Lecture.3

Restriction enzymes

Restriction enzymes (also known as restriction endonucleases) are enzymes that recognize specific short sequence, usually palindromic, of DNA sequences. These enzymes break the phosphodiester bond of double-strand DNA(dsDNA)near or within a recognition site. Bacterial cells protect their DNA from the action of their own restriction enzymes action by adding methyl group through a process called methylation. This methylation restricts the enzymes from targeting and breaking the DNA.

A palindromic sequence is a nucleic acid sequence in a double-stranded DNA or RNA molecule whereby reading in a certain direction (e.g. 5' to 3') on one strand is identical to the sequence in the same direction (e.g. 5' to 3') on the complementary strand. This definition of palindrome thus depends on complementary strands being palindromic of each other.

Function of restriction and modification enzyme in the nature

Bacteriophages invade bacteria as their host cell, and for any invader a defense mechanism will be developed. For bacteria, this mechanism is the host restriction/ modification system, composed not only of the restriction endonuclease but also a methylating enzyme. Restriction endonucleases cut or cleave target sequences on phage DNA as part of this defense mechanism, whereby the phage will be rendered harmless. DNA of the invading phage will be digested at the recognition sites; however, the host DNA itself should be somehow protected from this digestion, which is achieved by modifying the bacterial DNA by addition of a methyl group to target recognition sites (modification). In other words, for an E. coli EcoRI restriction enzyme that recognizes the sequence 5′-GAATTC-3′ on phage DNA, there has to be an EcoRI methylase that modifies the same sequence in the bacterial genome (Figure.3.1).

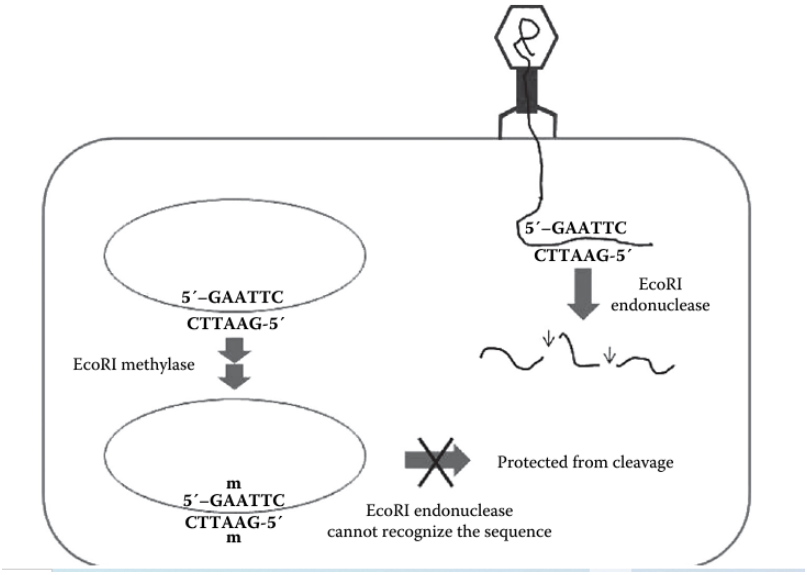


Figure.3.1: A schematic summary of the restriction/ modification system. In this example, the recognition motifs for the EcoRI restriction endonuclease in the host genome are modified by the EcoRI methylase, which covalently adds a methyl group to the adenine nucleotide. This modification does not affect the structure of the host DNA, but simply disables the endonuclease from recognizing the motif, thus the host genome is protected from cleavage.

Types of restriction enzymes:

Four categories of restriction enzymes were classifies based on their complexity, cofactor requirements, and enzymes recognition sequence.

1. Type I RE

A Type I restriction enzyme is a complex enzyme that recognizes specific sequences of DNA and cuts the DNA at locations that can be quite a distance away from the recognition site ((usually quite some distance away, up to 1000 bp), producing fairly random fragments.

They are less commonly used in molecular biology laboratories for DNA manipulation because their cutting sites are unpredictable, making them less practical for precise DNA cloning or gene editing tasks.

Ex: EcoDR2 TCANNNNNN GTCG

Requirements of Type I enzyme activity:

1. Recognition Sequence: Type I enzymes recognize specific DNA sequences. These recognition sequences are not where the cleavage occurs but are essential for enzyme binding.

2. S-Adenosylmethionine (SAM): SAM is required as a cofactor for the methyltransferase activity of Type I enzymes, which methylates specific adenine residues within the recognition sequence to protect the host DNA from being cleaved.

3. **ATP**: ATP is essential for the motor activity of the enzyme, providing the energy required for the enzyme to translocate along the DNA after binding to its recognition sequence and before cleaving the DNA at sites that can be thousands of base pairs away from the recognition site.

4. **Mg^{2+} Ions**: Magnesium ions are crucial for the enzymatic activity, as they are involved in the catalysis of both the cleavage and methylation reactions.

5. **DNA Substrate**: A DNA molecule containing the specific recognition sequence for the Type I restriction enzyme is necessary for the enzyme to bind and initiate its activity.

The application of Type I

Type I restriction-modification systems serve primarily as a bacterial defense mechanism against foreign DNA, such as phages, rather than tools for genetic engineering due to their complex and less predictable nature compared to Type II restriction enzymes.

TypeII restriction enzyme

Type II restriction enzymes are a class of enzymes that are widely used in molecular biology for their ability to precisely cut DNA at specific recognition sites. These enzymes recognize short, specific sequences of DNA (usually 4 to 8 base pairs in length) and cleave the DNA at or near these recognition sites, producing predictable and reproducible DNA fragments. Unlike Type I and Type III restriction enzymes, Type II enzymes do not require ATP for their activity and typically cut within or very close to their recognition sequences without significant sequence-specific movement along the DNA.

Characteristics of Type II restriction enzymes include:

1. **Specificity**: Each Type II enzyme has a specific recognition sequence, often a palindromic sequence, where the sequence reads the same backward and forward on complementary strands. For example, the EcoRI enzyme recognizes the sequence 5'-GAATTC-3' and cuts between G and A on both strands.

2. Cleavage Site: These enzymes cleave DNA at specific sites within or very close to their recognition sequences. The cuts can produce "blunt" ends (straight cuts across both DNA strands) or "sticky" ends (staggered cuts that produce overhanging ends).

3. No ATP Requirement: Type II enzymes work without the need for ATP, differentiating them from Type I and Type III enzymes, which require ATP for their cleavage activity.

4. Simplicity and Efficiency: Due to their simplicity and the predictability of their action, Type II restriction enzymes are essential tools in molecular biology for cloning, DNA mapping, and various genetic engineering applications.

Because of these properties, Type II restriction enzymes have become fundamental tools in genetic engineering, allowing scientists to cut and paste DNA fragments precisely and with high specificity. This has enabled the development of recombinant DNA technology, the construction of DNA libraries, and the analysis and manipulation of genes in a wide variety of research and applied fields.

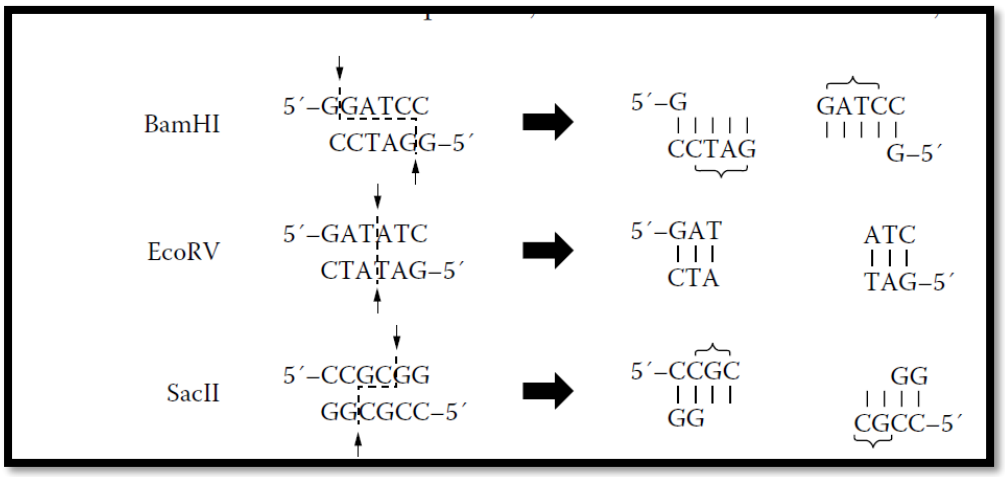


Figure: Different cut positions of restriction enzymes. BamHI cuts toward the 5' end and generates a sticky end with a 3' overhang, EcoRV cuts right in the middle of the sequence and generates a blunt end, and SacII cuts toward the 3' end to generate a sticky end with a 5' overhang.

Whats the meaning of Sticky and Blunt ends

- Sticky ends are produced when a restriction enzyme makes a staggered cut in the DNA, leaving overhanging, single-stranded portions at the ends of each DNA fragment. These overhangs are complementary to each other, meaning they can pair with any DNA molecule that has a compatible overhang.
- **Properties**: The single-stranded overhangs can easily form hydrogen bonds with complementary overhangs on other DNA fragments, facilitating the annealing of fragments cut with the same enzyme or different enzymes that produce compatible overhangs.

- **Use in Molecular Biology**: Sticky ends are particularly useful in genetic engineering because they increase the efficiency of DNA ligation, where DNA fragments are joined together. The specificity of base pairing between sticky ends helps ensure that fragments are joined in the correct

Blunt ends

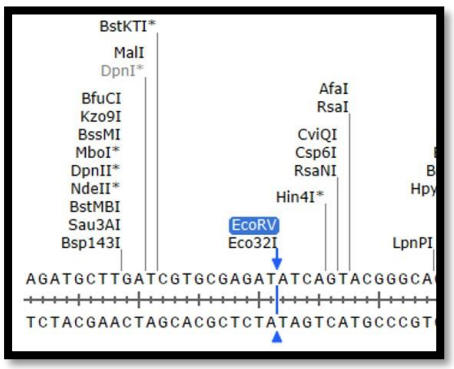
Definition: Blunt ends are produced when a restriction enzyme cuts straight through both DNA strands at the same location, leaving no overhangs. The ends of each DNA fragment are flush with each other, with no single-stranded DNA protruding.

Properties: Blunt ends can be more challenging to ligate than sticky ends because they lack the natural tendency to anneal to complementary sequences. The ligation of blunt-ended fragments often requires higher concentrations of DNA, more ligase enzyme, and sometimes specialized conditions to achieve efficient joining.

Use in Molecular Biology: Despite being somewhat less convenient for ligation, blunt ends are still useful in various molecular cloning strategies. They allow for the joining of DNA fragments regardless of sequence, providing flexibility in assembling fragments that do not have compatible sticky ends.

In summary, sticky ends and blunt ends represent two different types of DNA fragment ends generated by restriction enzyme cleavage. Sticky ends, with their single-stranded overhangs, facilitate easy and specific joining of DNA fragments. In contrast, blunt ends, without overhangs, offer a more general but less efficient approach for ligating DNA fragments.

A



B

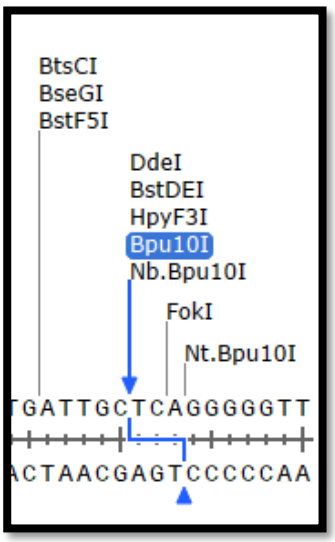


Figure: Digestion with RE. A. EcoRV producing blunt end. Enzyme recognize and cut within recognition sequence GAT/ATC. B. Bpu10I recognition site CCTNAGC

Ex: Examine the following sequences and answer questions?

5-GGTCTGTAGC TGCCATTAAAC ATTTAC TGCAGTCGGG-3

A. **Sequences digested with PStI RE, which recognise TGTAG (T ↓GCAG)**
B. **Sequences digested with ECoRII, which recognise TGCC (T ↓TAA)**
C. **Sequences digested (incubated) with double restriction enzymes PStI and BglI (T TAA)**
D. **Sequences digested with AciI C↓CGC**

GGTCTGTAGC T ↓GCAGTTAAAC ATTTAC TGCCTT ↓AAGTCG

A. 2

B. 2

C.(3)

D. 0