

# rDNA Construction Roadmap



## **BASIC OUTLINE OF rDNA CONSTRUCTION**

Recombinant DNA (rDNA) molecules are artificially produced DNA molecules where DNA 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

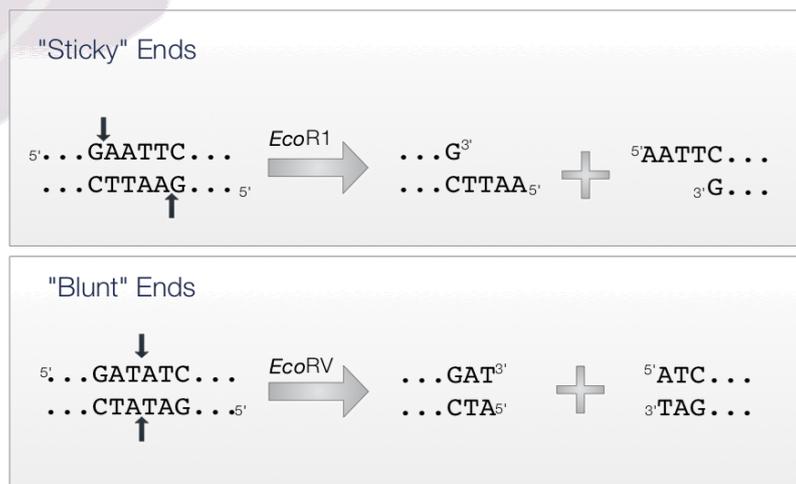
a. Isolation of the gene of interest:

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  $\lambda$  phage, cosmids, phagemids and artificial chromosomes are also used as vectors.

b. Restriction digestion:

Restriction digestion of donor and vector DNA molecules is a crucial step in construction of recombinant DNA. Restriction digestion is brought about by restriction enzymes which are 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 types of ends can be produced: sticky ends and blunt ends. 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.



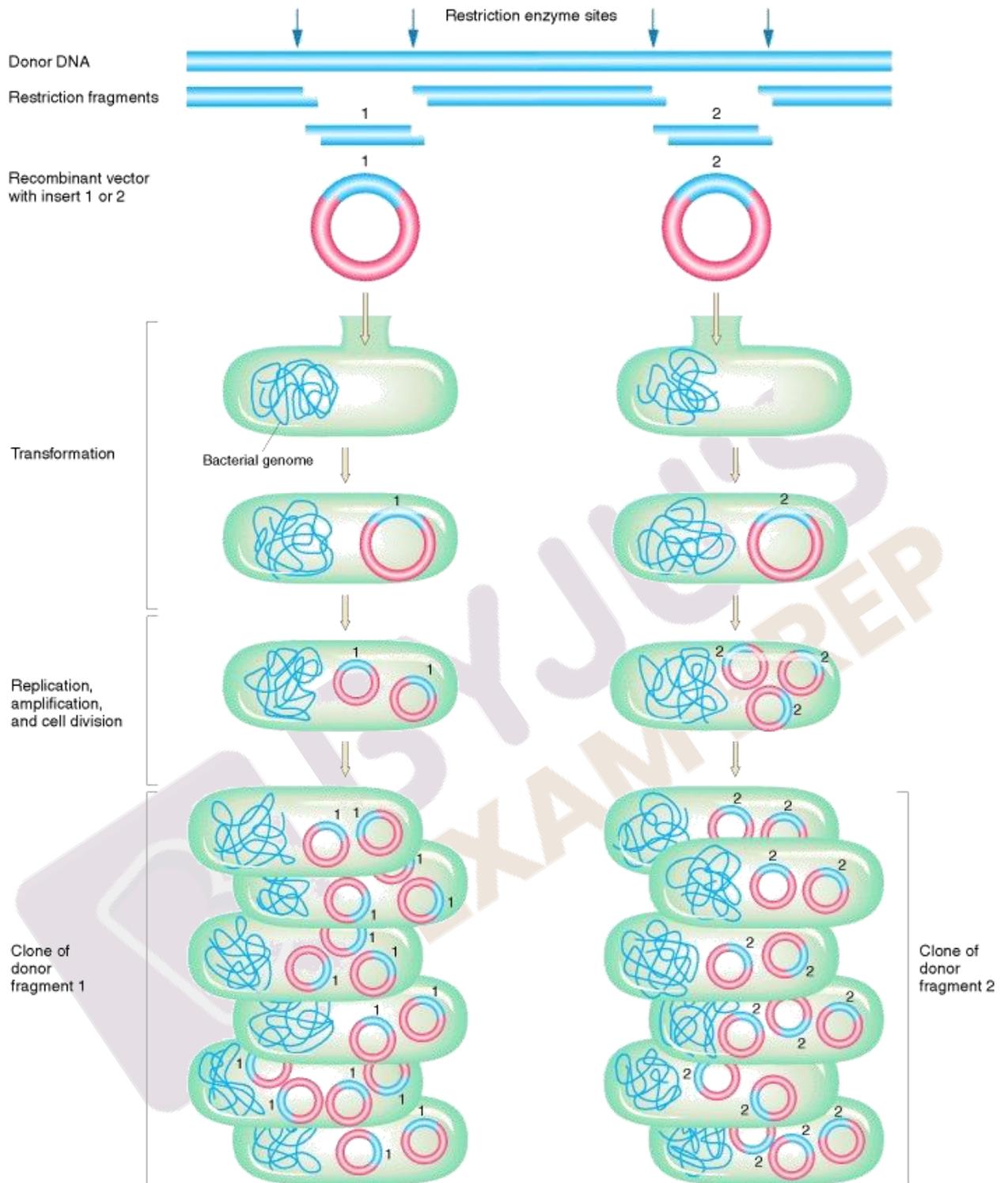
Name of the enzyme	Source	Recognition site and cleavage site	Nature of cut ends
Eco R1	<i>E. coli</i> RY13	5'-G AATTC-3' 3'-CTTAA G-5'	Sticky
Hind III	<i>Haemophilus influenzae</i> Rd	5'-A AGCTT-3' 3'-TTCGA A-5'	Sticky
Bam HI	<i>Bacillus amyloliquifaciens</i> H	5'-G GATCC-3' 3'-CCTAG G-5'	Sticky
Sal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

c. Ligation:

In most cases, both vector and donor DNA molecules are cut using the same restriction enzyme that produces sticky ends. When mixed together, 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.

d. Amplification of rDNA

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



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