the target sequence.

Type I restriction enzymes

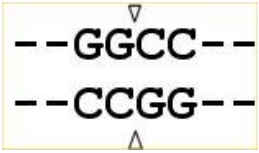
- Capable of both restriction and modification activities.
- The co factors S-Adenosyl Methionine(AdoMet), ATP, and Mg^{2+} are required for their full activity.
- **Contain:**
  - Two restriction (R) subunits.
  - Two methylation (M) subunits.
  - One specifity (S) subunits.
- Cleave DNA at random length from recognition sites.

Type II restriction enzymes

- These are the most commonly available and used restriction enzymes.
- They are composed of only one subunit.
- Their recognition sites are usually undivided and palindromic and 4-8 nucleotides in length.
- They recognize and cleave DNA at the same site.
- They do not use ATP for their activity.
- They usually require only Mg^{2+} as a cofactor.

Type II cut frequency:

The frequency by which a Type II restriction enzyme will cut DNA is mainly a function of the length of the sequence it is sensitive to. For instance:



4-base cutter

- **4-base cutter:**

  BsuR1 has a recognition sequence of 4 base-pairs. Out of sheer probability, we see that with 4 positions, and each position having potentially 4 different values, there are $\mathbf{4^4 = 256}$ different possibilities for any given 4-base long strand. Therefore, theoretically (assuming completely random DNA), this enzyme will cut **1** in **256** 4-base-pair long sites.

Type II cut frequency:……. continue

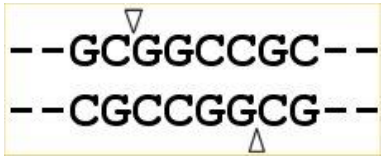
- **6-base cutter:**

  EcoR1, on the other hand, recognizes a sequence of 6 base-pairs. **4^6 = 4096** possible combinations with this length, and so EcoR1 will cut **1** in **4096** 6-base-pair long sites.

6-base cutter

- **8-base cutter:**

  Not1 has a recognition sequence of 8 base-pairs. **4^8 = 65536** possible combinations, and so Not1 will theoretically cut one out of 65536 8-base-pair long sites.



8-base cutter

As you can see, the frequency of cutting has much to do with the length of the sequence (because that is directly affected by probability).

Type III restriction enzymes

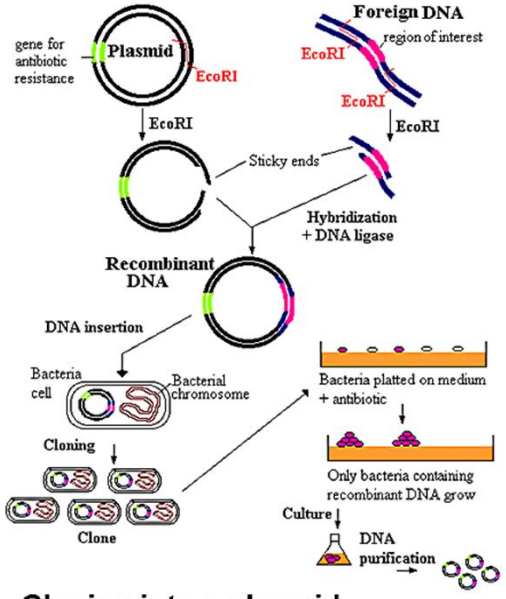
- Type III restriction enzymes recognize two separate non-palindromic sequences that are inversely oriented.
- They cut DNA about 20-30 base pairs after the recognition site.
- These enzymes contain more than one subunit.
- These enzymes are very large with many subunits and require S-Adenosyl methionine, and ATP cofactors for their roles in DNA methylation and restriction.

Type IV restriction enzymes

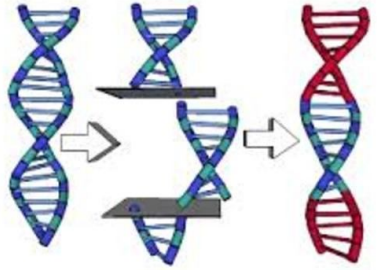
- Cleave only abnormal or modified DNA containing methylated, hydroxymethylated, and glucosylhydroxymethylated bases.
- Recognition sequences have not been well defined.
- Cleavage takes place ~30 bp away from one of the sites.

Applications and uses of restriction enzymes.

- Restriction Enzymes are used in the following areas:
  - DNA fingerprinting.
  - DNA typing/profiling.
  - DNA sequencing.
  - Gene splicing/recombinant DNA.
  - Transformation.
  - Human Genome Project.



Cloning into a plasmid



Question Time