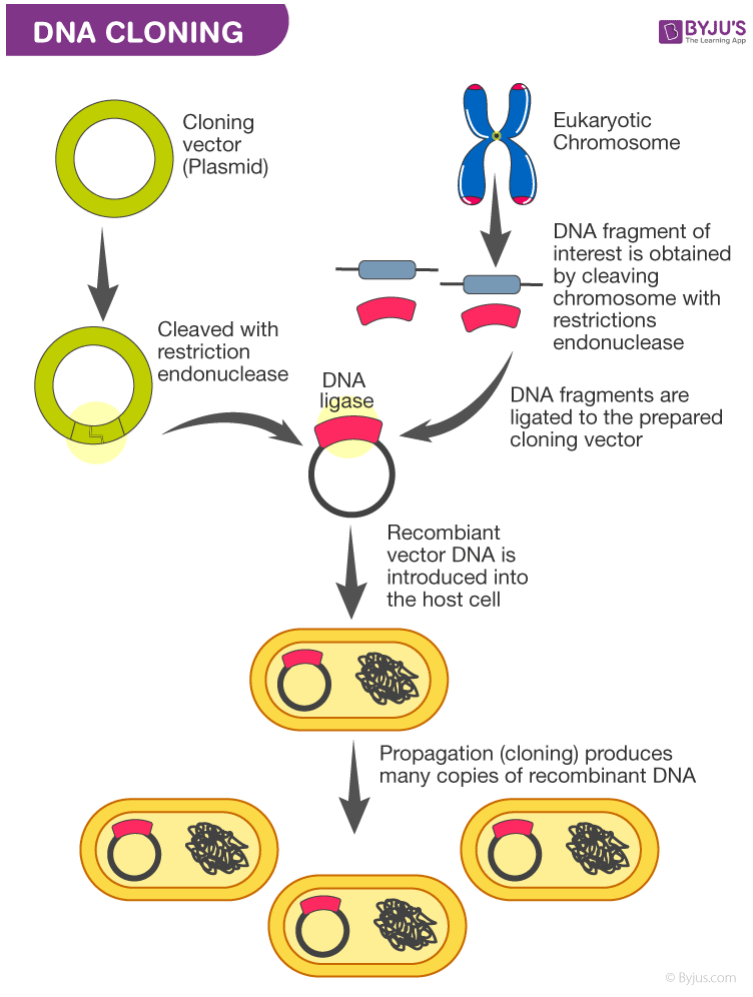


FDE 330
FOOD BIOTECHNOLOGY

Recombinant DNA Technology

Clone-DNA (or Gene or Molecular) Cloning

- **Clone:** A *clone* is an identical copy. This term originally applied to cells of a single type, isolated and allowed to reproduce to create a population of identical cells. **When applied to DNA, a clone represents many identical copies of a particular gene segment.**

- **DNA cloning:** Cloning means the production of an exact copy—specifically, an exact genetic copy—of a gene, cell, or organism.

  In brief, the researcher must cut the gene out of the larger chromosome, attach it to a much smaller piece of carrier DNA, and allow microorganisms to make many copies of it. This is the process of DNA cloning.

Recombinant DNA-Recombinant DNA Technology

- **Recombinant DNA** (rDNA) is a form of artificial DNA that is created by combining two or more sequences that would not normally occur together through the process of gene splicing.

- **Recombinant DNA Technology** is defined as “the joining together of DNA molecules from different organisms and inserting it into a host organism to produce new genetic combinations that are of value to science, medicine, agriculture and industry.”

  (by the Encyclopedia Britannica)

- **Recombinant DNA (rDNA) molecules** are DNA molecules formed by laboratory methods of genetic recombination (such as molecular cloning) that bring together genetic material from multiple sources, creating sequences that would not otherwise be found in the genome.

- **Recombinant DNA** is the general name for a piece of DNA that has been created by combining at least two fragments from two different sources. Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure, and differ only in the nucleotide sequence within that identical overall structure.

Chimera of Arezzo

- **Recombinant DNA molecules** (the vector-foreign DNA complex which is introduced into the host DNA) are sometimes called **chimeric DNA (or DNA chimera)**, because they can be made of material from two different species, like the mythical chimera.
  - (Chimera of classical Greek mythology had the head of lion, the body of a goat and the tail of a snake).

- In molecular biotechnology, recombinant DNA technology, gene cloning, and genetic engineering, in a broad sense, have the same meaning.

Recombinant DNA Technology

- As DNA is ubiquitous, found in living organisms across taxonomic classes, genes from any organism (of animal, plant, bacterial, or viral origin) could be cloned in bacteria. With the discovery of more cloning vectors, such as plasmids that could integrate into plant genomes, and several viruses, it was soon possible to clone genes in plant and animal hosts also. Thus, potentially, “designer” chimeric organisms containing specific genes conferring characteristics of choice could be created in the laboratory. This technology is referred to as **recombinant DNA (rDNA) technology** or **genetic engineering (GE)**.

Recombinant DNA Technology

- **Recombinant DNA technology**, which is also called **gene cloning** or **molecular cloning**, is a general term that encompasses a number of experimental protocols leading to the transfer of genetic information (DNA) from one organism to another.

- There is no single set of methods that can be used to meet this objective; however, a recombinant DNA experiment often has the following format:

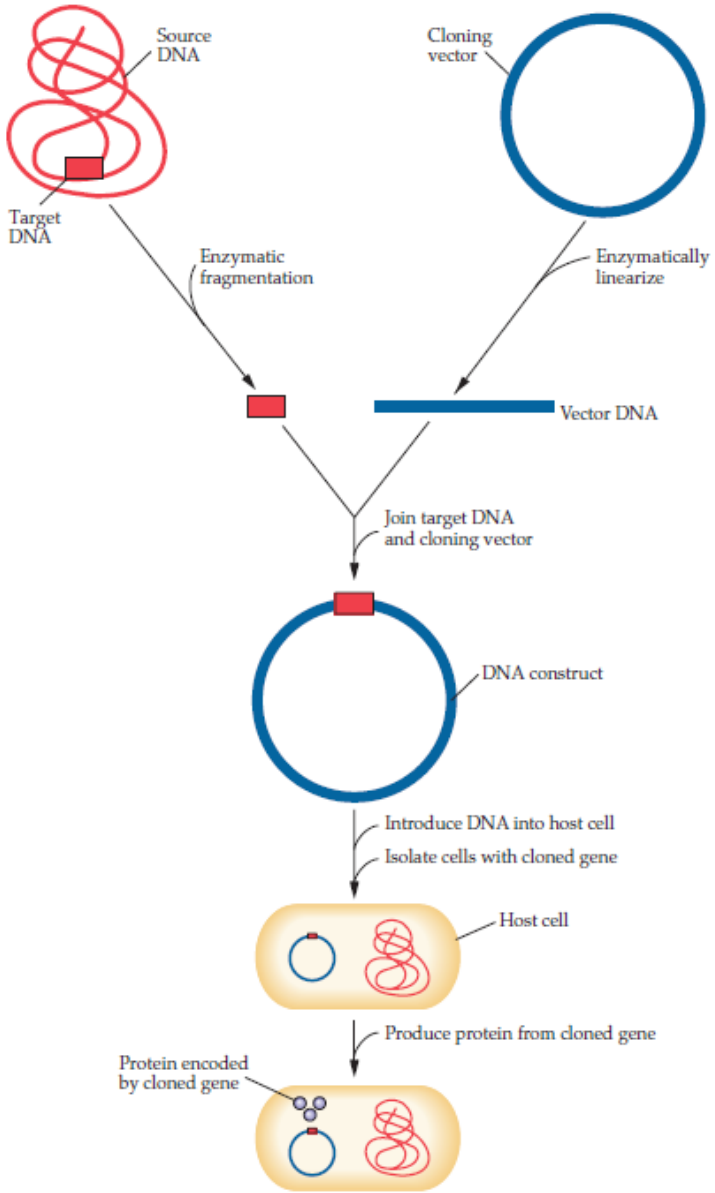
1. **The DNA (cloned DNA, insert DNA, target DNA, or foreign DNA) from a donor organism is extracted, enzymatically cleaved (cut, or digested), and joined (ligated) to another DNA entity (a cloning vector) to form a new, recombined DNA molecule (cloning vector- insert DNA construct, or DNA construct).**

2. **This cloning vector-insert DNA construct is transferred into and maintained within a host cell. The introduction of DNA into a bacterial host cell is called transformation.**

3. **Those host cells that take up the DNA construct (transformed cells) are identified and selected (separated, or isolated) from those that do not.**

4. **If required, a DNA construct can be created so that the protein product encoded by the cloned DNA sequence is produced in the host cell.**

Recombinant DNA-cloning procedure



Recombinant DNA-cloning procedure

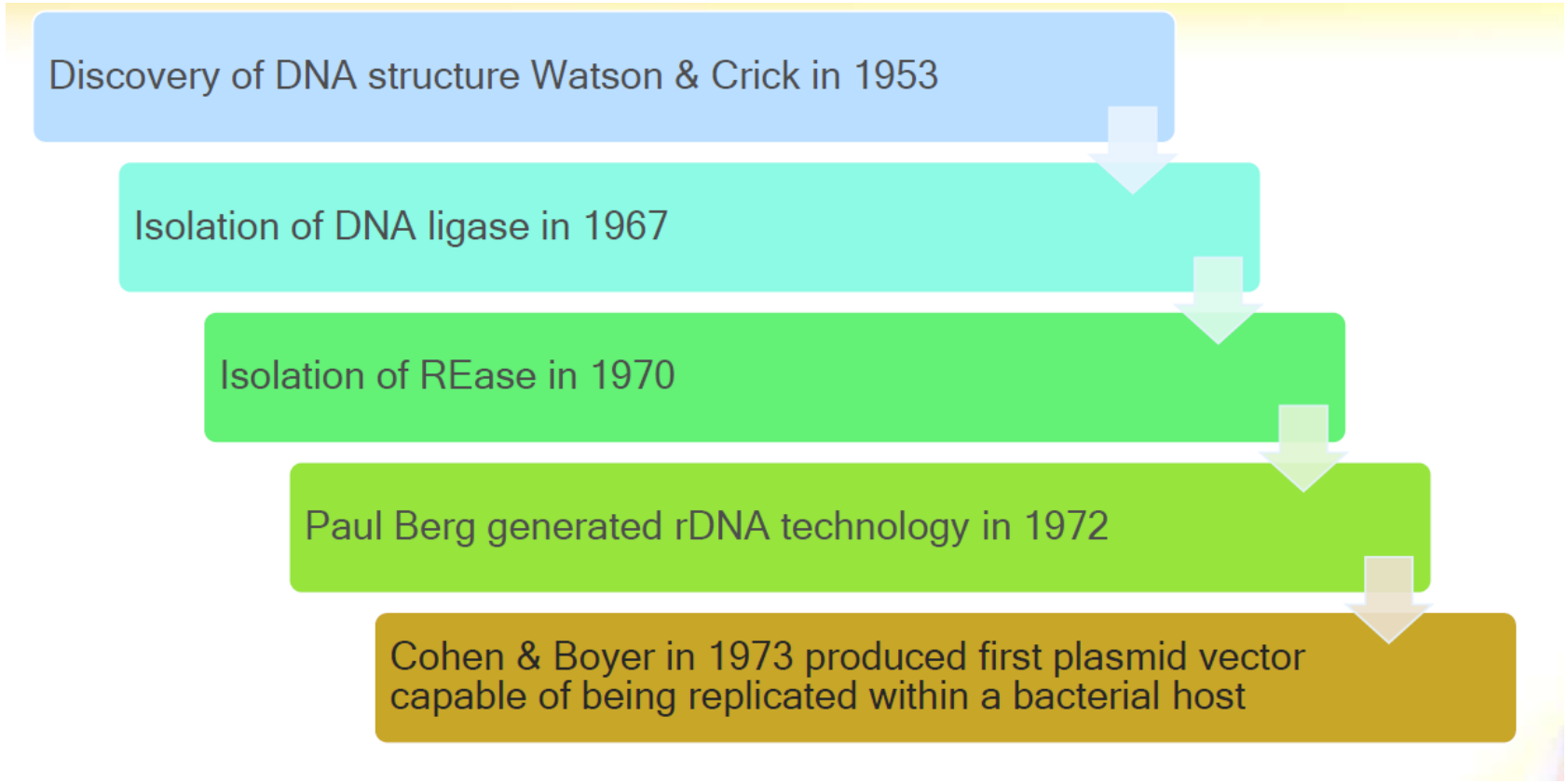
- DNA from a source organism is cleaved with a restriction endonuclease and inserted into a cloning vector.
- The cloning vector–insert (target) DNA construct is introduced into a host cell, and those cells that carry the construct are identified and grown.
- If required, the cloned gene can be expressed (transcribed and translated) in the host cell, and the protein (recombinant protein) can be harvested.

Discovery of Recombinant DNA Technology

- **Recombinant DNA technology** was developed from discoveries in molecular biology, nucleic acid enzymology, and the molecular genetics of both bacterial viruses (bacteriophages) and bacterial extrachromosomal DNA elements (plasmids).

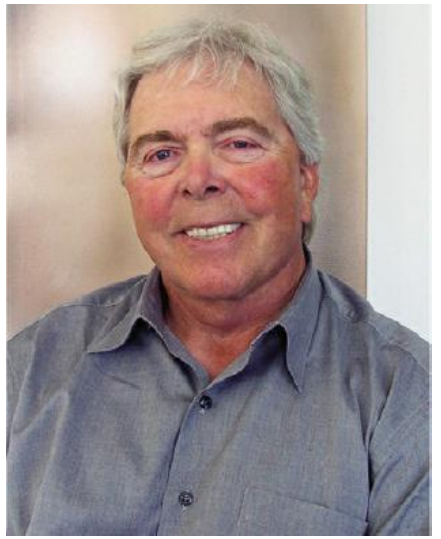
- **Recombinant DNA technology** would not exist without the availability of enzymes that recognize specific double-stranded DNA sequences and cleave the DNA in both strands at these sequences (restriction enzymes, or restriction endonucleases). Nucleases that cut nucleic acid molecules internally are endonucleases, and those that degrade from the ends of nucleic acids are exonucleases.

Discovery of Recombinant DNA Technology

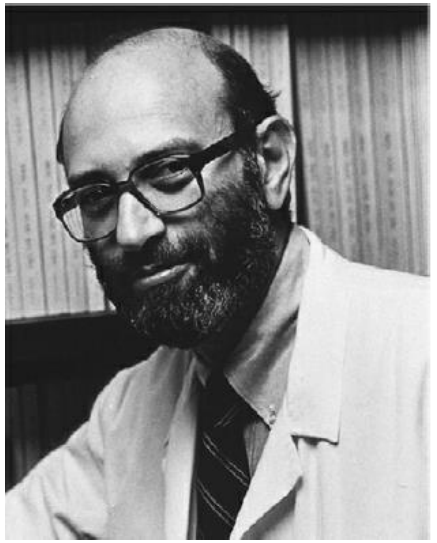


- Recombinant DNA technology is one of the recent advances in biotechnology, which was developed by two scientists named **Stanley N. Cohen** and **Herbert Boyer** in 1973.

- **Stanley N. Cohen** and **Herbert Boyer** constructed the first recombinant DNA using bacterial DNA and plasmids.



Herbert Boyer



Stanley N. Cohen

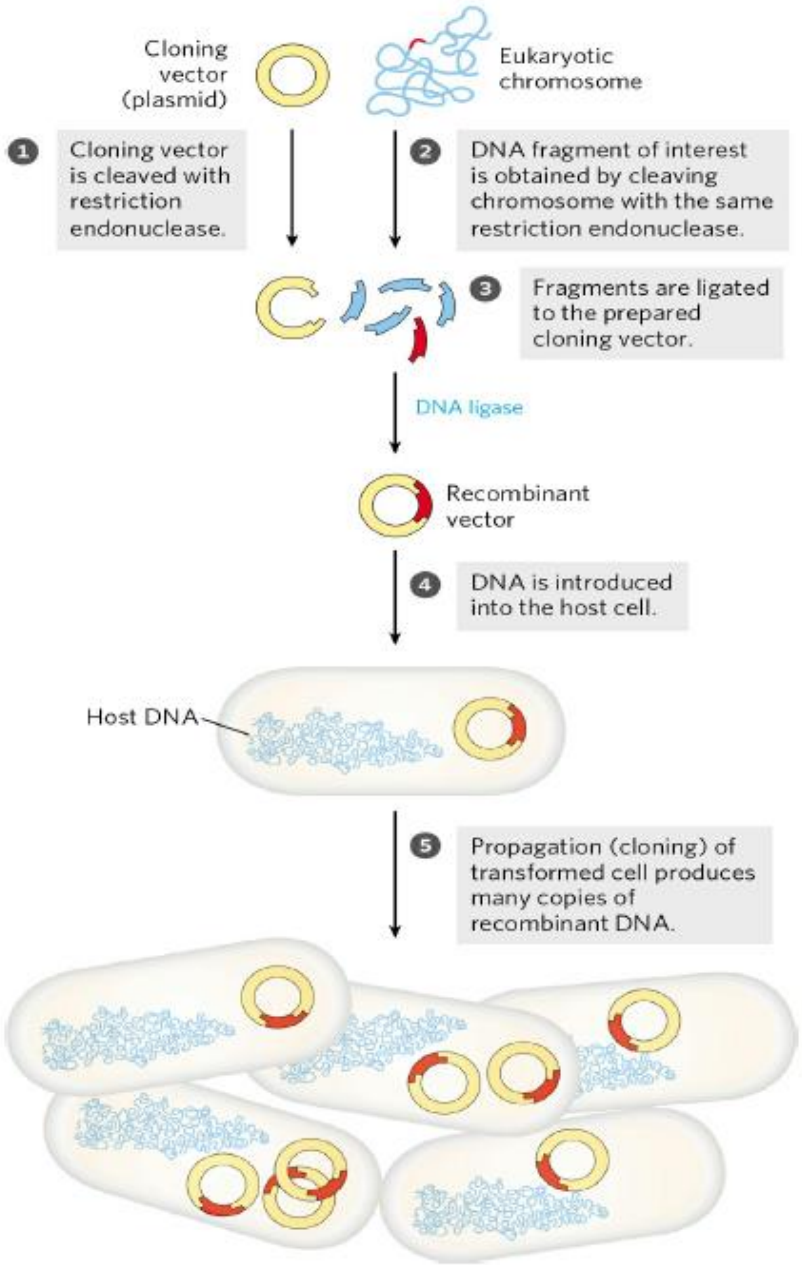
Gene Cloning: The Big Picture

- **Cloning** entails cutting DNA into fragments with enzymes; selecting and possibly modifying a fragment of interest; inserting the DNA fragment into a suitable cloning vector; transferring the vector with the DNA insert into a host cell for replication; and identifying and selecting cells that contain the DNA fragment.

Steps of DNA Cloning

Classically, the cloning of DNA from any organism entails five general procedures:

1. ***Obtaining the DNA segment to be cloned***
2. ***Selecting a small molecule of DNA capable of autonomous replication***
3. ***Joining two DNA fragments covalently***
4. ***Moving recombinant DNA from the test tube to a host organism***
5. ***Selecting or identifying host cells that contain recombinant DNA***



Schematic illustration of DNA cloning

- A cloning vector and eukaryotic chromosomes are separately cleaved with the same restriction endonuclease. (A single chromosome is shown here for simplicity.)
- The fragments to be cloned are then ligated to the cloning vector.
- The resulting recombinant DNA (only one recombinant vector is shown here) is introduced into a host cell, where it can be propagated (cloned).
- (Note that this drawing is not to scale: the size of the *E. coli* chromosome relative to that of a typical cloning vector (such as a plasmid) is much greater than depicted here).

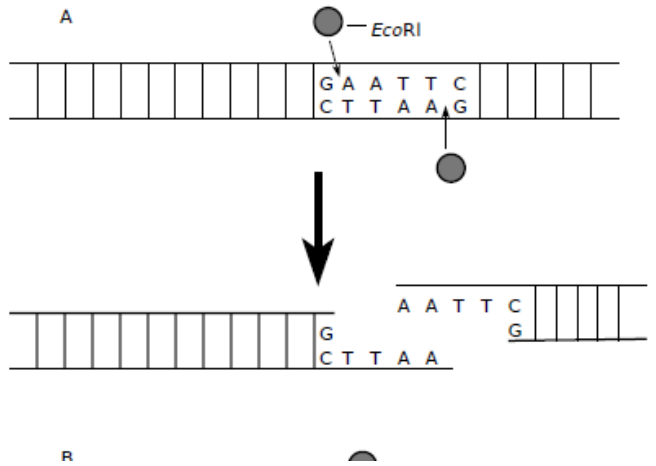
Gene Cloning: The Detailed Picture

Key enzymes in gene cloning: restriction endonucleases and DNA ligase

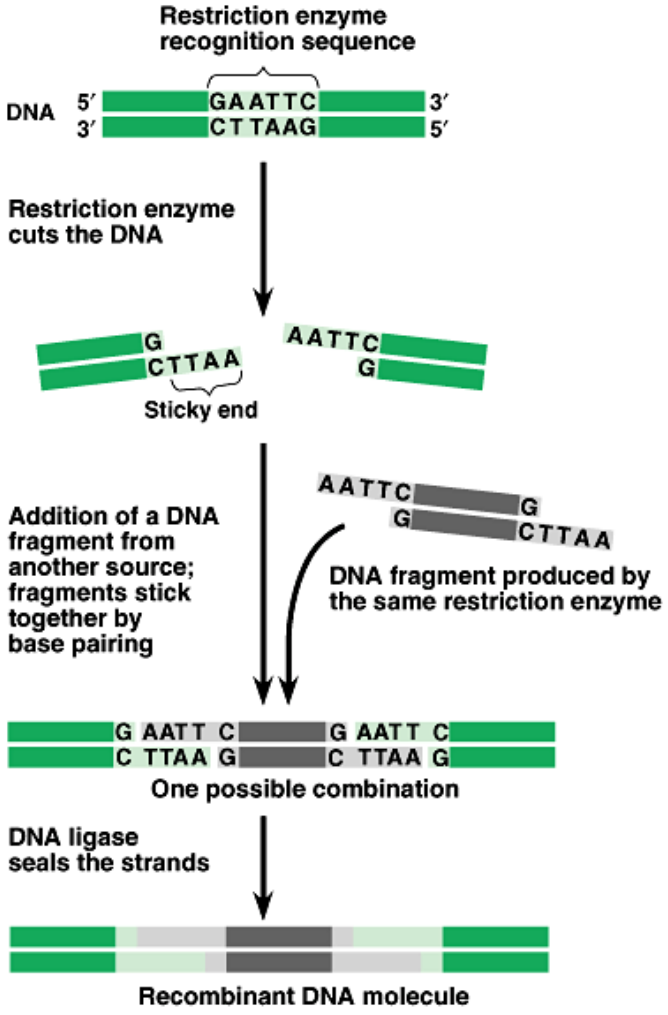
- Restriction Endonucleases and DNA Ligases Yield Recombinant DNA.
- Two classes of enzymes lie at the heart of the classic approach to generating and propagating a recombinant DNA molecule.
- First, **restriction endonucleases** (also called restriction enzymes) recognize and cleave DNA at specific sequences (recognition sequences or restriction sites) to generate a set of smaller fragments.
- Second, the DNA fragment to be cloned is joined to a suitable cloning vector by using **DNA ligases** to link the DNA molecules together. The recombinant vector is then introduced into a host cell, which amplifies the fragment in the course of many generations of cell division.
- Restriction enzymes and DNA ligases allow insertion of DNA fragments into cloning vectors.

Restriction Endonucleases

- Restriction enzymes are classified as endonucleases because they cut DNA at points within the molecule.
- They can be thought of as "molecular scissors," cutting the DNA at specific target sequences.
- The crucial advantage they have over other endonucleases is that they do not cut DNA at *random* points. Instead, they cut at *specific* DNA sequences.
  - For example, EcoRI will cut DNA whenever the enzyme encounters the sequence GAATTC.
- Therefore, the catalytic action of the enzyme is *restricted* to certain sequences of DNA.



Restriction enzymes cleave DNA



- The same sequence of bases is found on both DNA strands, but in opposite orders.

GAATTC
CTTAAG

- This arrangement is called a **palindrome**. *Palindromes are words or sentences that read the same forward and backward.*

- form **sticky ends:** single stranded ends that have a tendency to join with each other (the key to recombinant DNA)

Restriction Enzymes Cut DNA Chains at Specific Locations

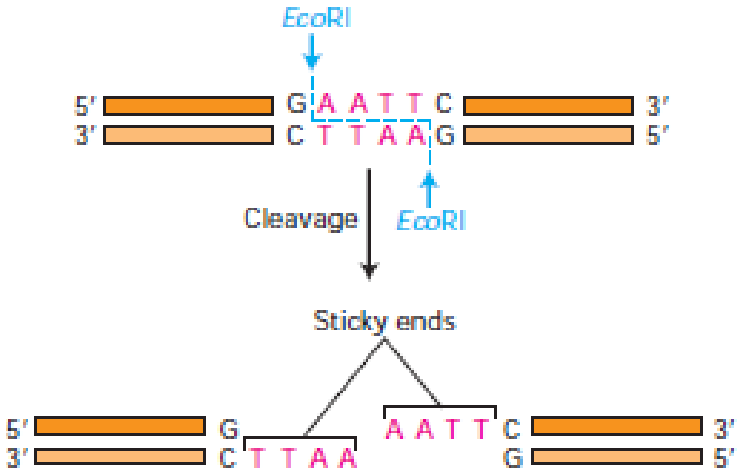
- Restriction enzymes are endonucleases produced by bacteria that typically recognize specific 4 to 8 bp sequences, called restriction sites, and then cleave both DNA strands at this site.

- Restriction sites are commonly short palindromic sequences; that is, the restriction site-sequence is the same on each DNA strand when read in the 5’ → 3’ direction.

Sticky ends or Blunt ends

- Each enzyme recognizes and cleaves a specific double-stranded DNA sequence that is 4-7 bp long. These DNA cuts result in blunt ends (eg, *Hpa*I) or overlapping (sticky) ends (eg, *Bam*H I) , depending on the mechanism used by the enzyme.

- Sticky ends are particularly useful in constructing hybrid or chimeric DNA molecules .



Cleavage of DNA by restriction enzyme EcoRI.
This restriction enzyme from *E. coli* makes staggered cuts at the specific 6-bp inverted repeat (palindromic) sequence shown, yielding fragments with single stranded, complementary «sticky» ends. Many other restriction enzymes also produce fragments with sticky ends.

Inserting DNA Fragments into Vectors

- DNA fragments with either sticky ends or blunt ends can be inserted into vector DNA with the aid of DNA ligases.
- For purposes of DNA cloning, purified DNA ligase is used to covalently join the ends of a restriction fragment and vector DNA that have complementary ends. The vector DNA and restriction fragment are covalently ligated together through the standard 3 → 5 phosphodiester bonds of DNA.
- DNA ligase “pastes” the DNA fragments together.

Vectors-Cloning Vehicles

- Cloning Vectors Allow Amplification of Inserted DNA Segments.

- **Cloning vectors can be plasmids, bacteriophage, viruses, or even small artificial chromosomes. Most vectors contain sequences that allow them to be replicated autonomously within a compatible host cell, whereas a minority carry sequences that facilitate integration into the host genome.**

- In molecular cloning, a **vector** is a DNA molecule used as a vehicle to artificially carry foreign genetic material into another cell, where it can be replicated and/or expressed (e.g., plasmid, cosmid, Lambda phages). A vector containing foreign DNA is termed recombinant DNA.

- The four major types of vectors are plasmids, viral vectors, cosmids, and artificial chromosomes. Of these, the most commonly used vectors are plasmids.

- Common to all engineered vectors have **an origin of replication**, **a multicloning site**, and **a selectable marker**.

  - All cloning vectors have in common at least one unique cloning site, a sequence that can be cut by a restriction endonuclease to allow site-specific insertion of foreign DNA. The most useful vectors have several restriction sites grouped together in a multiple cloning site (MCS) called a polylinker.

A useful vector for cloning must have;

(i) **an origin of replication in the plasmid** (i.e., a nucleotide sequence that directs and regulates replication so that each cell contains a reasonably consistent number of plasmid copies),

(i) **selectable auxotrophic or phenotypic markers to facilitate the identification of inserts apart from the classical antibiotic resistance genes,**

(ii) **marker genes with unique restriction sites for one or more different restriction endonucleases**, and

(iii) **lowest possible molecular weight plasmids**, because low molecular weight is normally accompanied by a high copy number, and because this property helps the material to avoid damage by shear forces during preparation.

What is the difference between PCR and recombinant DNA technology?

- Molecular cloning is the laboratory process used to create recombinant DNA. It is one of two most widely used methods, along with polymerase chain reaction (PCR), used to direct the replication of any specific DNA sequence chosen by the experimentalist.

- There are two fundamental differences between the methods.

- **One is that molecular cloning involves replication of the DNA within a living cell, while PCR replicates DNA in the test tube, free of living cells.**
- **The other difference is that cloning involves cutting and pasting DNA sequences, while PCR amplifies by copying an existing sequence.**