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CUET · BIOLOGY · CLASS XII · CODE 304

Biotechnology: Principles and Processes

CUET unit: Biotechnology and its Applications → Principles and Processes

By **UniDrill** · NCERT-grounded study material

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📌 Snapshot

- Chapter establishes the two core techniques of modern biotechnology: **genetic engineering** (rDNA technology) and **bioprocess engineering** (sterile large-scale cultivation).
- Builds the rDNA toolkit step by step: restriction enzymes, DNA ligase, vectors (pBR322, Ti plasmid, retroviruses), competent host cells, PCR, bioreactors and downstream processing.
- The Cohen-Boyer 1972 experiment (linking an antibiotic-resistance gene with a Salmonella plasmid, transferred into E. coli) is presented as the founding event of rDNA technology.
- CUET tests this chapter heavily for nomenclature (EcoRI = Escherichia coli, strain R-Y-13, first enzyme), component identification (pBR322 features, PCR steps, bioreactor parts) and palindromic-sequence recognition.

📖 Detailed Notes

2.1 Core concepts

- Biotechnology = techniques using live organisms / enzymes / cells to produce useful products and processes; modern restricted sense uses **genetically modified organisms** at large scale. (NCERT §9 intro, p. 163)
- The **European Federation of Biotechnology (EFB)** definition: "The integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services." (NCERT §9 intro, p. 163)
- Two core techniques enabled modern biotechnology: **(i) genetic engineering** — altering chemistry of genetic material (DNA/RNA) and introducing it into host to change phenotype; **(ii) bioprocess engineering** — sterile chemical-engineering ambience for desired microbe/eukaryotic-cell growth at large scale (antibiotics, vaccines, enzymes). (NCERT §9.1, p. 163-164)
- An alien piece of DNA cannot multiply in a host unless linked to an **origin of replication (ori)** — the specific chromosomal sequence that initiates replication. Cloning = making multiple identical copies of any template DNA. (NCERT §9.1, p. 164)
- The first artificial recombinant DNA was constructed by **Stanley Cohen and Herbert Boyer in 1972** by linking an antibiotic-resistance gene to a native plasmid

of Salmonella typhimurium using **restriction enzymes** ("molecular scissors") and **DNA ligase**, then transferring it into E. coli. (NCERT §9.1, p. 164-165)

- Three basic steps of GM: (i) identification of DNA with desirable genes; (ii) introduction into the host; (iii) maintenance of introduced DNA in host and transfer to progeny. (NCERT §9.1, p. 165)
- **Restriction endonucleases** — first such enzyme Hind II (characterised 1968) recognises a specific 6-bp **recognition sequence**. Over 900 restriction enzymes from 230+ bacterial strains are now known. (NCERT §9.2.1, p. 165)
- **Nomenclature**: first letter = genus, next two letters = species, then strain letter, then Roman numeral for order of isolation, e.g. **EcoRI** = Escherichia coli RY 13, first enzyme. (NCERT §9.2.1, p. 165-166)
- Nucleases come in two kinds: **exonucleases** (remove nucleotides from ends) and **endonucleases** (cut at specific internal positions). (NCERT §9.2.1, p. 166)
- Restriction enzymes recognise **palindromic** sequences — read same on both strands in 5'→3'; cut a little away from centre of palindrome between the same two bases on opposite strands, leaving overhanging single-stranded **sticky ends** which hydrogen-bond with complementary cut counterparts and facilitate DNA ligase action. (NCERT §9.2.1, p. 166-167)
- **Gel electrophoresis** — DNA fragments (negatively charged) move toward the **anode** through an **agarose** matrix (natural polymer from sea weeds); smaller fragments migrate farther; bands visualised by staining with **ethidium bromide** under **UV** light (bright orange bands); recovery of bands by cutting and **elution**. (NCERT §9.2.1, p. 168)
- **Cloning vector features**: (i) **ori** controls copy number; (ii) **selectable marker** (antibiotic resistance — amp, tet, kan, chloramphenicol) lets you eliminate non-transformants; (iii) **cloning sites** — preferably single recognition sites for common restriction enzymes; (iv) modified pathogen-derived vectors for plant/animal cells. (NCERT §9.2.2, p. 169-170)
- **pBR322** (E. coli cloning vector): carries **ori, rop** (replication-protein gene), **ampR** and **tetR** resistance genes, and unique restriction sites — Hind III, EcoR I, BamH I, Sal I, Pvu II, Pst I, Cla I. Foreign DNA ligated at BamH I (in tetR) inactivates tetR; recombinants grow on amp but not on tet (insertional inactivation of antibiotic resistance). (NCERT §9.2.2, p. 169)
- Alternative selectable marker — **insertional inactivation of β-galactosidase**: insertion-bearing colonies are colourless on chromogenic substrate; non-insert colonies are blue. (NCERT §9.2.2, p. 170)
- Vectors for eukaryotes: **Ti plasmid** of Agrobacterium tumefaciens (disarmed; for dicot plants) and **disarmed retroviruses** for animal cells. (NCERT §9.2.2, p. 170)
- **Competent host**: bacterial cells made competent by treatment with a **divalent cation (Ca^{2+})**; rDNA enters via **heat shock** (ice → 42 °C → ice). Other delivery

methods: **micro-injection** (animal nucleus), **biolistics / gene gun** (plant cells, gold/tungsten micro-particles), **disarmed pathogen** vectors. (NCERT §9.2.3, p. 170-171)

- **Processes of rDNA technology** (sequence): isolation of DNA → fragmentation with restriction enzymes → isolation of desired fragment → ligation into vector → transfer to host → culture → product extraction. (NCERT §9.3, p. 171)
- **DNA isolation**: cell-wall lysis with **lysozyme** (bacteria), **cellulase** (plants), **chitinase** (fungi); **ribonuclease** removes RNA; **protease** removes proteins; purified DNA precipitates as fine threads on adding **chilled ethanol** (collected by spooling). (NCERT §9.3.1, p. 171)
- **PCR** (Polymerase Chain Reaction) — three steps per cycle: (i) **Denaturation** (heat), (ii) **Primer annealing** (two short chemically-synthesised oligonucleotides complementary to template), (iii) **Extension** by thermostable **Taq polymerase** from Thermus aquaticus; ~30 cycles amplify ~1 billion times. (NCERT §9.3.3, p. 172-173)
- A protein-encoding gene expressed in a heterologous host gives a **recombinant protein**; large-scale production needs **bioreactors** processing 100-1000 L; bioreactors maintain optimal temperature, pH, substrate, salts, vitamins and oxygen. (NCERT §9.3.5, p. 173-174)
- **Stirred-tank bioreactor** — cylindrical or curved-base vessel with **agitator (stirrer)**, oxygen-delivery system, foam-control system, temperature- and pH-control systems and sampling ports. **Sparged stirred-tank** bioreactor passes sterile air bubbles for greater oxygen transfer area. (NCERT §9.3.5, p. 174)
- **Downstream processing** = post-biosynthetic separation, purification, formulation with preservatives, clinical trials and quality-control testing; varies from product to product. (NCERT §9.3.6, p. 174-175)

2.2 Definitions to memorise

Term	Definition	Page
Biotechnology (EFB)	Integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services	163
Genetic engineering	Techniques to alter chemistry of DNA/RNA and introduce into host to change phenotype	163-164
Bioprocess engineering	Maintenance of sterile ambience in chemical engineering for desired-microbe growth at large scale	164
Origin of replication (ori)	Specific DNA sequence from where replication starts; also controls copy number	164, 169
Plasmid	Autonomously replicating circular extra-chromosomal DNA	164
Restriction endonuclease	Enzyme that recognises a specific sequence in DNA and cuts both strands at specific sugar-phosphate-backbone positions	165-166
Recognition sequence	Specific 6-bp palindromic sequence recognised by a restriction endonuclease (e.g., GAATTC for EcoRI)	165, 167

Term	Definition	Page
Palindrome (in DNA)	A sequence of base pairs that reads the same on the two strands when the orientation of reading is kept the same	166-167
Sticky ends	Overhanging single-stranded stretches left after cutting; H-bond with complementary cut counterparts	167
Exonuclease	Nuclease that removes nucleotides from the ends of DNA	166
Endonuclease	Nuclease that cuts at specific positions within the DNA	166
Selectable marker	Vector gene (often antibiotic resistance) that lets you identify and select transformants from non-transformants	169
Insertional inactivation	Loss of function of a vector gene (e.g., tetR or β-galactosidase) because foreign DNA is inserted into it; used to select recombinants	169-170
Transformation	Procedure by which a piece of DNA is introduced into a host bacterium	169
Competent cell	Bacterial cell treated (with Ca^{2+}) to make its membrane permeable to DNA	170-171
Biolistics / gene gun	Plant-cell DNA-delivery by bombarding with high-velocity gold/ tungsten micro-particles coated with DNA	171
PCR	Polymerase Chain Reaction — in vitro amplification of a DNA segment via repeated denaturation, primer annealing and extension by Taq polymerase	172-173
Primers	Small chemically-synthesised oligonucleotides complementary to regions of template DNA	173
Recombinant protein	Protein produced when a protein-encoding gene is expressed in a heterologous host	173
Bioreactor	Vessel (100-1000 L) in which raw materials are biologically converted into specific products under controlled conditions	174
Downstream processing	Post-biosynthesis separation, purification, formulation and QC of the recombinant product	174-175

2.3 Diagrams / processes to remember

- **Figure 9.1, p. 166** — Action of EcoRI: cuts between G and A of the palindrome GAATTC / CTTAAG on vector and foreign DNA, generates sticky ends, which ligate to form recombinant DNA.
- **Figure 9.2, p. 167** — Flowchart of rDNA technology: same restriction enzyme cuts both foreign DNA and vector plasmid → ligase joins → recombinant DNA → transformation into E. coli (cloning host) → cells divide.

- **Figure 9.3, p. 168** — Agarose gel electrophoresis: wells at the cathode end (top); bands of largest fragments stay near wells, smallest run farthest toward anode; bright orange bands under UV after ethidium-bromide staining.
- **Figure 9.4, p. 169** — pBR322 map: ori, rop, ampR, tetR, and unique restriction sites Hind III, EcoR I, BamH I, Sal I, Pvu II, Pst I, Cla I.
- **Figure 9.5, p. 171** — DNA spooling: fine thread-like precipitate of purified DNA after addition of chilled ethanol, lifted out with a rod.
- **Figure 9.6, p. 172** — PCR cycle: (i) Denaturation (heat) → ssDNA; (ii) Annealing of two primers; (iii) Extension by Taq DNA polymerase; repeated for ~30 cycles to amplify ~1 billion times.
- **Figure 9.7, p. 174** — (a) Simple stirred-tank bioreactor: motor, foam-breaker, acid/base for pH, steam for sterilisation, flat-bladed impeller, culture broth, sterile-air inlet; (b) Sparged stirred-tank: sterile air bubbles sparged for increased oxygen-transfer area.

2.4 Common confusions / NTA trap points

- **EcoRI nomenclature**: the "R" stands for the **strain (RY 13)**, NOT for "restriction." NTA loves to swap "R = restriction" as a distractor. (p. 165-166)
- **Sticky ends are produced because the enzyme cuts** away **from the centre of the palindrome** but between the same two bases on opposite strands — students sometimes write "cut at the centre" which would give blunt ends.
- **pBR322 selection logic**: insertion at BamH I in tetR → recombinants grow on **amp** but NOT on **tet**; non-recombinants grow on **both**. Don't flip the resistance pattern.
- **Taq polymerase** is from Thermus aquaticus, not from E. coli. Its key property is **thermostability** so it survives 94 °C denaturation.
- **Lysozyme = bacteria, cellulase = plants, chitinase = fungi** — these three are routinely tested in match-the-following items.
- **Ti plasmid is from** Agrobacterium tumefaciens and is for **dicot plants**; **retroviruses** are the parallel vector for **animal** cells. Don't swap.
- **Gel electrophoresis**: DNA moves to **anode (positive)**, not cathode — because DNA is negatively charged due to phosphate backbone.
- **PCR step order**: Denaturation → Annealing → Extension. NTA sometimes reverses annealing and extension as a distractor.

🎯 Practice MCQs

Q1. In the name "EcoRI," the letter "R" stands for:

A. Restriction

B. The strain name (RY 13) of the source bacterium

C. Recognition

D. Recombinant

Q2. The first restriction endonuclease characterised, which depended on a specific six-base-pair recognition sequence, was:

A. EcoRI

B. BamHI

C. Hind II

D. Hind III

Q3. Which of the following sequences is a typical palindromic recognition sequence for EcoRI?

A. 5'-GAATTC-3' / 3'-CTTAAG-5'

B. 5'-AAGCTT-3' / 3'-TTCGAA-5'

C. 5'-GGATCC-3' / 3'-CCTAGG-5'

D. 5'-ATGCAT-3' / 3'-TACGTA-5'

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📊 PYQ Alignment

This chapter is one of the highest-yielding for CUET Biology: ~15-20 MCQs per year across 2023-25 papers, dominated by (a) nomenclature/naming of restriction enzymes (EcoRI = E. coli, RY13 strain), (b) component identification of pBR322 (ori, ampR/tetR, restriction sites) and the PCR cycle, and (c) match-the-following items pairing enzymes

(lysozyme/cellulase/chitinase, ligase, Taq polymerase) with their function or source. Diagrams of bioreactors and gel electrophoresis are also recurrent visual-identification targets.

CUET 2025 — Actual PYQs from this chapter

Q.18 (CUET 2025) Arrange steps involved in gel electrophoresis: (A) Exposure to UV light (B) Staining with ethidium bromide (C) Movement of DNA fragments toward anode (D) Elution

- A) [option not extracted — see source]
- B) [option not extracted — see source]
- C) [option not extracted — see source]
- D) [option not extracted — see source]

Tests: aligns with §2 (rDNA tools & processes) Answer: Not in extracted key — verify against official NTA key

Q.20 (CUET 2025) EcoRI, a significant tool in rDNA technology, is a:

- A) Bacteria
- B) Plasmid
- C) Enzyme
- D) Purine

Tests: aligns with §2 (rDNA tools & processes) Answer: Not in extracted key — verify against official NTA key

Q.22 (CUET 2025) Which steps are related to PCR? (A) Extension (B) Annealing (C) Propagation (D) Denaturation

- A) [option not extracted — see source]
- B) [option not extracted — see source]
- C) [option not extracted — see source]
- D) [option not extracted — see source]

Tests: aligns with §2 (rDNA tools & processes) Answer: Not in extracted key — verify against official NTA key

Q.30 (CUET 2025) The first isolated restriction endonuclease was:

- A) Hind II
- B) EcoRI
- C) BamHI
- D) Pvu II

Tests: aligns with §2 (rDNA tools & processes) Answer: Not in extracted key — verify against official NTA key

Q.40 (CUET 2025) ELISA is based on principle of:

- A) Antigen-antibody interaction

- B) PCR
- C) Radioactive molecule
- D) Cloning of DNA

Tests: aligns with §2 (rDNA tools & processes) Answer: Not in extracted key — verify against official NTA key

CUET 2024 — Actual PYQs from this chapter

Q.26 (CUET 2024) Downstream processing involves:

- A) Identification
- B) Amplification
- C) Fermentation
- D) Purification

Tests: aligns with §2 (rDNA tools & processes) Answer: Not in extracted key — verify against official NTA key

Q.27 (CUET 2024) Incorrect pair of organism and cell-wall degrading enzyme:

- A) Fungi – Chitinase
- B) Algae – Methylase
- C) Plant cells – Cellulase
- D) Bacteria – Lysozyme

Tests: aligns with §2 (rDNA tools & processes) Answer: Not in extracted key — verify against official NTA key

Q.28 (CUET 2024) Arrange steps in bacterial transformation: (A) Incubation with rDNA (B) Treatment with divalent cations (C) Heat shock (D) Selection on antibiotic agar (E) Placing again on ice

- A) [option not extracted — see source]
- B) [option not extracted — see source]
- C) [option not extracted — see source]
- D) [option not extracted — see source]

Tests: aligns with §2 (rDNA tools & processes) Answer: Not in extracted key — verify against official NTA key

Q.29 (CUET 2024) Identify incorrect statements about recombinant DNA technology. (A) DNA fragments separated by ELISA (B) Transformation introduces DNA into host bacterium (C) rDNA technology does not involve isolation of DNA (D) DNA ligase joins DNA fragments

- A) [option not extracted — see source]
- B) [option not extracted — see source]
- C) [option not extracted — see source]

- D) [option not extracted — see source]

Tests: aligns with §2 (rDNA tools & processes) Answer: Not in extracted key — verify against official NTA key

CUET 2023 — Actual PYQs from this chapter

Q.27 (CUET 2023) Agarose used in gel electrophoresis is extracted from:

- A) Soybean
- B) Sea weeds
- C) Sea anemone
- D) Sea corals

Tests: aligns with §2 (gel electrophoresis — agarose source) Answer: Not in extracted key — verify against official NTA key

Q.28 (CUET 2023) First instance of artificial recombinant DNA molecule construction was carried out on plasmid of:

- A) Bacillus thuringiensis
- B) Escherichia coli
- C) Agrobacterium tumefaciens
- D) Salmonella typhimurium

Tests: aligns with §2 (first rDNA — plasmid construction) Answer: Not in extracted key — verify against official NTA key

Q.29 (CUET 2023) Antibiotic resistance gene in vector helps in selection of:

- A) Recombinant cells
- B) Transformed cells
- C) Competent cells
- D) Totipotent cells

Tests: aligns with §2 (vector — antibiotic selection markers) Answer: Not in extracted key — verify against official NTA key