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CHAPTER NOTES

Biotechnology Principles and Processes Class 12 Notes — CBSE Biology Chapter 11

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Biotechnology Principles and Processes Class 12 Notes — CBSE Biology Chapter 11

Chapter 11 — **Biotechnology: Principles and Processes** — covers rDNA technology, tools (restriction enzymes, vectors, host organisms), and processes (PCR, gel electrophoresis, gene cloning). Carries **6-8 marks**.

Key Concepts

Principles of Biotechnology

1. **Genetic Engineering:** Altering DNA/RNA to modify an organism → recombinant DNA (rDNA) technology
2. **Bioprocess Engineering:** Maintaining sterile conditions for large-scale production (bioreactors)

Tools of rDNA Technology

Restriction Enzymes ("Molecular Scissors")

> Cut DNA at specific **palindromic recognition sequences**
> Example: EcoRI recognises 5′-GAATTC-3′ / 3′-CTTAAG-5′
> Cuts between G and A → creates **sticky ends** (overhanging single-stranded regions)
> Sticky ends are useful because they can join with complementary sticky ends from another DNA molecule using **DNA ligase**

Vectors (Vehicles for Gene Transfer)

Vector	Features
pBR322 (plasmid)	Origin of replication (ori), amp^R and tet^R genes (selectable markers), restriction sites
Ti plasmid	From Agrobacterium tumefaciens; T-DNA integrates into plant genome
Bacteriophage λ	Higher capacity than plasmid
BAC, YAC	For large DNA inserts (genomic libraries)

Features of an ideal vector: (1) Origin of replication (ori), (2) Selectable marker (antibiotic resistance), (3) Cloning site (restriction enzyme sites), (4) Small size for easy manipulation.

Selectable Markers — Insertional Inactivation

If foreign gene is inserted into a selectable marker gene (e.g., amp^R), that gene is inactivated. Recombinants can be identified because they lose that antibiotic resistance.

Newer method: Blue-white screening using lacZ gene and X-gal — recombinants are **white** (lacZ disrupted), non-recombinants are **blue**.

Processes of rDNA Technology

PCR (Polymerase Chain Reaction)

Amplifies a specific DNA segment in vitro

Steps (each cycle):

1. **Denaturation (94°C):** DNA strands separate
2. **Annealing (55-65°C):** Primers bind to template
3. **Extension (72°C):** Taq polymerase extends primers → new DNA

After n cycles: 2^n copies of target DNA

Taq polymerase: Thermostable DNA polymerase from Thermus aquaticus (works at high temp)

Gel Electrophoresis

- Separates DNA fragments by size in agarose gel
- DNA is negatively charged → moves towards anode (+)
- Smaller fragments move faster/farther
- Visualised using ethidium bromide (stains DNA → orange under UV)
- Separated DNA cut from gel = **elution**

Gene Cloning Steps

1. Isolation of DNA (gene of interest)
2. Cutting with restriction enzymes (same enzyme for gene and vector)
3. Ligation (gene + vector → rDNA using DNA ligase)
4. Transfer into host cell (transformation, microinjection, biolistics/gene gun)
5. Selection of recombinants (selectable markers)
6. Expression of gene → desired protein

Bioreactors

- Large vessels (100-1000L) for growing host cells at industrial scale
- Stirred-tank bioreactor: most common; agitator for mixing, O_2 supply, temperature/pH control
- Downstream processing: separation, purification of protein product

Quick Revision Points

- Restriction enzymes: cut at palindromic sequences → sticky ends; EcoRI (GAATTC)
- Vectors: pBR322 (ori, amp^R, tet^R); Ti plasmid (Agrobacterium, for plants)
- Selectable markers identify recombinants (insertional inactivation, blue-white screening)
- PCR: denature → anneal → extend; Taq polymerase; 2^n copies
- Gel electrophoresis: DNA moves to +ve electrode; smaller = faster
- Gene cloning: isolate → cut → ligate → transform → select → express
- Bioreactors: stirred-tank; downstream processing for purification

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