FREE NOTES · NCERT + NEET LEVEL

Biotechnology: Principles & Processes

Principles of biotechnology, tools of recombinant DNA technology (restriction enzymes, cloning vectors, competent host), and processes: isolation of DNA, cutting, PCR, insertion, selection, culturing in bioreactors and downstream processing.

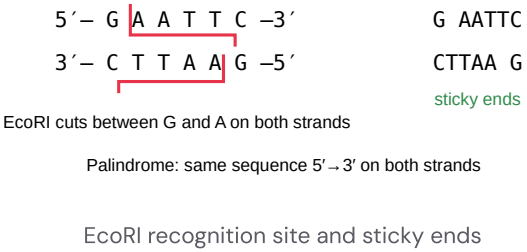
01 Principles of biotechnology
02 Restriction enzymes
03 Cloning vectors
04 Competent host and gene transfer
05 Processes of rDNA technology and PCR
06 Bioreactors and downstream processing
07 Extra + tricks
08 Important Q&A
09 Common mistakes
10 Practice (NEET style)
11 Quick revision

01 Principles of biotechnology

- EFB definition: integration of natural science and organisms, cells, parts thereof and molecular analogues for products and services.
- Two core techniques: **genetic engineering** (alter genetic material) and **bioprocess engineering** (sterile conditions for growing only desired microbe/cells in large quantities).
- First recombinant DNA: **Stanley Cohen and Herbert Boyer** (1972): antibiotic resistance gene linked with native plasmid of *Salmonella typhimurium*.
- Three basic steps: identify DNA with desirable genes; introduce it into host; maintain it in host and transfer to progeny.
- **Origin of replication (ori)**: sequence where replication starts; needed for alien DNA to multiply.

02 Restriction enzymes

- "Molecular scissors". First: **Hind II** (always cuts at a specific 6 bp sequence = recognition sequence). Now 900+ known from 230+ strains of bacteria.
- Naming: **EcoRI** = *E*scherichia *co*li strain **R**Y13, first (I) enzyme isolated.
- **Nucleases**: exonucleases (remove from ends) and endonucleases (cut within). Restriction endonucleases recognise **palindromic** sequences, cut a little away from centre ⇒ **sticky ends** (help DNA ligase join).
- **Gel electrophoresis**: DNA (negative) moves to anode through **agarose** (from seaweed); smaller fragments move farther. Stain **ethidium bromide**, see orange bands in UV; cut out = **elution**.



EcoRI recognition site and sticky ends

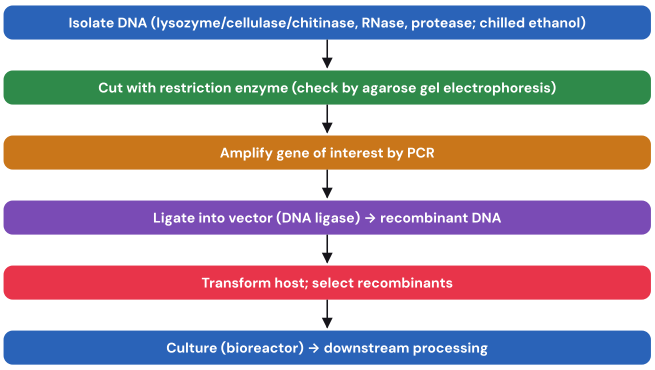
03 Cloning vectors

- Plasmids and bacteriophages replicate independently in bacteria; high copy number gives many copies of the inserted gene.
- Features: **ori** (controls copy number), **selectable marker** (e.g. antibiotic resistance: ampicillin, chloramphenicol, tetracycline, kanamycin), **cloning sites** (preferably single recognition site for commonly used enzymes), small size.
- **pBR322**: ori, rop (codes proteins for replication), amp^R and tet^R, sites for Hind III, EcoRI, BamHI, SalI, PvuII, PstI, ClaI. Insert in tet^R (at BamHI) ⇒ recombinants lose tetracycline resistance: **insertional inactivation**.
- Alternative selection: **blue-white screening**: insert in lacZ (β-galactosidase) gene; recombinants give **white** (colourless) colonies on chromogenic substrate, non-recombinants blue.
- Plant vector: ***Agrobacterium tumefaciens*** Ti plasmid (tumour inducing) modified to deliver genes without disease. Animal vectors: disarmed retroviruses.

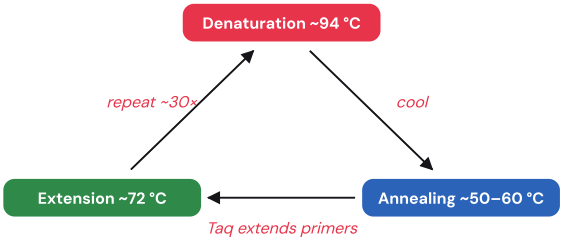
04 Competent host and gene transfer

- DNA is hydrophilic, cannot pass membranes. Make cells **competent**: treat with divalent cation (**calcium**) ⇒ incubate with rDNA on ice ⇒ **heat shock** (42 °C) ⇒ back on ice.
- **Micro-injection**: rDNA directly into nucleus of animal cell.
- **Biolistics / gene gun**: plant cells bombarded with high-velocity micro-particles of gold or tungsten coated with DNA.
- **Disarmed pathogen** vectors: allowed to infect cells, transfer rDNA.

05 Processes of rDNA technology and PCR



Steps of recombinant DNA technology



One PCR cycle

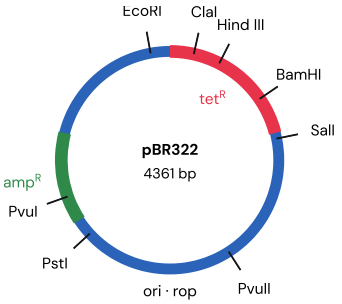
- **PCR** (polymerase chain reaction): two primers + thermostable **Taq polymerase** (from *Thermus aquaticus*) + dNTPs; repeated ~30 cycles ⇒ about 1 billion copies.
- Alien DNA in host is "expressed" to make **recombinant protein**.

06 Bioreactors and downstream processing

- Small-scale culture in flasks; large-scale in **bioreactors** (100–1000 L): optimal temperature, pH, substrate, salts, vitamins, O_2.
- **Stirred-tank bioreactor**: cylindrical with curved base for mixing; agitator, oxygen delivery (sparger), foam control, temperature and pH control, sampling ports. Sparged stirred-tank: sterile air bubbles increase O_2 transfer.
- **Continuous culture**: used medium drained and fresh added ⇒ cells in log/exponential phase ⇒ more biomass.
- **Downstream processing**: separation and purification; formulation with preservatives; clinical trials (for drugs) and strict quality control.

07 Extra + tricks

Enzyme	Site (5′→3′)	Ends
EcoRI	G↓AATTC	Sticky
BamHI	G↓GATCC	Sticky
Hind III	A↓AGCTT	Sticky
SmaI	CCC↓GGG	Blunt



pBR322 cloning vector (simplified map)

- **BAC** (bacterial artificial chromosome) and **YAC** (yeast artificial chromosome) carry very large DNA (used in HGP).
- Host cell walls broken by: lysozyme (bacteria), cellulase (plants), chitinase (fungi).
- pBR322: **p** = plasmid, **BR** = Bolivar and Rodriguez; insert at BamHI ⇒ tet^R lost; at PstI/PvuI ⇒ amp^R lost.

08 Important Q&A

Q1. What is insertional inactivation?

Inserting foreign DNA within a marker gene (e.g. tet^R or lacZ) inactivates it, so recombinants can be identified by loss of that function.

Q2. Why is Taq polymerase used in PCR?

It is thermostable (from *Thermus aquaticus*), remains active during high-temperature denaturation.

Q3. How is a bacterial cell made competent?

Treat with calcium ions, incubate with rDNA on ice, give heat shock at 42 °C, put back on ice.

09 Common mistakes

- Saying DNA moves to cathode (it moves to anode, being negative).
- Calling blue colonies recombinant (white are recombinant).
- Thinking restriction enzymes cut randomly.
- Confusing exonuclease and endonuclease.

10 Practice (NEET style)

1. First restriction endonuclease?
2. Stain for DNA in gel?
3. Source of Taq polymerase?
4. Plant vector bacterium?
5. First rDNA made by?

ANSWERS

1) Hind II · 2) Ethidium bromide · 3) *Thermus aquaticus* · 4) *Agrobacterium tumefaciens* · 5) Cohen and Boyer

11 Quick revision: 5 minutes before the exam

TOOLS

- EcoRI: GAATTC, sticky ends
- Ligase joins
- Agarose gel, EtBr

VECTORS

- ori, marker, cloning site
- pBR322 amp^R tet^R
- Blue-white screening

TRANSFER

- Ca^{2+} + heat shock
- Micro-injection
- Gene gun

PROCESS

- PCR: denature-anneal-extend
- Bioreactor 100–1000 L
- Downstream processing

Have a doubt? Ask the AI Doubt Solver at padhaiadda.netlify.app, or take the mock test on this chapter.