Ch 9 · Biotechnology: Principles & Processes

अध्याय 9 · जैव प्रौद्योगिकी: सिद्धांत व प्रक्रम

Class 12 Biology · NCERT based · English + हिंदी · ✍️ revision notes

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Introduction / परिचय

- **Biotechnology** (जैव प्रौद्योगिकी) deals with techniques of using **live organisms or enzymes from organisms** to produce products & processes useful to humans.
- Examples: making curd, bread, wine (microbe-mediated processes); **in vitro fertilisation** leading to "test-tube" baby; synthesising a gene & using it; developing a **DNA vaccine**; correcting a **defective gene**.

EFB definition (European Federation of Biotechnology): Biotechnology is "the integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services".

EFB के अनुसार – उत्पादों व सेवाओं हेतु प्राकृतिक विज्ञान तथा जीवों, कोशिकाओं, उनके भागों एवं आणविक अनुरूपों का समन्वय।

I. Principles of Biotechnology / जैव प्रौद्योगिकी के सिद्धांत

Two core techniques that enabled birth of modern biotechnology:

① Genetic engineering (आनुवंशिक इंजीनियरी)	② Bioprocess engineering (जैव प्रक्रम इंजीनियरी)
Techniques to **alter the chemistry of genetic material (DNA & RNA)**, introduce these into host organisms and thus change the phenotype of the host organism	Maintenance of **sterile** (microbial contamination-free) ambience in chemical engineering processes to enable growth of **only the desired microbe/eukaryotic cell in large quantities** for manufacture of biotech products (antibiotics, vaccines, enzymes)

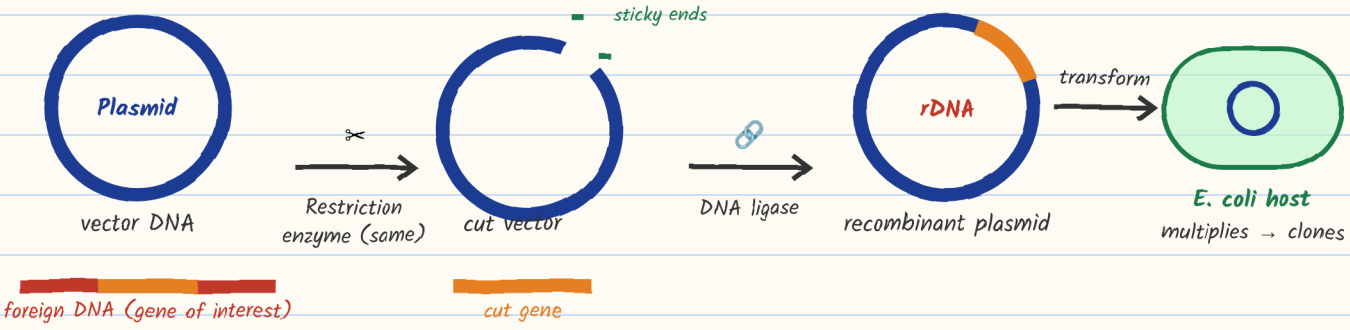
- **Asexual reproduction** preserves genetic information; **sexual reproduction** permits variation.
- Traditional **hybridisation** very often leads to inclusion & multiplication of **undesirable genes** along with desired genes.
- Genetic engineering techniques – **recombinant DNA (rDNA)**, **gene cloning** & **gene transfer** – overcome this limitation & allow us to isolate & introduce **only one or a set of desirable genes** without introducing undesirable genes into target organism.
- A piece of DNA transferred into an alien organism **would not be able to multiply itself** in progeny cells. It must be integrated into a chromosome that has a specific DNA sequence called 'origin of replication' (ori), which is responsible for initiating replication. Thus, an alien DNA is linked with ori so that it can multiply & make copies in host = cloning.

📌 **First recombinant DNA (1972):** Stanley Cohen & Herbert Boyer isolated an **antibiotic resistance gene** by cutting out a piece of DNA from a plasmid of *Salmonella typhimurium*, and linked it with the plasmid of *E. coli*.

· Cutting – by **restriction enzymes** ("molecular scissors") · Linking – by **DNA ligase** · Carrier – **plasmid vector** (autonomously replicating circular extra-chromosomal DNA).

When transferred into *E. coli*, it replicated using the host's DNA polymerase → many copies → **cloning of antibiotic resistance gene in *E. coli***.

Making recombinant DNA · पुनर्योगज डीएनए का निर्माण



Infographic: Basic steps of making recombinant DNA

Three basic steps in genetically modifying an organism:

1. **Identification** of DNA with desirable genes.
2. **Introduction** of the identified DNA into the host.
3. **Maintenance** of introduced DNA in the host & **transfer** of the DNA to its progeny.

2. Tools of rDNA Technology – Restriction Enzymes / प्रतिबंधन एंजाइम

Key tools: **restriction enzymes, polymerase enzymes, ligases, vectors & host organism**.

- In **1963**, two enzymes responsible for **restricting the growth of bacteriophage** in *E. coli* were isolated: one **added methyl groups** to DNA, other **cut DNA** = restriction endonuclease.
- First restriction endonuclease = Hind II – always cut DNA molecules at a particular point by recognising a specific sequence of **six base pairs** = **recognition sequence**.
- Today more than **900 restriction enzymes** isolated from over **230 strains of bacteria**, each recognising different recognition sequences.

Naming of restriction enzymes नामकरण

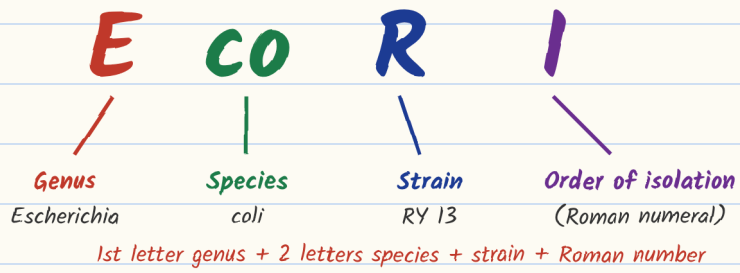


Fig: How EcoRI is named · नामकरण

Types of nucleases न्यूक्लिएज़

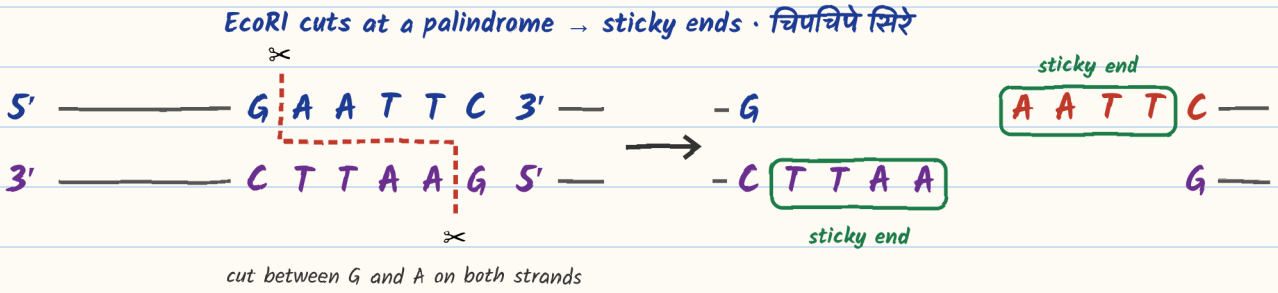
Exonucleases (बाह्य न्यूक्लिएज़)	Endonucleases (अंतः न्यूक्लिएज़)
Remove nucleotides from the **ends** of DNA	Make cuts at **specific positions within** the DNA

Restriction enzymes belong to a larger class of enzymes called **nucleases**.

How restriction endonucleases work कार्यविधि

- Each restriction endonuclease functions by **"inspecting"** the length of a DNA sequence. Once it finds its specific **recognition sequence**, it binds to DNA & cuts each of the two strands of the double helix at specific points in their **sugar-phosphate backbones**.
- Each RE recognises a specific palindromic nucleotide sequence in DNA.

- **Palindrome in DNA** = sequence of base pairs that reads **same on the two strands** when orientation of reading is kept the same (e.g. both 5′ → 3′).



- Reads **GAATTC** 5′ → 3′ on both strands = palindrome
- Overhanging single-stranded ends form H-bonds with complementary cut ends
- Same enzyme on vector & foreign DNA → same sticky ends → joined by **DNA ligase**

Infographic: Action of EcoRI · EcoRI की क्रिया

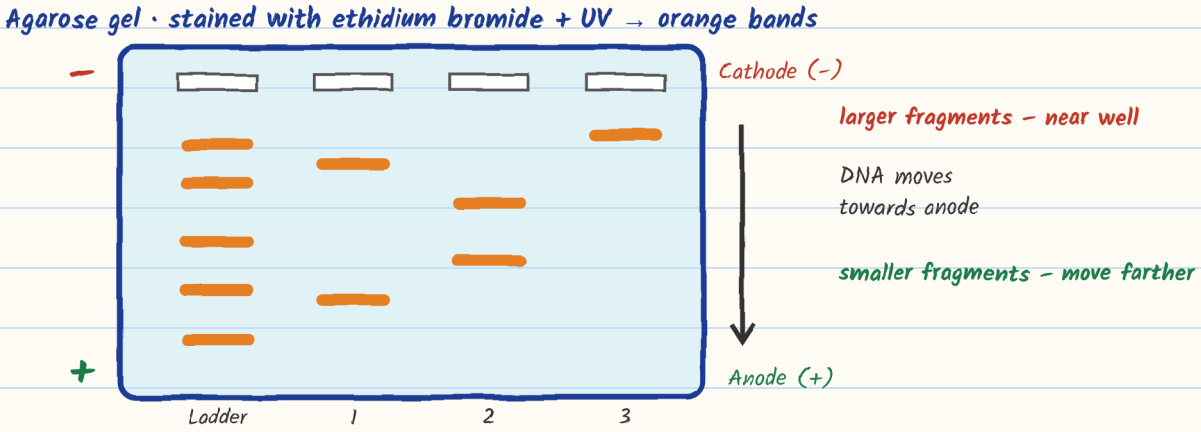
- Restriction enzymes cut the strand of DNA **a little away from the centre** of the palindrome sites, but **between the same two bases** on the opposite strands → leaves single-stranded portions at the ends.
- These overhanging stretches = sticky ends (चिपचिपे सिरे) on each strand. Named so because they form **hydrogen bonds** with their complementary cut counterparts.
- This stickiness facilitates the action of enzyme **DNA ligase**.
- When cut by the **same restriction enzyme**, resultant DNA fragments (vector & source DNA) have the **same kind of sticky ends** → can be joined together (end-to-end) using DNA ligases.

📌 **Palindrome example (words):** MALAYALAM reads same forwards & backwards. In DNA, palindrome is read on **both strands in same direction**:

5′ —— GAATTC —— 3′

3′ —— CTTAAG —— 5′

Separation & isolation of DNA fragments – Gel electrophoresis जेल वैद्युत कण संचलन



Infographic: Agarose gel electrophoresis · एगारोज़ जेल वैद्युत कण संचलन

- Cutting of DNA by restriction endonucleases results in fragments of DNA, separated by technique called gel electrophoresis.
- DNA fragments are **negatively charged** → separated by forcing them to move towards the **anode** under an electric field through a medium/matrix.
- Most commonly used matrix = agarose – a natural polymer extracted from **sea weeds**.
- DNA fragments separate (resolve) according to their **size** through **sieving effect** of agarose gel. Smaller the fragment, farther it moves.
- Separated DNA fragments visualised only after staining with ethidium bromide followed by exposure to **UV radiation** → bright **orange coloured bands**.
- Separated bands cut out from agarose gel & extracted from gel piece = elution (निक्षालन). Purified DNA fragments used in constructing recombinant DNA by joining with cloning vectors.

3. Tools – Cloning Vectors / क्लोनिंग संवाहक

- **Plasmids & bacteriophages** have ability to replicate within bacterial cells **independent of control of chromosomal DNA**.
- **Bacteriophages** – high number per cell → very high copy numbers of their genome. Some **plasmids** have only one or two copies per cell; others may have **15–100 copies** per cell.
- If an alien piece of DNA is linked with bacteriophage or plasmid DNA, we can multiply its numbers equal to the copy number of the plasmid or bacteriophage.
- Vectors used at present are engineered to easily link foreign DNA & select recombinants from non-recombinants.

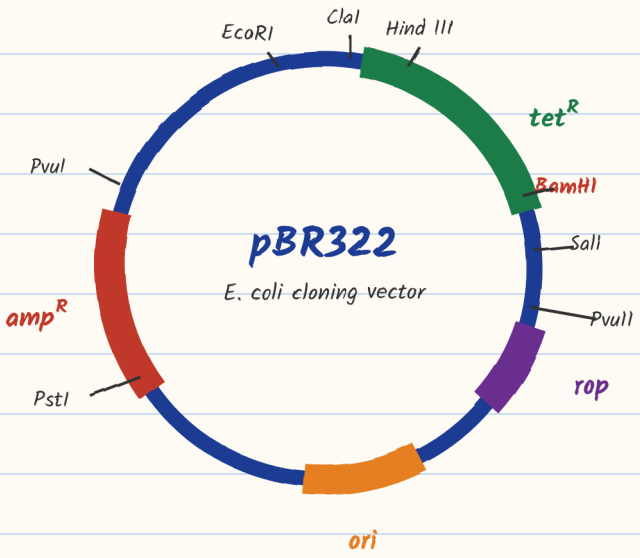


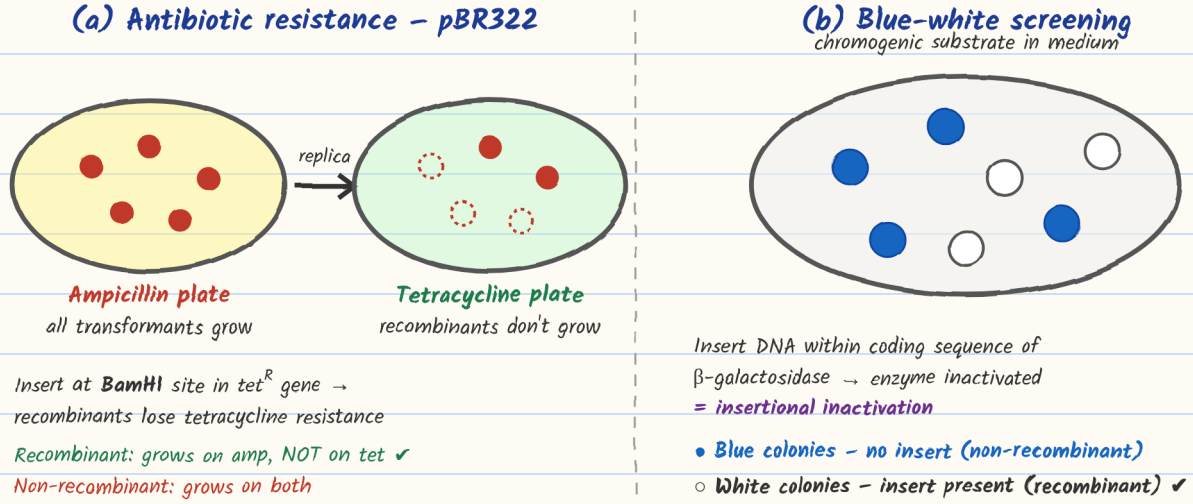
Fig: E. coli cloning vector pBR322 · pBR322 संवाहक

pBR322 has restriction sites for **Hind III, EcoRI, BamHI, SalI, PvuII, PstI, ClaI**; **ori**; antibiotic resistance genes **amp^R** & **tet^R**; and **rop** (codes for proteins involved in replication of the plasmid).

Features required to facilitate cloning into a vector संवाहक के आवश्यक लक्षण

1. **Origin of replication (ori)** (प्रतिकृतीयन का उद्भव): sequence from where replication starts; any piece of DNA linked to this sequence can be made to replicate within host cells. Also **responsible for controlling the copy number** of the linked DNA. So, to recover many copies of target DNA, clone it in a vector whose origin supports **high copy number**.
2. **Selectable marker** (वरण योग्य चिह्नक): helps in identifying & eliminating **non-transformants** and selectively permitting growth of **transformants**.
   - Transformation = procedure through which a piece of DNA is introduced in a host bacterium.
   - Normally, genes encoding resistance to antibiotics such as **ampicillin, chloramphenicol, tetracycline or kanamycin** are considered useful selectable markers for *E. coli*. Normal *E. coli* cells don't carry resistance against any of these antibiotics.
3. **Cloning sites** (क्लोनिंग स्थल): to link the alien DNA, vector needs to have **very few, preferably single, recognition sites** for commonly used restriction enzymes. Presence of more than one recognition site will generate several fragments → complicates gene cloning.
4. **Vectors for cloning genes in plants & animals** (see below).

Selection of recombinants पुनर्योगजों का चयन



Infographic: Selecting recombinants · पुनर्योगजों का चयन

- ***pBR322 & antibiotic resistance***: *Ligation of foreign DNA at* ***BamHI*** *site of* ***tetracycline resistance gene*** *→ recombinant plasmids* ***lose tetracycline resistance****, but still can be selected by plating transformants on* ***ampicillin****-containing medium. Transformants growing on ampicillin are then transferred on* ***tetracycline*** *medium. Recombinants grow in ampicillin but* ***not*** *on tetracycline. Non-recombinants grow on both.*
- *Here one antibiotic resistance gene helps select transformants; the other gets "inactivated due to insertion" of alien DNA & helps in selection of recombinants.*
- *Selection of recombinants due to inactivation of antibiotics is cumbersome – requires* ***simultaneous plating on two plates*** *with different antibiotics.*
- *Alternative selectable markers – differentiate recombinants from non-recombinants on the basis of their ability to produce* ***colour*** *in presence of a* ***chromogenic substrate****.*
- *Insertional inactivation (निवेशी निष्क्रियता): recombinant DNA inserted within coding sequence of enzyme* ***β-galactosidase*** *→ enzyme inactivated. Presence of chromogenic substrate gives* ***blue****-coloured colonies if plasmid has* ***no insert****. Presence of insert → no β-galactosidase → colonies* ***do not produce colour*** *(white) → identified as* ***recombinant colonies****.*

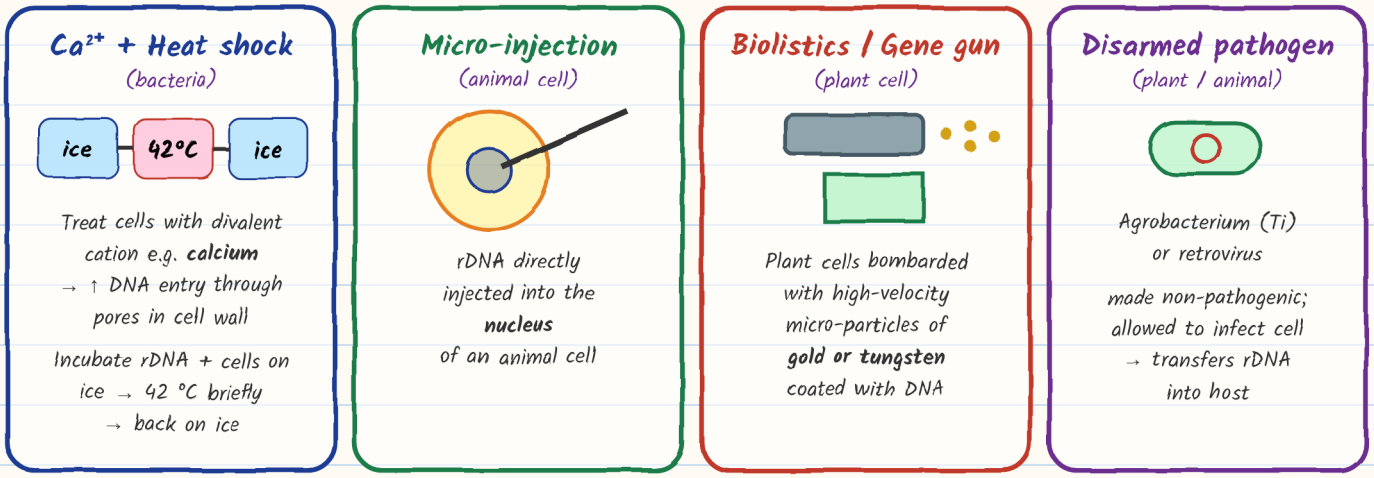
Vectors for cloning genes in plants & animals पादप व जंतुओं हेतु संवाहक

Plants	Animals
Agrobacterium tumefaciens – a pathogen of several **dicot plants** – delivers a piece of DNA known as **'T-DNA'** to transform normal plant cells into a **tumour** & direct these cells to produce chemicals required by the pathogen. **Tumour inducing (Ti) plasmid** of *A. tumefaciens* has been modified into a cloning vector which is **no more pathogenic** to plants but still able to use mechanisms to deliver genes of interest into plants.	**Retroviruses** in animals (including humans) have the ability to transform normal cells into **cancerous cells**. *Disarmed retroviruses* are now used to deliver desirable genes into animal cells.

📌 **Natural genetic engineers:** We learnt the lesson of transferring genes from pathogens – they have been doing this for ages. Once a gene/DNA fragment is ligated into a suitable vector, it is transferred into a bacterial, plant or animal host, where it multiplies.

4. Competent Host & Gene Transfer / सक्षम परपोषी

- DNA is a **hydrophilic** molecule → it **cannot pass through cell membranes**.
- To force bacteria to take up plasmid, bacterial cells must first be made *'competent'* to take up DNA.



Infographic: Methods of introducing rDNA into host cells · rDNA स्थानांतरण की विधियाँ

- **Chemical method**: treat bacterial cells with a specific concentration of a **divalent cation** such as *calcium* → increases efficiency with which DNA enters the bacterium through **pores in its cell wall**.
- rDNA then forced into such cells by incubating cells with rDNA **on ice**, followed by placing them briefly at *42 °C (heat shock)*, then putting back on ice → bacteria take up rDNA.

- **Micro-injection** (सूक्ष्म अंतक्षेपण): recombinant DNA directly injected into the **nucleus of an animal cell**.
- **Biolistics or gene gun** (जीन गन): suitable for **plants**; cells bombarded with high-velocity micro-particles of **gold or tungsten** coated with DNA.
- **Disarmed pathogen vectors**: when allowed to infect the cell, transfer the recombinant DNA into the host.

5. Processes of Recombinant DNA Technology / पुनर्योगज डीएनए प्रौद्योगिकी के प्रक्रम

① Isolation of DNA → ② Cutting at specific locations (RE) →
③ Amplification (PCR) → ④ Ligation into vector (ligase) →
⑤ Insertion into host (transformation) → ⑥ Culturing host & obtaining product →
⑦ Downstream processing

(a) Isolation of the genetic material (DNA) आनुवंशिक पदार्थ का पृथक्करण

- DNA is enclosed within membranes along with other macro-molecules (RNA, proteins, polysaccharides, lipids). To get pure DNA, cells must be broken open.

Cell type	Enzyme used to break cell
Bacteria	Lysozyme
Plant cells	Cellulase
Fungus	Chitinase

- Genes are located on long DNA molecules intertwined with proteins like histones. **RNA** removed by treatment with **ribonuclease**; **proteins** removed by treatment with **protease**. Other molecules removed by appropriate treatments.
- Purified DNA finally **precipitates after addition of chilled ethanol** → seen as a collection of **fine threads** in the suspension (**spooling**).

📌 **Trick:** **L**ysozyme – **L**ittle bacteria · **Cellu**lase – **Cellu**lose wall of plants · **Chiti**nase – **Chiti**n wall of fungi.

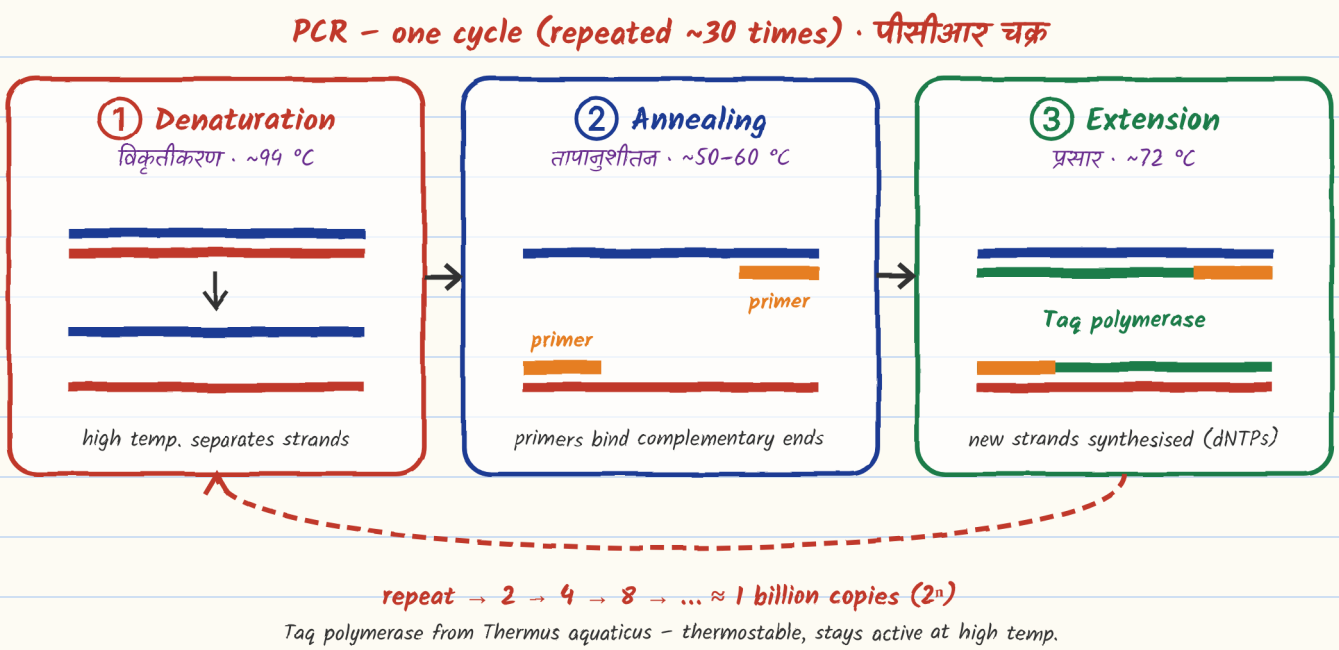
(b) Cutting of DNA at specific locations विशिष्ट स्थलों पर डीएनए काटना

- Restriction enzyme digestion: incubating purified DNA molecules with the restriction enzyme at **optimal conditions** for that specific enzyme.

- **Agarose gel electrophoresis** employed to check progression of a restriction enzyme digestion (DNA is negatively charged → moves towards positive electrode/anode).
- Process repeated with **vector DNA** too (cut with same enzyme).
- After cutting source DNA & vector DNA with specific RE, cut-out "gene of interest" from source DNA & cut vector with space are mixed, and **ligase** is added → **recombinant DNA**.

(c) Amplification of gene of interest using PCR पीसीआर द्वारा प्रवर्धन

PCR = Polymerase Chain Reaction (पॉलीमरेज़ शृंखला अभिक्रिया). Multiple copies of gene (or DNA) of interest synthesised **in vitro** using **two sets of primers** (small chemically synthesised oligonucleotides complementary to regions of DNA) and enzyme **DNA polymerase**.



Infographic: Polymerase Chain Reaction (PCR) · पॉलीमरेज़ शृंखला अभिक्रिया

Step	What happens
Denaturation	Double-stranded DNA heated to high temperature → strands separate
Annealing	Two sets of **primers** bind to complementary regions of DNA
Extension	DNA polymerase extends primers using nucleotides provided in reaction & genomic DNA as template

- If the process of replication of DNA is repeated many times, the segment of DNA can be amplified to approximately billion times (1 billion copies).
- Repeated amplification achieved by use of a **thermostable DNA polymerase** (isolated from bacterium *Thermus aquaticus*) – **Taq polymerase** – remains active during high temperature induced denaturation of double-stranded DNA.
- Amplified fragment, if desired, can now be used to ligate with a vector for further cloning.

(d) Insertion of rDNA into host cell/organism परपोषी में निवेशन

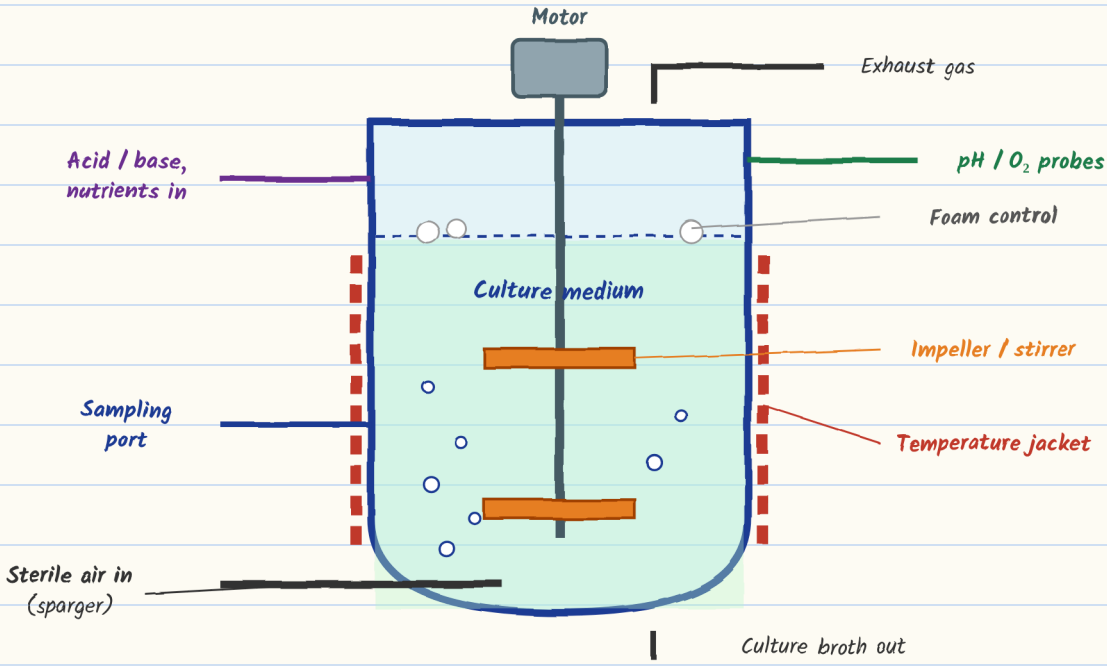
- Several methods to introduce ligated DNA into recipient cells. Recipient cells, after making them 'competent', take up DNA present in their surroundings.
- If a recombinant DNA bearing gene for resistance to an antibiotic (e.g. **ampicillin**) is transferred into *E. coli* cells, host cells become **transformed into ampicillin-resistant cells**.
- If transformed cells are spread on agar plates containing ampicillin, **only transformants will grow**; untransformed recipient cells will die. Since due to ampicillin resistance gene one is able to select a transformed cell in presence of ampicillin, the ampicillin resistance gene = selectable marker.

(e) Obtaining the foreign gene product बाह्य जीन उत्पाद प्राप्त करना

- When you insert a piece of alien DNA into a cloning vector & transfer it into a bacterial, plant or animal cell, the alien DNA gets multiplied. Ultimate aim of rDNA technology = to **produce a desirable protein**.
- If any protein-encoding gene is expressed in a heterologous host, it is called a recombinant protein (पुनर्योगज प्रोटीन).
- Cells harbouring cloned genes of interest may be grown on a **small scale in laboratory**; cultures used for extracting desired protein & purifying it.
- Cells can also be multiplied in a continuous culture system: used medium drained out from one side while fresh medium added from the other → maintains cells in their physiologically most active **log/exponential phase** → **larger biomass** → higher yields of desired protein.

6. Bioreactors & Downstream Processing / जैव रिएक्टर व अनुप्रवाह संसाधन

- Small volume cultures can't yield appreciable quantities of products. To produce in large quantities, development of bioreactors was required, where large volumes (**100–1000 litres**) of culture can be processed.
- Bioreactors = **vessels** in which raw materials are biologically converted into specific products, individual enzymes, etc., using microbial, plant, animal or human cells.
- Bioreactor provides **optimal conditions** for achieving desired product by providing optimum growth conditions: **temperature, pH, substrate, salts, vitamins, oxygen**.
- Most commonly used = stirred-tank reactors.



Infographic: Sparged stirred-tank bioreactor (schematic) · हिलाने वाला टैंक जैव रिएक्टर

- ***Stirred-tank reactor*** *is usually* ***cylindrical*** *or with a* ***curved base*** *to facilitate mixing of reactor contents.*
- ***Stirrer*** *facilitates even mixing &* ***oxygen availability*** *throughout the bioreactor. Alternatively, air can be bubbled through the reactor.*
- *In a* ***sparged stirred-tank bioreactor****, sterile air bubbles are sparged → increases* ***surface area for oxygen transfer****.*

Components of a bioreactor *(जैव रिएक्टर के भाग):*

1. ***Agitator system***
2. ***Oxygen delivery system***
3. ***Foam control system***
4. ***Temperature control system***
5. ***pH control system***
6. ***Sampling ports*** *– so that small volumes of culture can be withdrawn periodically*

Downstream processing अनुप्रवाह संसाधन

- *After completion of biosynthetic stage, product has to be subjected through a series of processes before it is ready for marketing as a finished product.*
- *Processes include* ***separation & purification*** *= collectively referred to as downstream processing.*
- *Product formulated with suitable* ***preservatives****. Such formulation has to undergo thorough* ***clinical trials*** *as in case of drugs.*
- ***Strict quality control testing*** *for each product is also required.*
- *Downstream processing & quality control testing vary from product to product.*

⚡ Quick Revision / त्वरित पुनरावृत्ति

Tool / Term	Remember
Molecular scissors	Restriction endonucleases
Molecular glue	DNA ligase
First RE	Hind II (6 bp recognition sequence)
EcoRI recognition site	5'-GAATTC-3' (cuts between G & A)
Agarose	From sea weeds; matrix for electrophoresis
Ethidium bromide + UV	Orange DNA bands
Elution	Extracting DNA bands from gel
pBR322	ori, rop, amp^R, tet^R; BamHI in tet^R
Insertional inactivation	β-galactosidase; blue = non-recombinant, white = recombinant
Ti plasmid	*Agrobacterium tumefaciens* → plant vector
Disarmed retrovirus	Animal vector
Competent cells	Ca^{2+} + heat shock (ice → 42 °C → ice)
Gene gun	Gold / tungsten particles – plants
Lysozyme / cellulase / chitinase	Bacteria / plant / fungus cell breaking
Chilled ethanol	Precipitates DNA (fine threads)
Taq polymerase	*Thermus aquaticus*; thermostable
Bioreactor volume	100–1000 litres
Cohen & Boyer	First rDNA (1972) – Salmonella typhimurium plasmid

⭐ Exam Corner / परीक्षा उपयोगी प्रश्न

1-Mark Questions 1M

Q1. What are "molecular scissors"? / "आणविक कैंची" क्या हैं?

→ Restriction endonucleases (enzymes).

Q2. Expand PCR. / PCR का पूर्ण रूप?

→ Polymerase Chain Reaction.

Q3. Name the source of Taq polymerase.

→ Bacterium *Thermus aquaticus*.

Q4. Write the palindromic sequence recognised by EcoRI.

→ 5'-GAATTC-3' / 3'-CTTAAG-5'.

Q5. Why is DNA isolated by adding chilled ethanol?

→ DNA precipitates in chilled ethanol (seen as fine threads).

Q6. What is the function of 'rop' in pBR322?

→ Codes for proteins involved in replication of the plasmid.

Q7. Which stain is used to visualise DNA in gel electrophoresis?

→ Ethidium bromide (under UV light).

Q8. Name the enzyme used to break fungal cell walls during DNA isolation.

→ Chitinase.

2/3-Mark Questions 2–3M

Q9. Explain how EcoRI got its name.

→ E – Escherichia, co – coli, R – strain RY13, I – first enzyme isolated from this strain.

Q10. What are sticky ends? Why are they important?

→ Overhanging single-stranded ends after RE cut; form H-bonds with complementary ends; help DNA ligase join vector & foreign DNA.

Q11. Why must the vector and source DNA be cut with the same restriction enzyme?

→ To produce identical, complementary sticky ends that can pair & be ligated.

Q12. Describe the role of ori, selectable marker and cloning sites in a vector.

→ Replication & copy number; identify transformants; single RE sites for inserting foreign DNA.

Q13. What is insertional inactivation? How does it help in selecting recombinants?

→ Insertion within β-galactosidase gene inactivates it; recombinants give white colonies, non-recombinants blue.

Q14. How are bacterial cells made competent to take up DNA?

→ Ca^{2+} treatment; incubate with rDNA on ice → heat shock 42 °C → ice.

Q15. Why is Agrobacterium called a "natural genetic engineer"? How is Ti plasmid used?

→ Delivers T-DNA into dicot plant cells causing tumours; Ti plasmid disarmed → vector for plant genes.

Q16. What is downstream processing?

→ Separation, purification, formulation with preservatives, clinical trials & quality control before marketing.

5-Mark Questions (5M) ★★★

Q17. Describe the process of PCR with a labelled diagram. Why is Taq polymerase used?

→ Denaturation, annealing, extension; repeated cycles; ~1 billion copies; Taq is thermostable.

Q18. Draw a labelled diagram of pBR322 and explain how recombinants are selected using antibiotic resistance genes.

→ ori, rop, ampR, tetR, RE sites; insert at BamHI in tetR; grow on ampicillin, not on tetracycline.

Q19. Explain gel electrophoresis and its use in rDNA technology.

→ Agarose matrix, DNA moves to anode, smaller fragments move farther, ethidium bromide + UV, elution.

Q20. Describe the processes of rDNA technology in sequence.

→ Isolation → cutting → amplification (PCR) → ligation → insertion → culture & product → downstream processing.

Q21. What is a bioreactor? Describe a stirred-tank bioreactor with its components.

→ Large vessel (100–1000 L), optimal conditions; agitator, O_2 delivery, foam, temperature, pH control, sampling ports; sparged type.

✏ **Diagrams to practise / अभ्यास हेतु चित्र:**

1. Steps in formation of rDNA by EcoRI ⭐
2. Agarose gel electrophoresis
3. pBR322 vector ⭐
4. PCR ⭐
5. Stirred-tank bioreactor (simple & sparged)

🎯 **Last-minute tips:** Always mark **5′ and 3′** ends on palindromes · In pBR322 questions, remember **BamHI is in tet^R** & **PstI/PvuI are in amp^R** · Blue = no insert, White = recombinant · PCR steps in order: **D-A-E** (Denaturation, Annealing, Extension) · Enzyme for each cell wall: **lysozyme, cellulase, chitinase**.

~ All the best! / शुभकामनाएँ! ~