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Recombination DNA Technology

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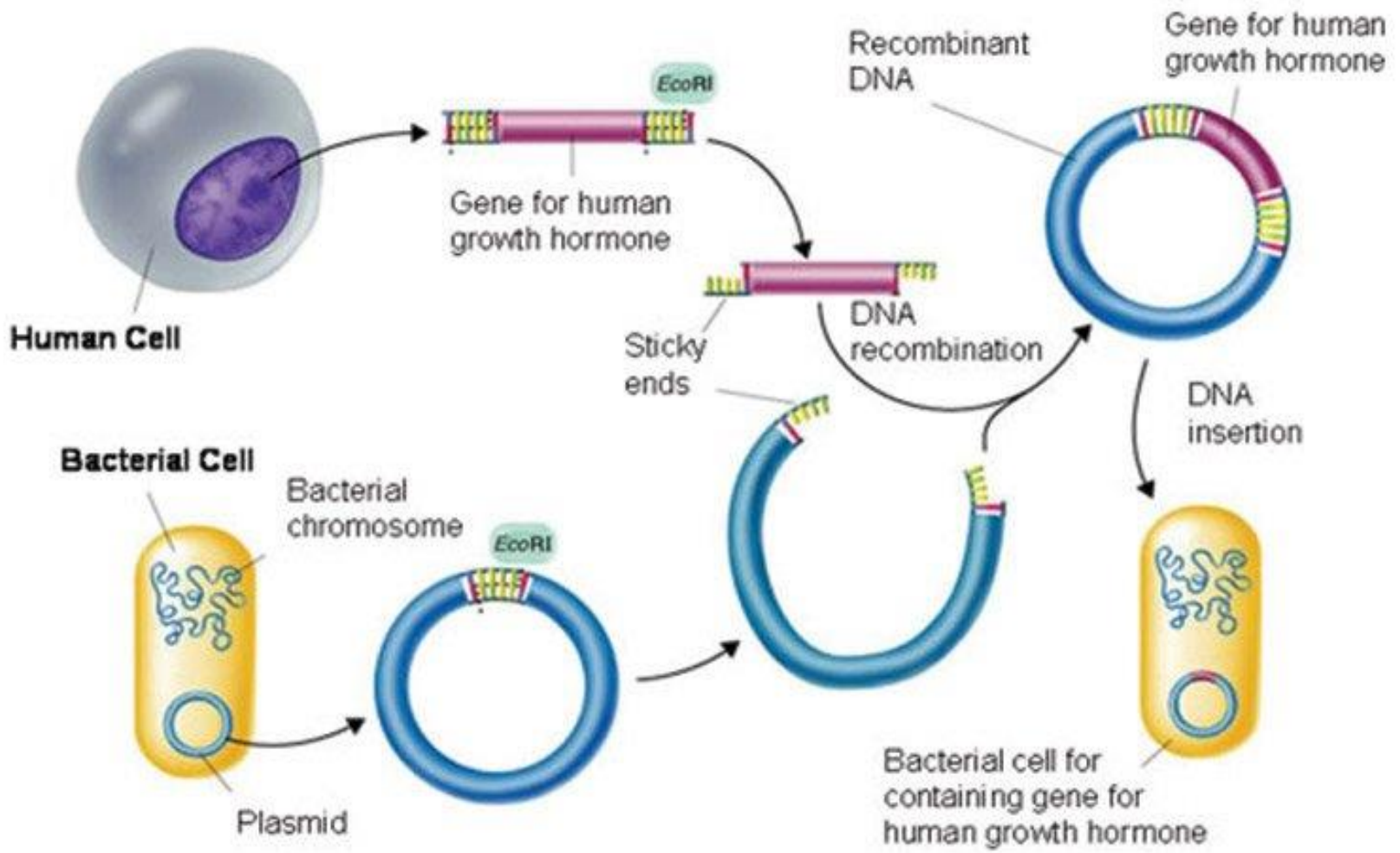
- Recombinant DNA (rDNA) technology refers to the joining together of DNA molecules from two different species that are inserted into a host organism to produce new genetic combinations that are of worth to science, medicine, agriculture, and industry.
- Recombinant DNA in a living organism was first achieved in 1973 by Herbert Boyer.
- This technique is mainly used to change the phenotype of an organism (host) when a genetically altered vector is introduced and integrated into the genome of the organism.
- So, basically, this process involves the introduction of a foreign piece of DNA structure into the genome which contains our gene of interest. This gene which is introduced is the recombinant gene and the technique is called the recombinant DNA technology.


Human Cell
EcoRI
Gene for human growth hormone
Sticky ends
Recombinant DNA
DNA recombination
Gene for human growth hormone
DNA insertion
Bacterial Cell
Bacterial chromosome
EcoRI
Plasmid
Bacterial cell for containing gene for human growth hormone

Steps of Genetic Recombination Technology

The complete process of recombinant DNA technology includes multiple steps, maintained in a specific sequence to generate the desired product.

Step-1. Isolation of Genetic Material

The first and the initial step in Recombinant DNA technology is to isolate the desired DNA in its pure form i.e. free from other macromolecules.

Step-2. Restriction Enzyme Digestion

Restriction enzymes act as molecular scissors that cut DNA at specific locations. These reactions are called restriction enzyme digestions. The restriction enzyme belong to a larger class of enzymes called exonucleases and endonucleases. Exonucleases remove nucleotide from ends whereas endonuclease cuts at specific position within the DNA.

Step-3. Amplifying the gene copies through Polymerase chain reaction (PCR)

It is a process to amplify a single copy of DNA into thousands to millions of copies once the proper gene of interest has been cut using the restriction enzymes.

Step-4. Ligation of DNA Molecules

In this step of Ligation, joining of the two pieces – a cut fragment of DNA and the vector together with the help of the enzyme DNA ligase.

Step-5. Insertion of Recombinant DNA into Host

In this step, the recombinant DNA is introduced into a recipient host cell. This process is termed as transformation. Once after the insertion of the recombinant DNA into the host cell, it gets multiplied and is expressed in the form of the manufactured protein under optimal conditions.

Enzymes used in DNA Technology

1. DNA ligase

DNA ligase is isolated from E.coli and Bacteriophage. It joins the DNA fragments with cloning vector.

2. Reverse transcriptase

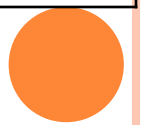
Reverse transcriptase is isolated from retrovirus and used to synthesize complementary strand (cDNA) from mRNA template. It is also known as RNA dependent DNA polymerase.

3. Restriction endonuclease

Restriction endonuclease enzyme recognize and cut DNA strand at specific sequence called restriction site.

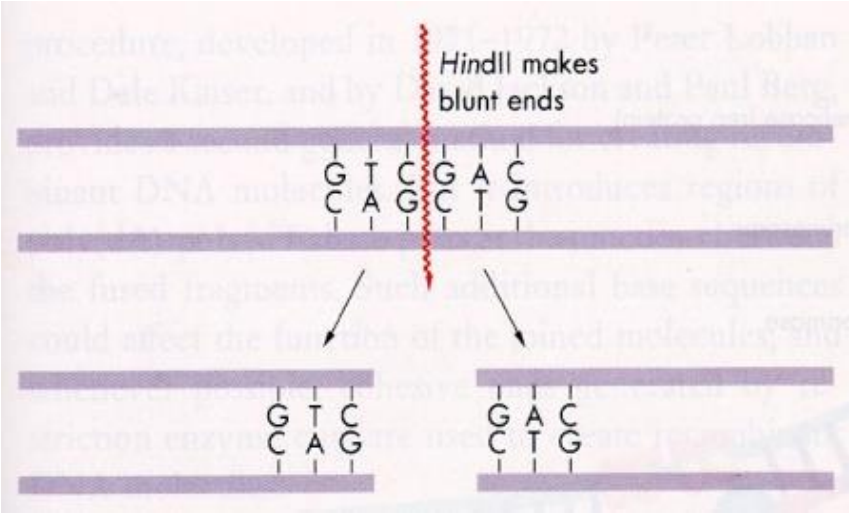
There are 3 types of restriction endonuclease

Type I	Type II	Type III
It has both methylation and endonuclease activity	-	-
It require ATP to cut the DNA	It does not require ATP to cut DNA	It requires ATP to cut DNA
It cuts DNA about 1000bp away from its restriction site	It cuts DNA at restriction site itself	It cuts DNA about 25bp away from restriction site.
Example EcoKI	Example EcoRI, Hind III	Example EcoPI

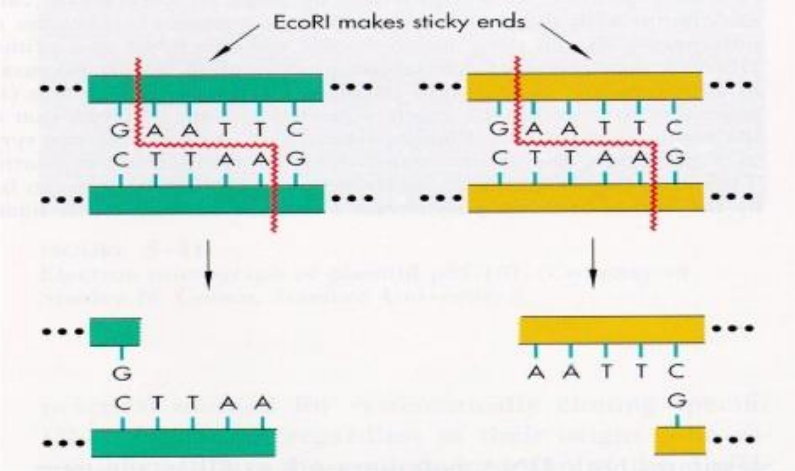


4. Terminal transferase

- It is the enzyme that converts blunt end of DNA fragments into sticky end.
- If the restriction enzyme cuts DNA forming blunt ends, then efficiency of ligation is very low. So the enzyme terminal transferase converts bunt end into sticky end.
- Terminal transferase enzyme synthesize short sequence of complementary nucleotide at free ends of DNA, so that blunt end is converted into sticky end.



Blunt ends



Sticky end

5. Nuclease

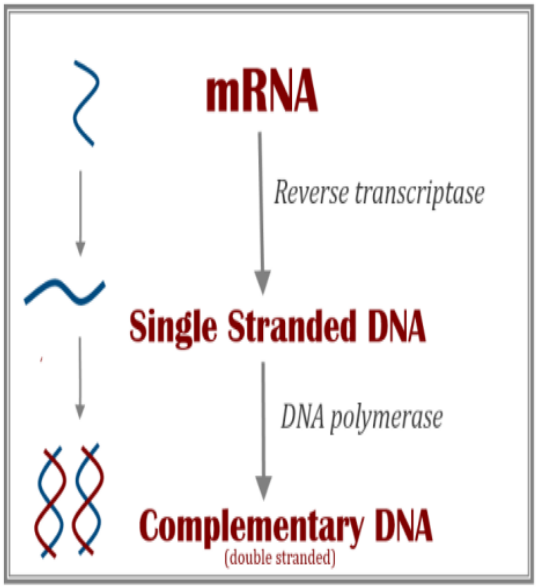
- The enzyme nucleases hydrolyses the phosphodiester bond on DNA strand creating 3'-OH group and 5'-P group.
- Nuclease are of two types; endonuclease and exonuclease

6. DNA polymerase

- DNA polymerase is a complex enzyme which synthesize nucleotide complementary to template strand.
- It adds nucleotide to free 3′ OH end and help in elongation of strand
- It also helps to fill gap in double stranded DNA.

7. Ribonuclease-H (RNase H)

RNase-H removes mRNA from DNA-RNA heteroduplex and that mRNA is used to synthesize cDNA.



8. Alkaline phosphatase

The enzyme Alkaline phosphatase helps in removal of terminal phosphate group from 5′ end.

It prevents self annealing of vector DNA soon after cut open by restriction endonuclease.

9. Polynucleotide kinase

It adds phosphate group from ATP molecule to terminal 5'end after dephosphorylation by alkaline phosphatase.

Application of Recombinant DNA technology

1. Recombinant DNA is widely used in biotechnology, medicine and research. It can also be used to detect the presence of HIV in a person.

2. Many additional practical applications of recombinant DNA are found in industry, food production, human and veterinary medicine (Insulin production by DNA recombinant technology), agriculture (manufacture of Bt-Cotton to protect the plant against ball worms), and bioengineering.

3. Recombinant DNA is used to identify, map and sequence genes, and to determine their function.

4. Recombinant proteins are widely used as reagents in laboratory experiments and to generate antibody probes for examining protein synthesis within cells and organisms.

5. Gene Therapy – It is used as an attempt to correct the gene defects which give rise to heredity diseases.

6. Clinical diagnosis – ELISA is an example where the application of recombinant DNA is possible.