CSIR NET Life Science Unit 3

Recombinant DNA (rDNA) Construction

Recombinant DNA (rDNA) molecules are artificially produced where strands from different sources are joined together to enhance or obtain a desired trait. The overall process of rDNA production is called rDNA technology.

Steps involved in rDNA construction

- Isolation of the gene of interest and vector DNA
- Restriction digestion
- Ligation
- Amplification

Isolation of the Gene of Interest

The gene of interest is first isolated from the donor organism. Various DNA isolation methods are available. Depending on the donor and the vector, the isolation method can be chosen. Plasmids are the most commonly used vectors. After isolation, plasmids must be purified from the genomic DNA. Apart from plasmids, bacteriophages such as λ phage, cosmids, phagemids and artificial chromosomes are also used as vectors.

Restriction Digestion

Restriction digestion of donor and vector DNA molecules is a crucial step in the construction of recombinant DNA. Restriction digestion is brought about by restriction enzymes produced by bacteria as a defence mechanism against bacteriophages. Restriction enzymes cut DNA at specific sequences. These sequences are called restriction sites. When cut by restriction enzymes, two ends can be produced: sticky ends and blunt ends. For example, EcoRI is a restriction enzyme that produces sticky ends, whereas EcoRV produces blunt ends.

Some of the restriction enzymes, the sequences they recognise and the type of cuts they make can be seen in the table given below.

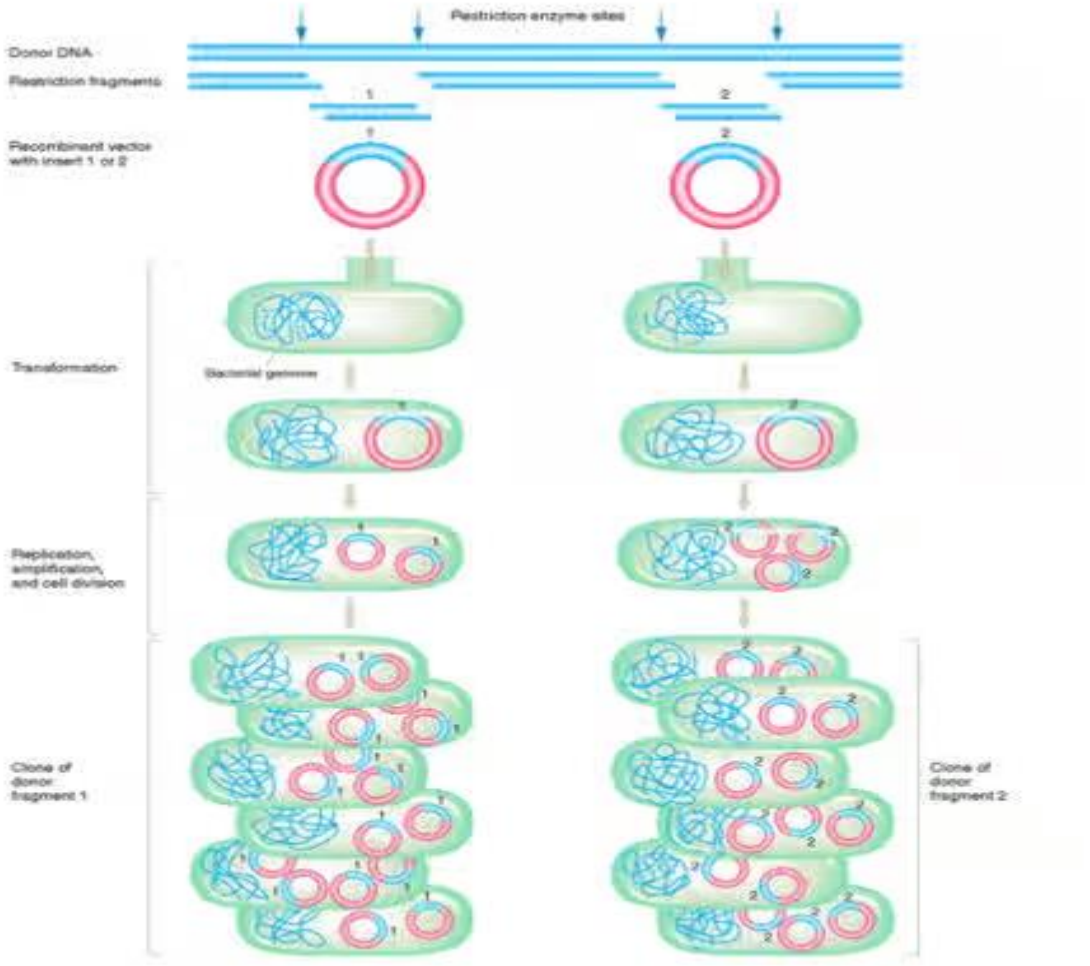
Name of the enzyme	Source	Recognition site and cleavage site	Nature of cut ends
Eco R1	*E. coli* RY13	5'–G|AATTC–3' 3'–CTTAA|G–5'	Sticky
Hind III	*Haemophilus influenzae* Rd	5'–A|AGCTT–3' 3'–TTCGA|A–5'	Sticky
Bam HI	*Bacillus amyloliquifaciens* H	5'–G|GATCC–3' 3'–CCTAG|G–5'	Sticky
Sal I	*Streptomyces albus* G	5'–G|TCGAC–3' 3'–CAGCT|G–5'	Sticky
Bal I	*Brevibacterium albidum*	5'–TGG|CCA–3' 3'–ACC|GGT–5'	Blunt
Hae III	*Haemophilus aegiptius*	5'–GG|CC–3' 3'–CC|GG–5'	Blunt
Sma I	*Serratia marcescens*	5'–CCC|GGG–3' 3'–GGG|CCC–5'	Blunt

Ligation

In most cases, vector and donor DNA molecules are cut using the same restriction enzyme that produces sticky ends. When mixed, they unite to form a recombinant DNA molecule. If the DNA molecules carry blunt ends, then specialized linkers and adapters are used for conjoining the donor and vector DNA molecules. The backbones of these recombinant DNA molecules are sealed by an enzyme called DNA ligase that creates phosphodiester bonds.

Amplification of rDNA

The rDNA molecule enters bacterial cells through transformation. The plasmids carrying rDNA replicate inside the host cell. After many replication cycles, the entire bacterial colony will carry the recombinant DNA, and therefore, the rDNA molecule would be amplified.


Restriction enzyme sites
Donor DNA
Restriction fragments
1
2
Recombinant vector with insert 1 or 2
1
2
Transformation
Bacterial genome
Replication, amplification, and cell division
Clone of donor fragment 1
Clone of donor fragment 2