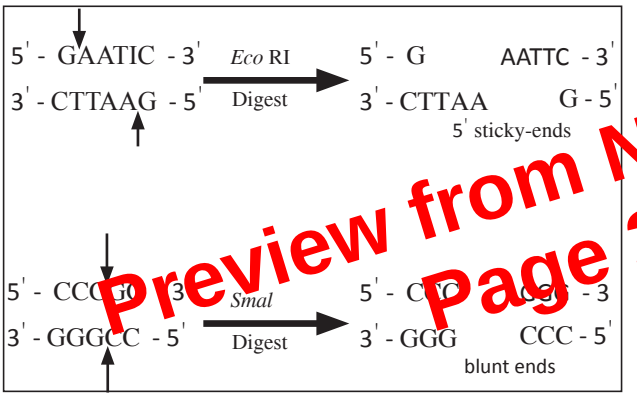
TOOLS OF RECOMBINANT DNA TECHNOLOGY

1. Restriction Enzymes ('Molecular Scissors')

- Restriction enzymes are molecular scissors used in molecular biology for cutting DNA sequences from a specific site.
- It plays an important role in gene manipulation.
- The enzyme recognises a specific six box pair sequence known as the recognition sequence and cut the sequence at a specific site.
- The first restriction endonuclease is **Hind II.** It always cuts DNA molecules at a particular point by recognizing a specific sequence of six base pairs. This is known as the **recognition sequence** for Hind II.
- Today more than 900 restriction enzymes have been isolated from over 230 strains of bacteria.
- Some restriction enzymes digest DNA into fragments with "sticky ends".These DNA fragments will hydrogen bond to each other due to their complementary sequences.
- Other restriction enzymes generate blunt ends.



- Restriction enzymes belong to a class of enzymes called **nucleases**. They are categorized into two types: **Exonucleases** and **endonucleases**
  - (i) **Exonucleases:** It is a type of restriction enzymes that remove the nucleotide from either 5'or 3'ends of the DNA molecules.
  - (ii) **Endonucleases:** It is a type of restriction enzymes that make a cut within the DNA at a specific site. This enzyme acts as an important tool in genetic engineering. It is commonly used to make a cut in the sequence to obtain DNA fragments with sticky ends. **Sticky ends are** single stranded portions of DNA which can form hydrogen bonds with their complementary cut DNA segments. These ends are later on joined by enzyme ligase.

➢ **Ligase** are enzymes that form phosphodiester bonds between adjacent nucleotides and covalently links two individual fragments of double stranded DNA.

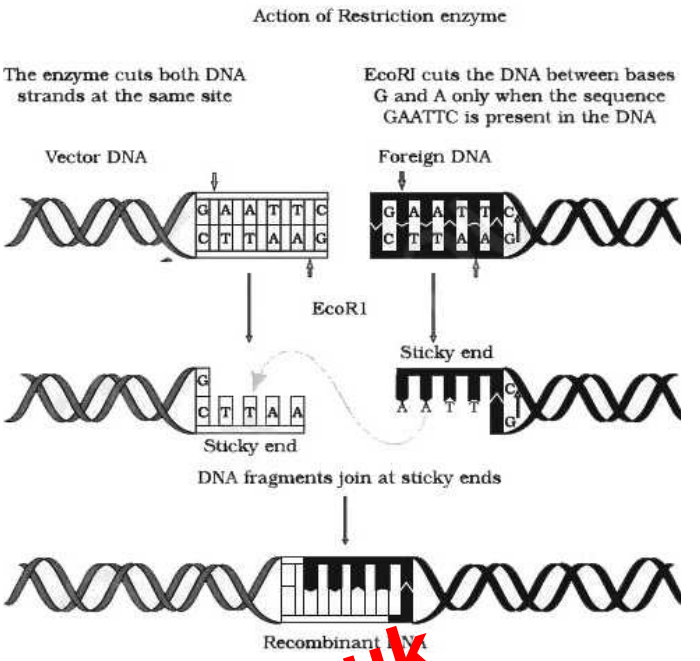


Fig. Steps in formation of recombinant DNA by action of restriction endonuclease enzyme- EcoRI

- Each restriction endonuclease recognizes a specific palindromic nucleotide sequences in the DNA.
- **Palindromes**: The palindrome in DNA is a sequence of base pairs that reads the same on the two strands in $5' \rightarrow 3'$ direction and in $3' \rightarrow 5'$ direction.
- *E.g.*

$$\begin{array}{l} 5' - - \text{GAATTC} - - 3' \\ 3' - - \text{CTTAAG} - - 5' \end{array}$$

$$\downarrow$$

$$\begin{array}{l} 5' - \text{G AATTC} \quad 3' \\ 3' - \text{CTTAA G} \quad 5' \end{array}$$

$$\uparrow$$

- Restriction enzymes cut the strand a little away from the centre of the palindrome sites, but between the same two bases on the opposite strands. This leaves single stranded overhanging stretches at the ends. They are called **sticky ends**. This stickiness facilitates action of the enzyme **DNA ligase.**
- When foreign DNA and the host DNA are cut by the same restriction enzyme, the resultant DNA fragments have the same kind of sticky-ends and these are joined together by DNA ligases.

Convention of naming restriction enzymes

- The first letter of the name comes from the **genus.**
- Second two letters come from the species of the prokaryotic cell from which the enzyme isolated
- The fourth letter is in capital form derived from the Strain of microbes.

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Alternative selectable marker

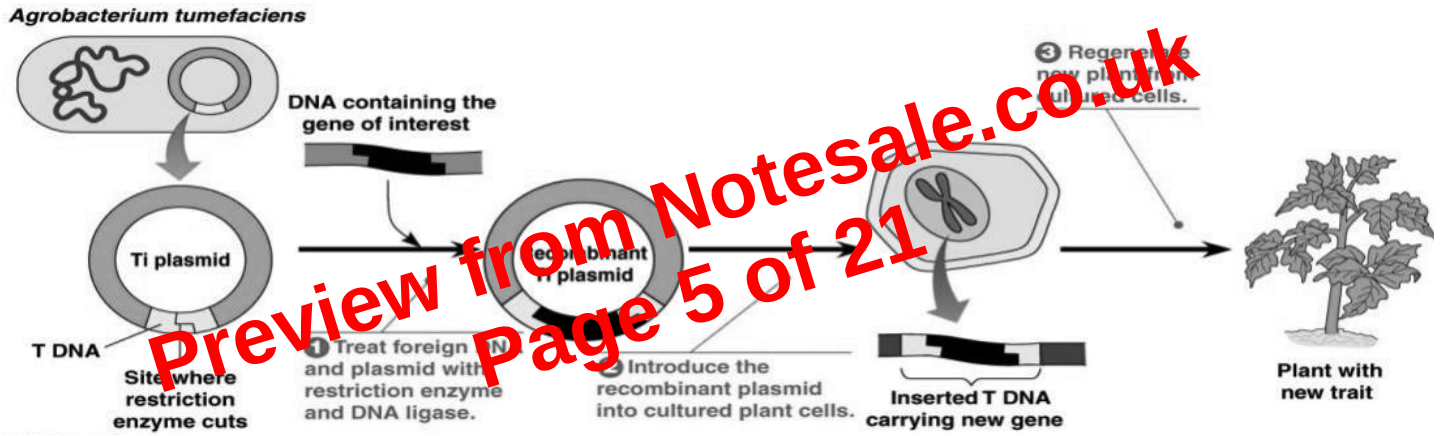
- A recombinant DNA is inserted within the coding sequence of an enzyme, β-galactosidase. As a result, the gene gets inactivated due to insertion of alien DNA. This results into inactivation of enzyme, which is referred to as insertional inactivation.
- The presence of chromogenic substrate gives blue-colour colonies if the plasmid in bacteria does not have an insert.
- Presence of insert results into insertional inactivation of the β-galactosidase and the colonies do not produce any colour, these are referred to as recombinant colonies.

(d) Vectors for cloning genes in plants and animals:

- The **tumor inducing (Ti) plasmid** of *Agrobacterium tumifaciens* is modified into a cloning vector which is not pathogenic to the plants but is able to use the mechanisms to deliver genes of interest into a variety of plants. *Agrobacterium tumifaciens is* a pathogen of many dicot plants.
- Similarly, Retroviruses have also been disarmed and are now used to deliver desirable genes into animal cells.
- So, once a gene or a DNA fragment had been ligated into a suitable vector it is transferred into a bacterial, plant or animal host, where it multiplies.

3. Competent Host (Introduction of DNA into Host Cells)

- Competent host is essential for transformation with recombinant DNA. In order to force bacteria to take up the plasmid, the bacterial cells must first be made competent to take up DNA.
- Cells that are able to take up DNA are called competent cells.
- **Methods to introduce alien DNA into host cells:**
  - ➢ This is done by treating bacterial cell with specific concentration of divalent cation such as calcium. Calcium increases the efficiency with which DNA enters the bacterium through pores in its cell wall.



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- ➢ Recombinant DNA can also be forced into cells by incubating the cells with recombinant DNA on ice followed by placing them briefly at 42°C (heat shock), and then putting them back on ice. This enables the bacteria to take up the recombinant DNA.
- ➢ **Micro-injection:** In this, recombinant DNA is directly injected into the nucleus of an animal cell.
- ➢ **Biolistics (gene gun):** In this, cells are bombarded with high velocity micro-particles of gold or tungsten coated with DNA. This method is suitable for plants.
- ➢ **'Disarmed pathogen' vectors** (*Agrobacterium tumifaciens*) which when infects the cell transfer the recombinant DNA into the host.

PROCESSES OF RECOMBINANT DNA TECHNOLOGY

- Recombinant DNA technology is a "cut and paste" technology.
- In this, specific nucleotide sequences are cut' from the DNA of an organism and "pasted" into plasmids. Hence, the DNA of the plasmid carrying nucleotide sequence of another organism is known as recombinant DNA.
- It is then inserted into bacteria. Bacteria divide repeatedly and a clone of bacteria with the recombinant DNA is obtained.
- It includes the following steps:
  1. Isolation of DNA.
  2. Fragmentation of DNA by **restriction endonuclease**.
  3. Isolation of desired DNA fragment by **gel electrophoresis**.
  4. Amplification of gene using PCR.
  5. Ligation of the DNA fragment into a vector.
  6. Culturing the host cells in a medium at large scale in a **bioreactor.**
  7. Extraction of desired product by **down stream processing.**

37. Expression vectors are different from other vectors because they
(a) contain drug resistance markers.
(b) contain telomeres.
(c) contain regulatory regions that permit the cloned DNA to produce a gene product.
(d) contain DNA origins.

38. Stirred tank bioreactors have been designed for
(a) addition of preservatives to the product
(b) purification of the product
(c) ensuring anaerobic conditions in the culture vessel
(d) availability of oxygen throughout the process

39. A genomic library is
(a) where you look to find out how to make recombinant DNA.
(b) a listing of the known nucleotide sequences for a particular species.
(c) all the genes contained in one kind of cell.
(d) a collection of cloned DNA pieces from an organism's genome.

40. Electrophoresis is used to
(a) separate fragments of DNA.
(b) clone genes.
(c) cut DNA into fragments.
(d) match a gene with its function.

41. Which of the following is not necessary to execute a polymerase chain reaction successfully?
(a) All four DNA bases
(b) Short DNA base primers
(c) DNA polymerase
(d) DNA library

42. Which of the following statements about restriction enzymes is false?
(a) They work on DNA extracted from all types of organisms.
(b) They are used to glue together short segments of DNA.
(c) They come in many varieties, each with its own DNA target sequence.
(d) They are highly specific for their DNA target sequences.

43. DNA ligases are enzymes that can be used to
(a) chop a large DNA molecule into small fragments.
(b) copy DNA fragments.
(c) insert the DNA from one species into the DNA of another species.
(d) separate DNA fragments based on their size.

44. Imagine a gel through which DNA fragments have moved in response to an applied electrical current. The band on this gel that is farthest from the top (that is, from the place where the DNA fragments were added to the "well") represents the
(a) shortest fragments of DNA.
(b) longest fragments of DNA.
(c) restriction enzyme used to cut the DNA into fragments.
(d) ligase used to bind the DNA fragments together.

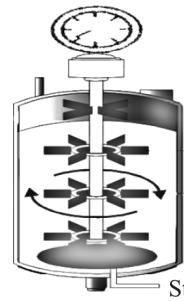
45. First restriction endonuclease enzyme was discovered in
(a) PPLO
(b) *E. coli*
(c) *Haemophilous influenzae*
(d) Bacteriophages

46. A biologist intends to use a polymerase chain reaction to perform a genetic task. The biologist probably is trying to
(a) discover new genes.
(b) clone a gene.
(c) cut DNA into many small fragments.
(d) isolate DNA from a living cell.

47. In genetic engineering, genes can be inserted from one organism into another or back into the original organism using which of the following techniques?
(a) Polymerase chain reaction
(b) Gene gun
(c) DNA hybridization
(d) Gel electrophoresis

48. Which of the following is not necessary to execute a polymerase chain reaction successfully?
(a) All four DNA bases (b) Short DNA base primers
(c) DNA polymerase (d) DNA library

49. Identify the correct match for the given apparatus.



	Apparatus	Function
(a)	Gene gun	Vectorless direct gene transfer
(b)	Column chromatograph	Separation of chlorophyll pigments
(c)	Stirred tank bioreactor	Carry out fermentation process
(d)	Respirometer	Finding out rate of respiration

50. Recombinant DNA molecules were first of all synthesized by Paul Bery using DNA of
(a) TMV and *Salmonella*
(b) Virus SB-40 and *E.coli*
(c) Influenza virus and *Diplococcus* bacterium
(d) TMV and *Agrobacterium tumefaciens*

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