FREE NOTES · NCERT + NEET LEVEL

Biotechnology: Principles & Processes

Biotechnology ke siddhant, recombinant DNA technology ke tools (restriction enzymes, cloning vectors, competent host), aur processes: DNA nikaalna, kaatna, PCR, insertion, selection, bioreactors me culture aur downstream processing.

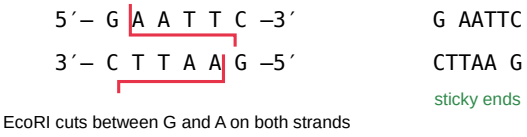
01 Biotechnology ke siddhant
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01 Biotechnology ke siddhant

- EFB paribhasha: natural science aur jeevon, cells, unke hisson aur molecular analogues ko milakar products aur services.
- Do mukhya techniques: **genetic engineering** (genetic material badalna) aur **bioprocess engineering** (sterile halat me sirf chaha microbe/cells bade paimaane pe).
- Pehla recombinant DNA: **Stanley Cohen aur Herbert Boyer** (1972): antibiotic resistance gene *Salmonella typhimurium* ke plasmid se joda.
- Teen basic steps: chahe genes wala DNA pehchaanna; host me daalna; host me banaye rakhna aur santaan tak.
- **Origin of replication (ori)**: jahan se replication shuru; alien DNA badhne ke liye zaroori.

02 Restriction enzymes

- "Molecular scissors". Pehla: **Hind II** (hamesha 6 bp ke khaas sequence pe kaata = recognition sequence). Ab 230+ bacteria strains se 900+ enzymes.
- Naam: **EcoRI** = *E*scherichia *co*li strain **R**Y13, pehla (I) enzyme.
- **Nucleases**: exonucleases (siron se hataate) aur endonucleases (beech me kaatte). Restriction endonucleases **palindromic** sequence pehchaante, centre se thoda hatkar kaatte ⇒ **sticky ends** (DNA ligase jodne me madad).
- **Gel electrophoresis**: DNA (negative) **agarose** (samundari ghaas se) me anode ki taraf; chhote tukde door tak. **Ethidium bromide** se stain, UV me orange bands; kaatkar nikaalna = **elution**.



EcoRI cuts between G and A on both strands

Palindrome: same sequence 5′→3′ on both strands

EcoRI site aur sticky ends

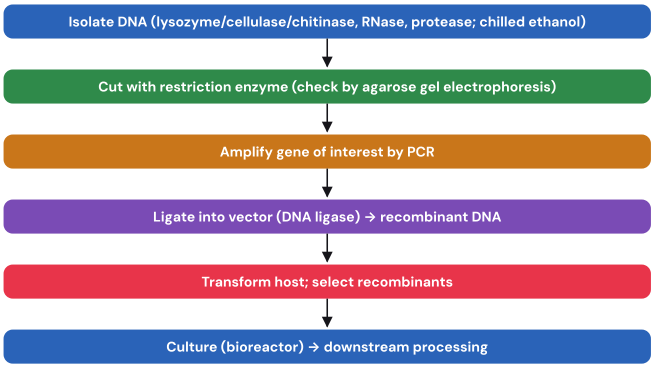
03 Cloning vectors

- Plasmids aur bacteriophages bacteria me khud replicate; zyada copy number se gene ki zyada copies.
- Gun: **ori** (copy number), **selectable marker** (antibiotic resistance: ampicillin, chloramphenicol, tetracycline, kanamycin), **cloning sites** (aam enzymes ke liye ek hi site), chhota size.
- **pBR322**: ori, rop (replication proteins), amp^R aur tet^R, Hind III, EcoRI, BamHI, SalI, PvuII, PstI, ClaI sites. tet^R me (BamHI pe) insert ⇒ recombinants tetracycline resistance kho dete: **insertional inactivation**.
- Doosra tareeka: **blue-white screening**: lacZ (β-galactosidase) gene me insert; chromogenic substrate pe recombinants **safed**, baaki neele.
- Plant vector: ***Agrobacterium tumefaciens*** ka Ti plasmid (tumour inducing) badalkar bina rog gene dene wala. Animal vectors: disarmed retroviruses.

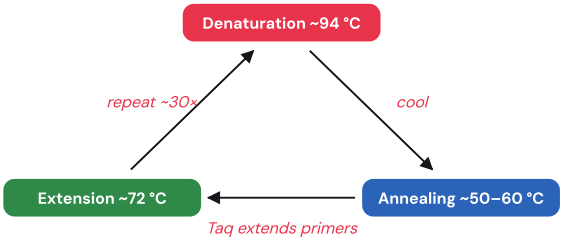
04 Competent host aur gene transfer

- DNA hydrophilic, membrane se nahi guzarta. Cells ko **competent** banana: divalent cation (**calcium**) ⇒ ice pe rDNA ke saath ⇒ **heat shock** (42 °C) ⇒ phir ice.
- **Micro-injection**: animal cell ke nucleus me seedha rDNA.
- **Biolistics / gene gun**: DNA lage gold ya tungsten ke chhote kanon se plant cells pe tez bombardment.
- **Disarmed pathogen** vectors: cells ko infect karke rDNA dete.

05 rDNA technology ke processes aur PCR



rDNA technology ke steps



Ek PCR cycle

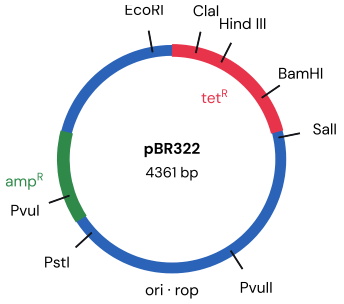
- **PCR**: do primers + garmi sahne wala **Taq polymerase** (*Thermus aquaticus* se) + dNTPs; ~30 cycles ⇒ lagbhag 1 arab copies.
- Host me alien DNA "express" hokar **recombinant protein** banata.

06 Bioreactors aur downstream processing

- Chhota culture flasks me; bada **bioreactors** me (100–1000 L): sahi temperature, pH, substrate, salts, vitamins, O_2.
- **Stirred-tank bioreactor**: gol, mixing ke liye mudi base; agitator, oxygen (sparger), foam control, temperature aur pH control, sampling ports. Sparged stirred-tank: sterile hawa ke bulbule O_2 badhate.
- **Continuous culture**: purana medium nikaalna aur naya daalna ⇒ cells log phase me ⇒ zyada biomass.
- **Downstream processing**: alag karna aur shuddh karna; preservatives ke saath formulation; dawaon ke clinical trials aur sakht 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 (saral map)

- **BAC** aur **YAC** bahut bada DNA le jaate (HGP me).
- Host ki cell wall todna: lysozyme (bacteria), cellulase (paudhe), chitinase (fungi).
- pBR322: **p** = plasmid, **BR** = Bolivar aur Rodriguez; BamHI pe insert ⇒ tet^R khatam; PstI/PvuI pe ⇒ amp^R khatam.

08 Important Q&A

Q1. Insertional inactivation kya?

Marker gene (jaise tet^R ya lacZ) me foreign DNA daalne se wo gene band, recombinants us kaam ki kami se pehchaane jaate.

Q2. PCR me Taq polymerase kyun?

Garmi sehne wala (*Thermus aquaticus* se), denaturation ke tez temperature me bhi active.

Q3. Bacterial cell ko competent kaise?

Calcium ions, ice pe rDNA ke saath, 42 °C heat shock, phir ice.

09 Common mistakes

- Kehna ki DNA cathode ki taraf (negative hai, anode ki taraf).
- Neeli colonies ko recombinant kehna (safed hain).
- Sochna ki restriction enzymes kahin bhi kaatte.
- Exonuclease aur endonuclease mix karna.

10 Practice (NEET style)

1. Pehla restriction endonuclease?
2. Gel me DNA ka stain?
3. Taq polymerase ka srot?
4. Plant vector bacterium?
5. Pehla rDNA kisne banaya?

ANSWERS

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

11 Quick revision: exam se 5 minute pehle

TOOLS

- EcoRI: GAATTC, sticky ends
- Ligase jodta
- 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

Koi doubt? padhaiadda.netlify.app pe AI Doubt Solver se poocho, ya is chapter ka mock test do.