

Solutions to Class 12 Biotechnology

CBSE NCERT





NCERT Biotechnology

Class XII

DETAILED EXERCISE SOLUTIONS

PART 1: Chapters 1 – 7

With explanatory notes for average students



<https://pagenest.in/>

CONTENTS

CHAPTER 1: An Overview of Recombinant DNA Technology	1
CHAPTER 2: Host–Vector System	3
CHAPTER 3: Gene Cloning	7
CHAPTER 4: Applications of Recombinant DNA Technology	12
CHAPTER 5: Genome Technology and Engineering	18
CHAPTER 6: Microbial Culture	24
CHAPTER 7: Plant Tissue Culture.....	28
CHAPTER 8: Animal Cell Culture	33
CHAPTER 9: Stem Cell Culture and Organ Culture	37
CHAPTER 10: Bioprocessing and Biomanufacturing	41
CHAPTER 11: Bioremediation	45
CHAPTER 12: Recent Innovations in Biotechnology	49
CHAPTER 13: Entrepreneurship	52

CHAPTER 1: An Overview of Recombinant DNA Technology

Recombinant DNA (rDNA) technology involves combining DNA from different sources to create new genetic combinations, revolutionising medicine, agriculture, and industry.

Q1. How was recombinant DNA technology initially developed?

Recombinant DNA technology developed through a series of landmark discoveries:

- 1869: Miescher isolated DNA (then called 'nuclein') from white blood cells.
- 1944: Avery, MacLeod, and McCarty proved DNA carries genetic information.
- 1953: Watson and Crick described the double helix structure of DNA.
- 1960s–70s: Discovery of restriction endonucleases (Werner Arber, Daniel Nathans, Hamilton Smith) provided the molecular 'scissors' to cut DNA at specific sequences.
- 1972: Paul Berg created the first recombinant DNA molecule by combining DNA from lambda phage with SV40 virus DNA — the birth of rDNA technology.
- 1973: Stanley Cohen and Herbert Boyer cloned a foreign gene into a bacterial plasmid — the first functional rDNA clone.
- 1975: Scientists convened the Asilomar Conference to discuss biosafety in rDNA research.

 **Note:** Think of restriction enzymes as molecular scissors that cut DNA at specific sequences, and ligase as molecular glue that joins pieces together. Paul Berg won the Nobel Prize (1980) for his pioneering work.

Q2. Applications of rDNA technology in crop improvement and therapeutics

In Crop Improvement:

- Development of Bt crops (e.g., Bt cotton, Bt brinjal) with insect resistance via cry genes from *Bacillus thuringiensis*.
- Herbicide-tolerant crops like Roundup Ready soybean.
- Improved nutritional content, e.g., Golden Rice with beta-carotene.
- Disease-resistant and drought-tolerant transgenic plants.

In Therapeutics:

- Insulin production: Human insulin (Humulin) made in *E. coli* – first therapeutic use.
- Recombinant vaccines: Hepatitis B vaccine (yeast-produced HBsAg).
- Growth hormones: Humatrope (somatotropin), Protropin.
- Clot-busting drugs: Tissue Plasminogen Activator (tPA).
- Monoclonal antibodies for cancer treatment (e.g., Herceptin).

Q3. Who discovered the Plasmid?

Answer: (c) Joshua Lederberg

 **Note:** Joshua Lederberg coined the term 'plasmid' in 1952. Paul Berg pioneered recombinant DNA. Kary Mullis invented PCR. Sir Alec Jeffreys developed DNA fingerprinting.

Q4. Plasminogen activator and Urokinase are used as:

Answer: (b) Blood clot dissolving drug

 **Note:** Both plasminogen activator (tPA) and urokinase are thrombolytic (clot-busting) agents used in treating heart attacks and strokes. They convert plasminogen to plasmin, which breaks down blood clots (fibrin).

Q5. Assertion–Reason: Restriction endonuclease

Assertion: Restriction endonuclease cuts DNA and is isolated mostly from bacteria.

Reason: Restriction endonuclease is a type of nuclease.

Answer: (b) Both assertion and reason are true but the reason is NOT the correct explanation.

Explanation: Both statements are individually true. Restriction enzymes ARE isolated from bacteria (they protect bacteria from foreign DNA — like a bacterial immune system). They ARE a type of nuclease (enzymes that cut nucleic acids). However, the fact that they are 'a type of nuclease' does not explain WHY they are isolated from bacteria. The correct explanation would be: bacteria produce them as a defense mechanism against bacteriophage DNA.

Q6. Assertion–Reason: E. coli multifork replication

Assertion: E. coli divides in 20 minutes while replicates its DNA in about 60 minutes.

Reason: E. coli follows multifork replication mechanism.

Answer: (a) Both assertion and reason are true and the reason IS the correct explanation.

Explanation: E. coli divides every ~20 minutes, but complete DNA replication takes ~60 minutes. This appears paradoxical — how can it divide faster than it replicates its DNA? The answer is multifork (or multiple origin) replication: a new round of replication starts before the previous one finishes. Multiple replication forks run simultaneously, so even though replication takes 60 min, a full copy is available every 20 min.

 **Remember:** Multifork replication is like a relay race where a new runner starts before the previous one finishes, ensuring the baton (genome) arrives on time.

CHAPTER 2: Host–Vector System

rDNA technology requires two components: a HOST (the organism that receives the DNA) and a VECTOR (the DNA vehicle that carries the gene of interest into the host).

Q1. Importance of host–vector system in rDNA technology

The host–vector system is the backbone of rDNA technology:

- The VECTOR acts as a vehicle to carry the gene of interest into the host cell, where it can replicate and be expressed.
- The HOST provides the cellular machinery (ribosomes, enzymes, energy) needed to replicate the vector and express the inserted gene.
- Without a compatible host-vector system, the foreign gene cannot survive, replicate, or produce the desired protein.
- Different host-vector combinations are used depending on the application: *E. coli* (bacteria) for simple proteins, yeast for eukaryotic proteins requiring glycosylation, insect/mammalian cells for complex proteins.

 **Note:** Example: The Humulin (human insulin) production system uses the *E. coli* bacterium as host and a plasmid as vector carrying human insulin gene.

Q2. Major characteristics of a vector

- Origin of Replication (ori): The vector must have its own ori to replicate autonomously inside the host.
- Selectable Marker: A gene (usually antibiotic resistance) that allows identification of host cells that have taken up the vector (transformed cells).
- Cloning Site (MCS – Multiple Cloning Site): A region with multiple restriction enzyme sites where foreign DNA can be inserted.
- Appropriate Size: Should be small (usually <10 kb) for efficient transformation and easy manipulation.
- Low Copy Number or High Copy Number depending on application.
- Stability: Should be stably maintained in the host cell.

 **Remember:** Memory trick for vector characteristics: 'ORI-SM-MCS-S-S' = Origin of replication, Selectable Marker, Multiple Cloning Site, Small size, Stable.

Q3. What is a plasmid and what are its different types?

A plasmid is a small, circular, double-stranded DNA molecule that exists independently of the chromosomal DNA in bacterial cells. It can self-replicate and is stably inherited by daughter cells.

Types of plasmids:

- Fertility (F) plasmids: Allow conjugation (transfer of genetic material between bacteria). Example: F factor in *E. coli*.
- Resistance (R) plasmids: Carry antibiotic resistance genes. Important in the spread of antibiotic resistance.
- Col plasmids: Produce colicins — bacteriocins that kill other bacteria of the same or related species.
- Ti plasmids (Tumor-inducing): Found in *Agrobacterium tumefaciens*; used extensively in plant transformation.
- Degradative plasmids: Encode enzymes to break down unusual organic compounds like toluene, salicylate, etc.

- Episomes: Plasmids capable of integrating into the bacterial chromosome (e.g., lambda phage, F factor).

Q4. Strategy for development of pBR322 plasmid cloning vector

pBR322 was the first widely used artificial cloning vector, developed by Bolivar and Rodriguez in 1977 (hence pBR, 322 = strain number):

- Started with natural plasmid pSF2124 as base material.
- Selectable markers included: ampicillin resistance (amp^R) gene and tetracycline resistance (tet^R) gene.
- Multiple restriction sites (BamHI, Sall, PstI, EcoRI, HindIII) were incorporated as cloning sites.
- Origin of replication from ColE1 plasmid was incorporated for autonomous replication.
- Insertional inactivation principle: Inserting foreign DNA into amp^R gene destroys it (colony grows on tet plates but not amp plates), allowing identification of recombinants.

 **Note:** pBR322 size is 4361 bp. The 'p' stands for plasmid, 'BR' for Bolivar and Rodriguez, '322' for the strain number. It was a landmark in rDNA technology.

Q5. Structure of lambda bacteriophage and lambda phage vectors

Lambda (λ) bacteriophage structure:

- Double-stranded linear DNA of ~48.5 kb.
- Head (icosahedral capsid): Contains the packaged DNA.
- Tail: Helps in host recognition and DNA injection.
- Tail fibres: Recognise and attach to E. coli host cells.
- Has cohesive ends (cos sites) which allow circularisation after infection.

Lambda phage vectors:

- The central region (non-essential for lytic growth) is replaced with foreign DNA insert.
- Can accommodate inserts of 9–23 kb — larger than plasmids.
- Two types: Replacement vectors (central stuffer DNA replaced with insert) and Insertion vectors (insert added at a specific site).
- Used for making genomic DNA libraries.

 **Note:** Diagram suggestion: Refer to the lambda phage structure diagram in Chapter 2 of the textbook.

Q6. M13 based vectors and applications

M13 is a filamentous bacteriophage that infects E. coli bearing the F-pilus.

- Genome: Single-stranded circular DNA of ~6.4 kb.
- M13 does not lyse the host cell; infected cells continuously secrete phage particles.
- M13mp series vectors have MCS (Multiple Cloning Site) inserted in the lacZ gene — allowing blue-white selection.

Applications:

- Production of single-stranded DNA templates for Sanger DNA sequencing.
- Site-directed mutagenesis (since it produces ssDNA).
- Phage display technology — displaying peptides/antibodies on phage surface for library screening.

Q7. Differentiate between cosmids and phagemids

Cosmid	Phagemid
Hybrid of plasmid + lambda phage cos sites.	Hybrid of plasmid + M13 or f1 phage origin.
Can accommodate large inserts: 35–45 kb.	Smaller inserts, similar to plasmids (~4–6 kb).
Packaged in lambda phage heads for efficient delivery.	Produces ssDNA or dsDNA depending on induction.
Used for genomic library construction with large fragments.	Used for site-directed mutagenesis and sequencing.

Q8. Why is a vector required for cloning of a gene?

A vector is essential because:

- A bare DNA fragment cannot replicate on its own inside a host cell — it lacks its own origin of replication.
- The vector provides an ori that drives replication of itself along with the inserted gene.
- The vector carries selectable markers to identify transformed cells.
- Without a vector, the foreign DNA fragment would be degraded by host nucleases.
- Vectors ensure stable maintenance and expression of the gene of interest in the host.

 **Remember:** A vector is like a postal envelope — the gene of interest is the letter, and the vector is the envelope that carries it safely to the destination (host cell).

Q9. A plasmid capable of getting integrated into host chromosome is called:

Answer: (b) Episome

 **Note:** An episome is a plasmid that can exist either as an autonomous extrachromosomal element OR integrate into the host chromosome. F factor (fertility factor) in *E. coli* is the classic example of an episome.

Q10. Why is replication of single copy plasmid called stringent replication?

Single copy plasmids (like F factor) are maintained at exactly ONE copy per cell. This is called stringent replication because:

- The plasmid copy number is tightly coupled/controlled to match the host chromosome replication.
- It replicates ONLY once per cell cycle, ensuring exactly one copy per daughter cell.
- This stringent control prevents over-replication which could be a metabolic burden to the cell.

In contrast, relaxed replication (multicopy plasmids like pBR322 with ~15–20 copies/cell) is not tightly coupled to chromosome replication.

Q11. Identify the incorrect match pair:

Options: (i) Multicopy plasmid – Stringent replication. (ii) Col plasmid – Kills bacteria. (iii) pBR322 – Plasmid. (iv) Prophage – Phage genome inserted into host genome.

Answer: (i) Multicopy plasmid – Stringent replication is INCORRECT.

Multicopy plasmids undergo RELAXED replication (many copies per cell). STRINGENT replication is characteristic of single-copy/low-copy plasmids.

Q12. How can a large size eukaryotic gene insert be cloned?

Large DNA inserts (>40 kb) cannot be cloned in ordinary plasmid or lambda vectors. Special vectors are used:

- Cosmids: Can carry 35–45 kb inserts; packaged in lambda phage heads.
- BACs (Bacterial Artificial Chromosomes): Based on F factor; can carry 100–300 kb inserts; used in Human Genome Project.
- YACs (Yeast Artificial Chromosomes): Can carry 200 kb – 2 Mb of foreign DNA; used for very large genomic fragments.
- PACs (P1-derived Artificial Chromosomes): 100–300 kb inserts.

 **Note:** *The Human Genome Project used a combination of BACs and YACs to clone large fragments of the human genome.*

Q13. Assertion–Reason: Selectable marker

Assertion: An ideal vector should have a selectable marker.

Reason: Selectable markers are required to screen out transformation.

Answer: (a) Both assertion and reason are true and the reason IS the correct explanation.

Explanation: A selectable marker (like antibiotic resistance) allows us to identify and select cells that have successfully taken up the vector (transformed cells). Non-transformed cells die on selective media, while transformed cells survive. So yes, selectable markers are essential to screen for transformation success.

Q14. Assertion–Reason: Cosmid

Assertion: Cosmid is a hybrid vector. Reason: Cosmid has properties of both plasmids and lambda phage vector.

Answer: (a) Both assertion and reason are true and the reason IS the correct explanation.

Explanation: A cosmid contains: (1) plasmid features — ori, antibiotic resistance gene, MCS; (2) lambda phage feature — cos sites for packaging DNA into phage heads. This dual nature is exactly WHY it is called a hybrid vector. The cos sites allow it to be packaged at high efficiency like lambda phage, while the plasmid components allow it to replicate in bacterial cells.

CHAPTER 3: Gene Cloning

Gene cloning is the process of making multiple identical copies of a specific gene or DNA fragment using biological systems.

Q1. Methods used for isolation of DNA

DNA isolation involves breaking open cells and purifying the DNA away from other cellular components. Steps:

- Cell lysis: Breaking cells open using detergents (SDS/CTAB), enzymes (lysozyme for bacteria), or mechanical disruption. This releases cellular contents.
- Protein removal: Treatment with proteinase K or phenol-chloroform extraction removes proteins.
- RNA removal: RNase treatment degrades RNA.
- DNA precipitation: Addition of cold ethanol or isopropanol precipitates DNA.
- Washing and resuspension: DNA pellet is washed with 70% ethanol, dried, and dissolved in TE buffer or water.

 **Note:** *Bacteria are easier to lyse (single layer cell wall). Plant cells have a tough cellulose wall requiring extra mechanical disruption. Animal cells (no cell wall) are easiest to lyse.*

Q2. Role of biological detergent in isolation of nucleic acid

Biological detergents (like SDS — Sodium Dodecyl Sulphate, or CTAB — Cetyltrimethylammonium bromide) play critical roles:

- Disrupt and dissolve cell membranes (plasma membrane and nuclear envelope) by solubilising lipids.
- Denature proteins, preventing nuclease activity that would degrade DNA.
- CTAB is particularly useful for plant DNA isolation as it precipitates polysaccharides which would otherwise contaminate the DNA prep.
- SDS is used for bacteria and animal cells; CTAB is preferred for plants.

Q3. DNA isolation from plant tissue vs bacterial cell

Plant DNA Isolation	Bacterial DNA Isolation
Tough cellulose cell wall requires extra mechanical grinding (pestle & mortar with liquid nitrogen) or enzymatic digestion (cellulase).	Thin peptidoglycan wall lysed easily by lysozyme enzyme.
CTAB buffer is used to precipitate polysaccharides and remove them.	SDS-based lysis buffer is sufficient.
Chloroplast and mitochondrial DNA also present as contaminants.	Only one type of DNA (chromosomal + plasmid).
High polyphenol content (in some plants) requires polyvinylpyrrolidone (PVP) to prevent oxidation.	Polyphenol contamination not a concern.

Q4. Types of restriction enzymes; can all be used in rDNA technology?

Types of Restriction Enzymes:

- Type I: Cut DNA far from recognition site (>1000 bp away). Require ATP and SAM. NOT used in rDNA technology.
- Type II: Cut DNA AT or very near the recognition site. Most produce sticky ends. Require only Mg^{2+} . USED in rDNA technology.
- Type III: Cut ~25 bp from recognition site. Require ATP. NOT commonly used in rDNA.

Only Type II restriction enzymes are used in rDNA technology because:

- They cut at precise, predictable locations (within or adjacent to their recognition sequence).
- They generate reproducible fragments with defined ends (sticky or blunt).
- They do not require ATP or SAM — simple Mg^{2+} cofactor only.

 **Note:** Examples of Type II enzymes: *EcoRI* (from *E. coli*), *HindIII* (from *Haemophilus influenzae*), *BamHI* (from *Bacillus amyloliquefaciens*).

Q5. Challenges during nucleic acid extraction

- Degradation by nucleases (DNases/RNases): These enzymes are everywhere (including on skin) and rapidly degrade DNA/RNA.
- Contamination with proteins, lipids, polysaccharides that inhibit downstream applications.
- RNA contamination in DNA preps (and vice versa).
- Mechanical shearing of large DNA molecules during extraction.
- For RNA: Highly unstable due to abundant RNases; requires special precautions (DEPC-treated water, RNase-free equipment).
- Chemical contaminants (phenol, CTAB, ethanol) if not properly washed can inhibit PCR or other applications.

Q6. Roles of alkaline phosphatase, DNA ligase, terminal transferase

- Alkaline Phosphatase: Removes 5' phosphate groups from DNA ends. Used to prevent self-ligation (re-circularisation) of vector — ensures that only recombinant clones (vector + insert) form, increasing cloning efficiency.
- DNA Ligase: Joins (ligates) DNA fragments by forming phosphodiester bonds between the 3'-OH and 5'-phosphate ends. It's the 'molecular glue' in cloning. T4 DNA ligase (from T4 phage) is most commonly used.
- Terminal Transferase (TdT): Adds nucleotides to the 3'-OH ends of DNA in a template-independent manner. Used to add homopolymeric tails (e.g., poly-dA and poly-dT) to vector and insert, allowing complementary tailing for cloning of blunt-ended fragments.

 **Note:** Analogy: Alkaline phosphatase = cap that prevents the vector from 'talking to itself'; Ligase = stapler that joins two pieces; Terminal Transferase = adding velcro to attach blunt-ended pieces.

Q7. Role of chelating agent in DNA extraction

Common chelating agents: EDTA (Ethylene Diamine Tetraacetic Acid)

- EDTA chelates (binds) divalent metal ions like Mg^{2+} and Ca^{2+} .
- DNases require Mg^{2+} as cofactor — by chelating Mg^{2+} , EDTA inhibits DNase activity, thus preventing DNA degradation.
- DNA is typically stored in TE buffer (10 mM Tris, 1 mM EDTA) to maintain long-term stability.

 **Remember:** Remember: EDTA acts like a magnet that grabs divalent metal ions, starving the DNA-destroying enzymes (DNases) of their required cofactors.