Recombinant DNA Technology

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Recombinant DNA technology

- **In the early 1970s, biochemists at stanford university showed that genetic traits could be transferred from one organism to another.**

- **In this experiment, the DNA of one microorganism recombined with the inserted DNA sequence of another, and thus had been edited to exhibit a very specific modification.**

- **The actual editing, or insertion process, is painstaking, as it involves manipulating incredibly tiny pieces of incredibly tiny organisms.**

Important definitions

- **The recombinant DNA:** the term given to the fused human gene DNA and the bacterial (plasmid) DNA.

- **Plasmid :** the extrachromosomal DNA present in some bacterial cells and normally gives the bacterial cells the power to resist the action of antibiotics.

- The recombinant DNA molecule is also known as **hybrid or chimeric molecule**.

Recombinant DNA technology

- **It is genetic engineering which causes artificial modifications of genetic constitution of a living cell by introduction of foreign DNA through experimental techniques.**

- **The techniques involves:**

➢ **Splicing of DNA by restriction endonucleases.**

➢ **Preparation of chimeric molecules.**

➢ **Cloning of large number of identical target DNA molecules**

Tools of recombinant DNA technology

Restriction endonucleases

Vectors or vehicle DNA

Passenger DNA (foreign DNA)

Hosts: they include
Bacterial-animal or plant cells

Restriction endonucleases

They are important class of DNA endonucleases that recognize specific sequence of bases in DNA (restriction sites) and have the ability to cleave DNA molecules at these sites so they serve as molecular scissors.

They are found in a wide range of bacteria, their function is to recognize and cleave foreign DNA and so prevent or restrict the infecting virus "bacteriophages" (so the name restriction). They are called endonucleases because they cut in the middle of the polynucleotide chain.

- **The cell's own DNA is protected from cleavage by these restriction enzymes by methylation as bacteria that contain these enzymes also contain a DNA methylase enzyme that methylates the cytosine bases of the bacterial DNA at the restriction site rendering the bacterial DNA resistant to the action of the restriction endonuclease.**

- **Over 3,000 restriction enzymes have been studied in detail, and more than 600 of these are available commercially. They are named according to the bacterial species from which they are isolated .**

- **The first letter indicates the genus name , and the other two indicate the species name and a roman numeral indicates the order of discovery of an enzyme from that species. e.g. EcoRI was the first enzyme isolated from Escherichia coli (E.coli).**

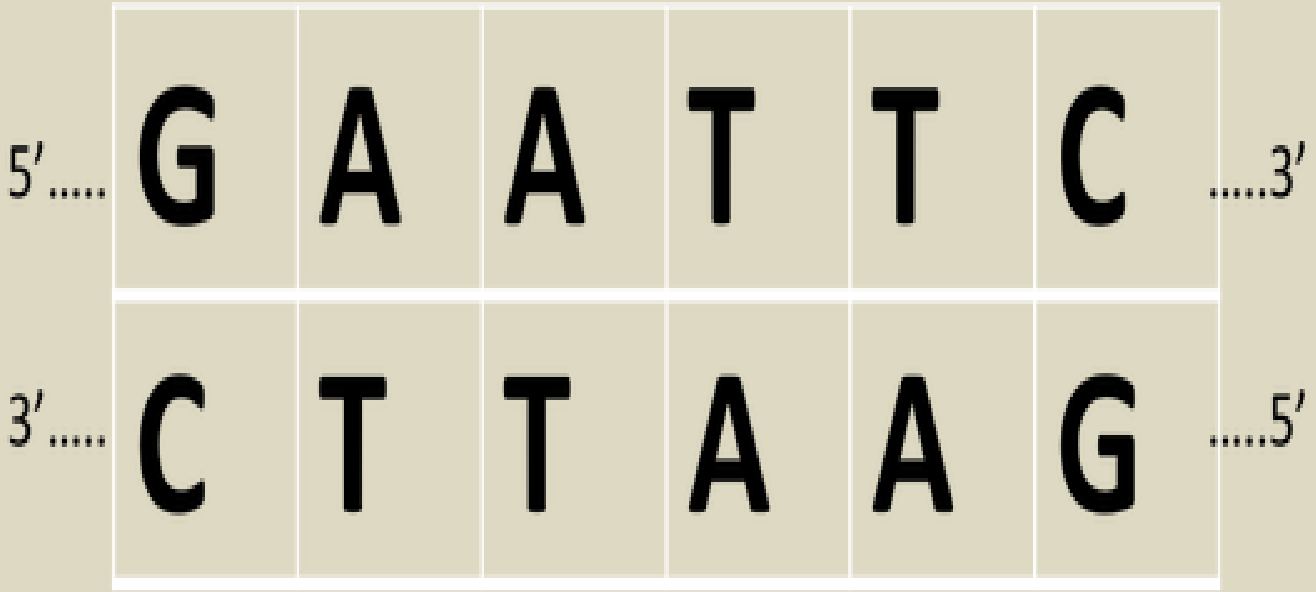
Derivation of the EcoRI name		
Abbreviation	**Meaning**	**Description**
E	*Escherichia*	genus
co	*coli*	specific species
R	RY13	strain
I	First identified	order of identification in the bacterium

Restriction sites

- They are sequences of four, six, eight or rarely more bases with palindrome arrangement.

- Palindrome in Greek means “to run backwards”. It is similar to a word that reads backwards or forwards similarly e.g. madam. These are also called inverted repeat sequences which means the nucleotide sequence in 5`to 3` direction is the same in both strands.

palindrome arrangement

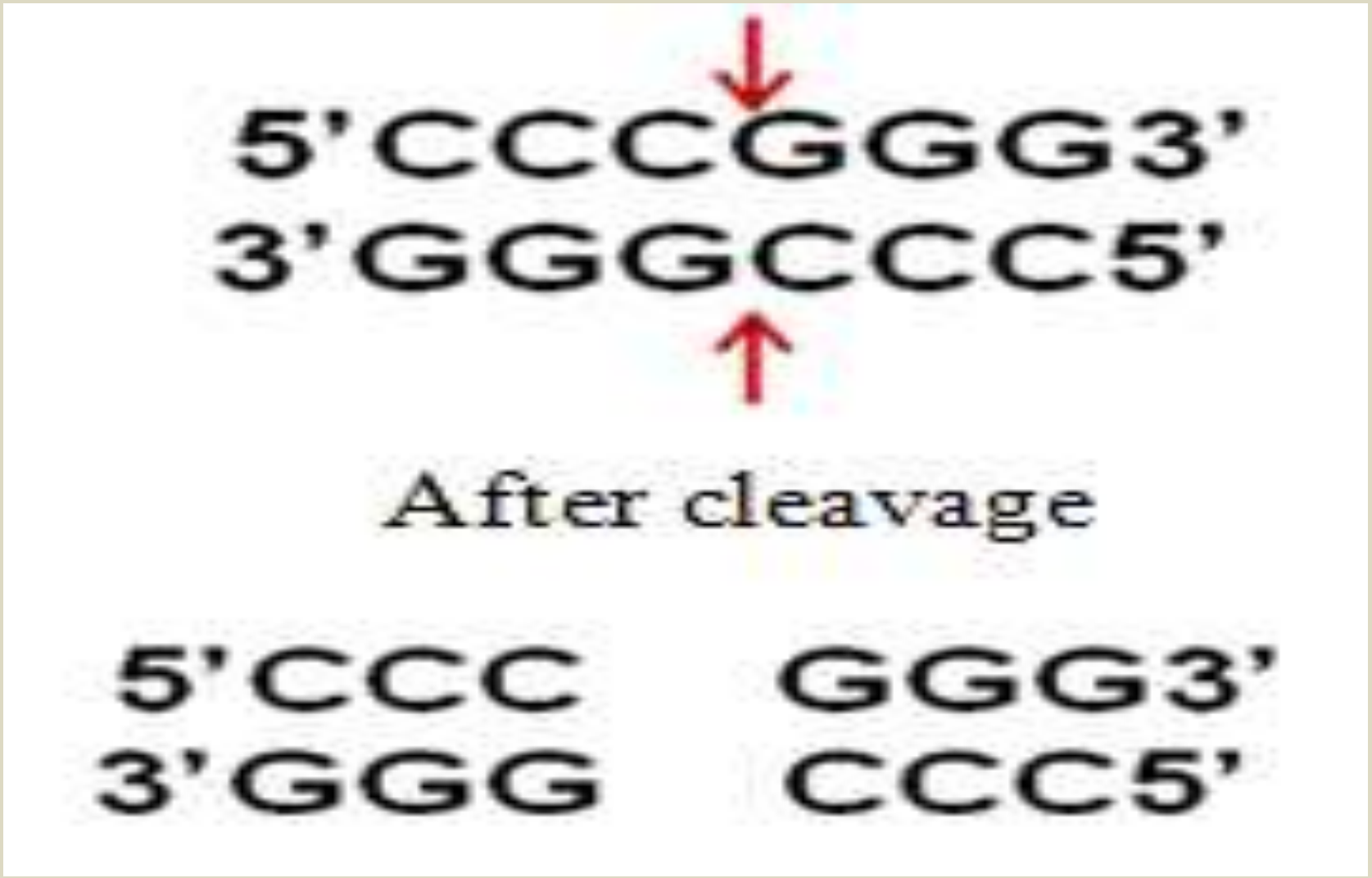


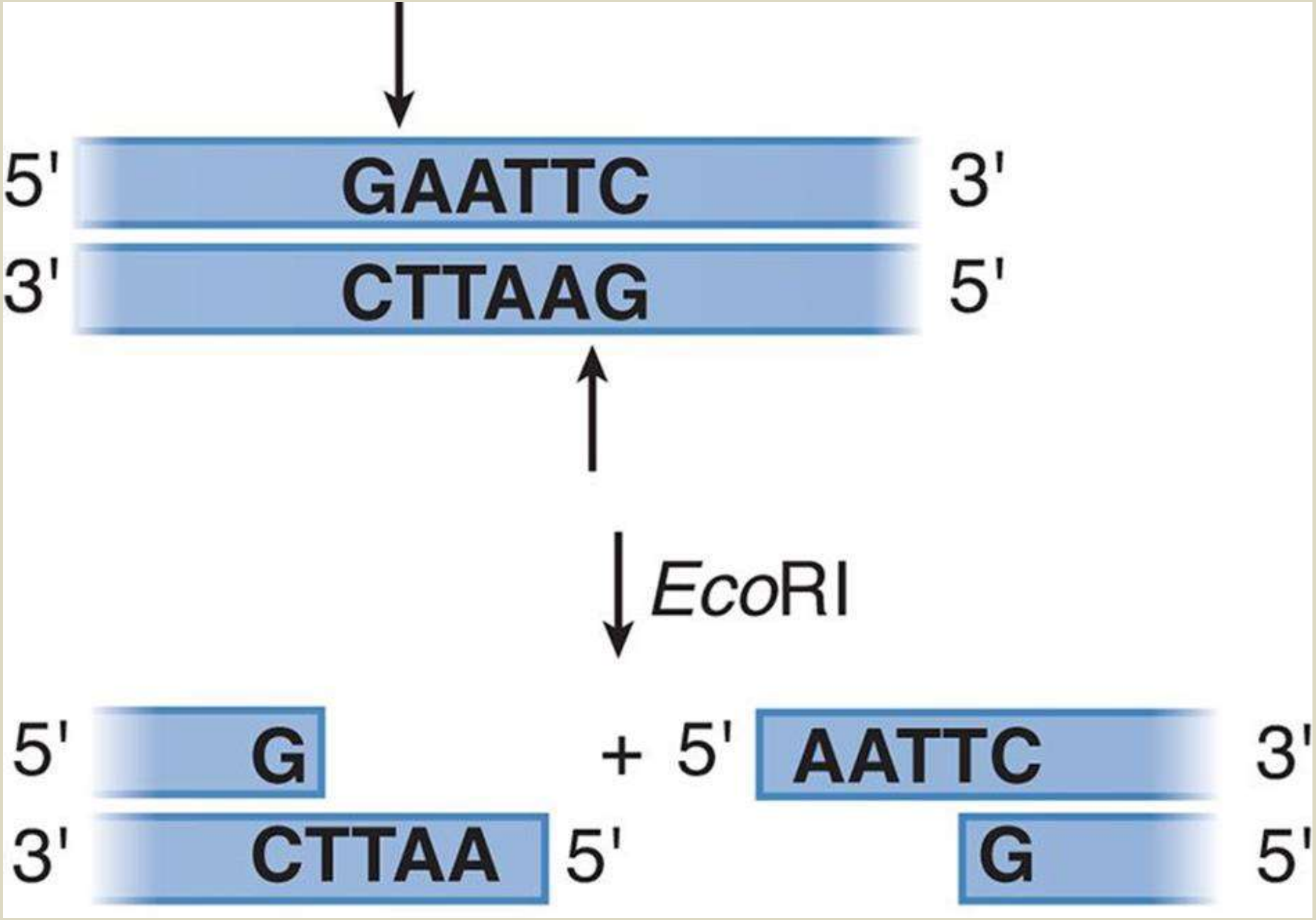
- **They cut either blunt end cut or sticky end cut.**

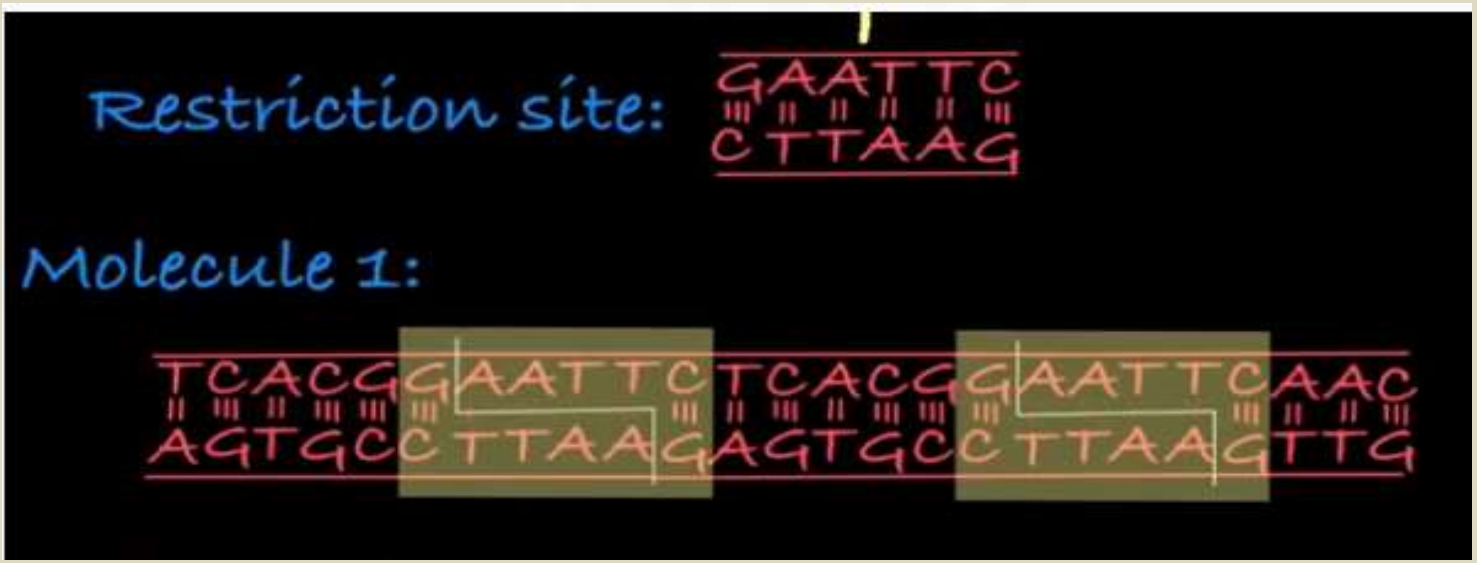
- **Blunt end cut cleaves both strands of DNA so as to leave no unpaired bases on either ends.**

- **Sticky end cut leaves unpaired ends which are called cohesive ends, sticky ends, or staggered ends.**

- **Blunt end of the DNA fragment are ligated at a low efficiency than those with complementary sticky end, while the sticky end of the DNA fragment facilitates the ligation of amplified DNA into cloning vector.**


5'CCCGGG3'
3'GGGCCC5'
After cleavage
5'CCC GGG3'
3'GGG CCC5'


5'
GAATTC
3'
3'
CTTAAG
5'
EcoRI
5'
G
+ 5'
AATTC
3'
3'
CTTAA
5'
G
5'


Restriction site:
GAATTC
CTTAAG
Molecule 1:
TCACGGAATTCTCACGGAATTCAAC
AGTGCCTTAAGAGTGCCTTAAGTTG


Molecule 1:
1
2
3
TCACGG
AATTCTCACGG
AATTCAAC
AGTGCCTTAA
GAGTGCCTTAA
GTTG


Molecule 2:
Mutation
TCACGGAATTCTCACGGAATTCAAC
AGTGCCTTAAGAGTGCCTTAAGTTG
*


Molecule 2:
1
2
TCACGG
AATTCTCACGGACTTCAAC
AGTGCCTTAA
GAGTGCCTGAAGTTG

Restriction map

- **If a piece of DNA from a species is made to react with a specific restriction enzyme, a characteristic array of cut pieces will be produced , this is called a restriction map. These fragments can be isolated by electrophoresis.**
- **A restriction map is a map of known restriction sites within a sequence of DNA.**

vectors

- In order to introduce the human gene into bacteria, at first, the gene is transferred into a carrier, known as a vector.

- Vectors show the following essential features:
  - They are able to replicate.
  - They must contain a site for insertion of target DNA.
  - They could be inserted into the host cell
  - They have a selectable marker to trace them after insertion.

Commonly used vectors

- **(1) plasmids:**

- **They are bacterial extra chromosomal circular double stranded DNA.**
- **They replicate independent of bacterial DNA.**
- **Foreign DNA "small pieces from 6-10 Kbp" could be incorporated in plasmid by using specific restriction endonuclease.**

- **Plasmids usually carry one or more of antibiotic resistance genes “which are utilized as selectable markers”. i.e. a method of selection of cells containing recombinant DNA molecule as growth in presence of antibiotic, only the bacteria containing the plasmid will grow.**

(2) Bacteriophage :

- Is a virus that infects and replicates within a bacterium.
- Plasmids can accept only about 6-10 kbp long foreign DNA. If a DNA segment of 10-20 kbp is to be introduced, bacteriophages may be the vectors of choice.

One Kb=1000 nocleotides base sequence.

- **(3) Cosmids :** *(artificially constructed cloning vectors)*

❖**They are plasmid that also contain some portion of bacteriophage DNA . They can take up still bigger fragments of DNA “up to 50 Kbp”.**

Preparation of Chimeric DNA Molecules

- **Chimera is the Greek mythological monster with a lion's head, goat's body and serpent's tail.**

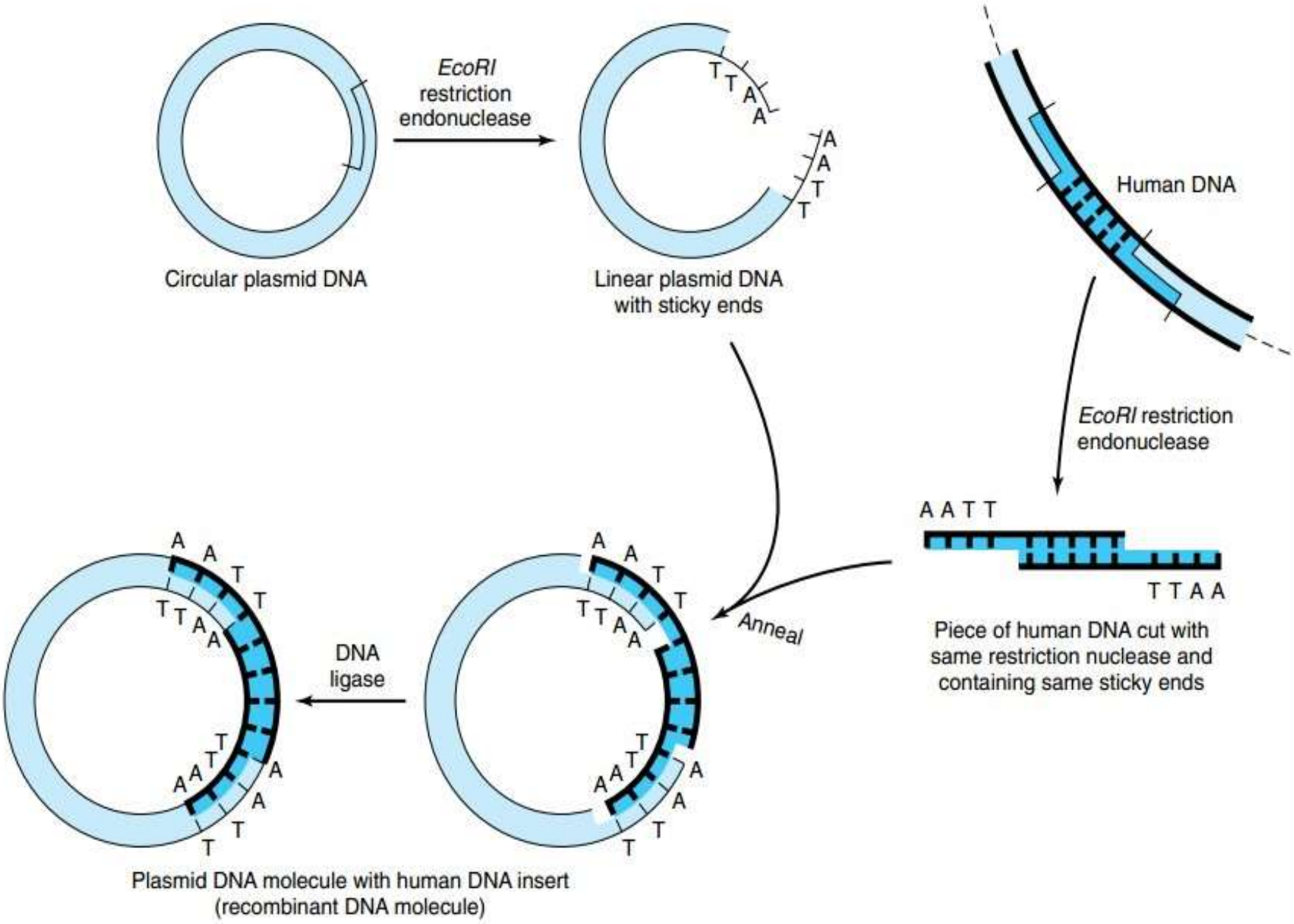
- **A vector carrying a foreign DNA is called Chimeric DNA or Hybrid DNA or Recombinant DNA.**

i. **A circular plasmid vector DNA is cut with a specific restriction endonuclease (RE). If EcoRI is used, sticky ends are produced with TTAA sequence on one DNA strand, and AATT sequence on the other strand**

- **ii. The human DNA is also treated with the same RE, so that the same sequences are generated on the sticky ends of the cut piece.**

- **iii. Then the vector DNA and human cut-piece DNA are incubated together so that annealing takes place. The sticky ends of both vector and human DNA have complementary sequences, and therefore they come into contact with each other.**

- **iv. Then DNA ligase enzyme is added, which introduces phosphodiester linkages between the vector and the insert molecules. Thus the chimeric DNA is finally produced.**


EcoRI restriction endonuclease
Circular plasmid DNA
Linear plasmid DNA with sticky ends
Human DNA
EcoRI restriction endonuclease
A A T T
T T A A
Piece of human DNA cut with same restriction nuclease and containing same sticky ends
Anneal
DNA ligase
Plasmid DNA molecule with human DNA insert (recombinant DNA molecule)

Cloning of Chimeric DNA

- **A clone is a large population of identical bacteria or cells that arise from a common ancestor molecule.**

- **Cloning allows the production of a large number of identical DNA molecules. The hybrid molecules are amplified by the cloning technique.**

- **DNA cloning is an in vivo DNA amplification.**

- **Only 5% of bacteria colonies contain the desired vector, so we have to select the desired colonies.**

- **The bacterial host cell containing the recombinant vector can be selected if the vector contains an antibiotic resistance genes.**

- **Bacteria without vector die in the presence of antibiotic medium.**

Isolation of cloned foreign DNA or its protein product:

Cells containing an appropriate chimeric plasmid are cultured then the plasmids are isolated from host cells (the bacteria are lysed and the hybrid plasmids are isolated) and treated with the same restriction enzyme to release the foreign DNA.

If the host cells are grown under conditions that permit the production of protein produced from target DNA, then the protein of interest can be isolated.

- **Hundreds of human proteins are now being synthesized by the recombinant technology.**

- **Recombinant human insulin is now available in market. Other useful products produced include; interferons, hepatitis B antigen and growth hormone.**