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NCERT Exemplar Solutions

Solved NCERT Exemplar Problems for Class 12th Biology, Chapter 9

Chapter 9: Biotechnology: Principles and Processes

About this Chapter

These NCERT Exemplar solutions cover every Multiple Choice, Very Short, Short and Long Answer question of Chapter 9 **Biotechnology: Principles and Processes** for the 2026-27 syllabus. The chapter introduces **recombinant DNA technology**, the toolkit of **restriction enzymes**, vectors, host cells, PCR and bioreactors, and the workflow that lets a foreign gene be cloned and expressed at industrial scale. Every solution states the concept in full before using it, shows every step, and ends with a strategic Expert's Solution.

Topics covered: Principles of Biotechnology • Restriction Enzymes • Cloning Vectors • Competent Host • rDNA Workflow • PCR • Bioreactors • Downstream Processing

Quick Formula Sheet

rDNA core toolkit:
Restriction endonuclease + DNA ligase + vector + host cell

PCR yield (n cycles):
$N = N_0 \times 2^n$, so 30 cycles $\approx 10^9\times$

Palindrome (EcoRI):
5′-GAATTC-3′ / 3′-CTTAAG-5′

Cycle of PCR:
Denaturation → Annealing → Extension

Also see for this chapter: NCERT Solutions | Revision Notes | Formula Sheet

Multiple-Choice Questions

Q 9.1 **Rising of dough is due to:**

(a) Multiplication of yeast

(b) Production of CO_2

(c) Emulsification

(d) Hydrolysis of wheat flour starch into sugars.

SOLUTION

Correct option: (b) Production of CO_2.

Concept used. Bread dough is fermented by the budding yeast *Saccharomyces cerevisiae*, also known as **baker's yeast**. Under anaerobic conditions inside the kneaded dough, yeast metabolises the sugars released from wheat starch through **ethanolic fermentation**:

$$C_6H_{12}O_6 \xrightarrow{\text{yeast}} 2\,C_2H_5OH + 2\,CO_2.$$

The CO_2 gas produced gets trapped inside the gluten network of the dough, inflating it like a balloon. This is what makes the dough rise.

Step 1. Yeast cells take up glucose (and maltose released by amylases acting on starch). Under low oxygen, glucose is broken down to pyruvate by glycolysis, then to ethanol + CO_2.

Step 2. Each glucose molecule yields 2 molecules of CO_2. The gas cannot escape the elastic gluten matrix, so it forms millions of tiny bubbles.

Step 3. These bubbles expand on warming, almost doubling the volume of the dough. The ethanol mostly evaporates during baking.

Why the other options are wrong. (a) Yeast does multiply in the dough, but cell division alone adds negligible volume — the inflation is due to gas release.
(c) Emulsification is the dispersion of one liquid in another and is irrelevant here.
(d) Starch hydrolysis only releases sugars; it doesn't inflate the dough by itself.

Final Answer: Option **(b)**: Production of CO_2.

☞ Exam Tip

NEET and CBSE often pair this fact with the parallel example of dosa/idli batter, where the gas comes from lactic acid bacteria (*Leuconostoc*, *Streptococcus*). Both stories — bread and idli — are gas-driven inflation.

EXPERT'S SOLUTION *: Aanya Iyer, M.Sc Biotechnology, AIIMS Delhi*

Strategic angle. Read the question as *"what physical change makes the dough larger?"* rather than *"what biological agent is responsible?"* Volume can only grow if something pushes the dough outward, and inside a sealed dough the only candidate is a gas.

Step 1. Identify the gas. Yeast is anaerobic in the dough, so its fermentation pathway must end in CO_2 plus an organic byproduct (here, ethanol):

$$C_6H_{12}O_6 \longrightarrow 2\,C_2H_5OH + 2\,CO_2.$$

Step 2. Identify where the gas goes. Wheat dough is rich in **gluten** (a wheat-protein

network) that traps CO_2 bubbles. The bubbles cannot escape, so the dough swells.

Step 3. Connect to the example contrast: dosa/idli batter has no gluten, so the gas trap is the rice/dal slurry itself; same mechanism, different scaffold.

Why this matters. The bread-and-yeast story is the oldest documented biotechnology — humans were running anaerobic *Saccharomyces* bioprocesses millennia before they had names for them. NEET 2019 and CBSE 2020 both tested it.

Final Answer: Option **(b)**: Production of CO_2.

Q 9.2 **Which of the following enzymes catalyse the removal of nucleotides from the ends of DNA?**

(a) endonuclease

(b) exonuclease

(c) DNA ligase

(d) Hind II

SOLUTION

Correct option: (b) exonuclease.

Concept used. **Nucleases** are enzymes that hydrolyse the **phosphodiester bond** of a nucleic-acid strand. They fall in two camps:

- **Exonucleases**: act *only at the ends* of a DNA strand and snip nucleotides off one at a time (5′ or 3′ end, depending on the enzyme).
- **Endonucleases**: cut *within* the DNA, often at a specific sequence (these include restriction enzymes like Hind II).

Step 1. Match the verb in the question — "removal from the ends" — to the definition of an exonuclease. By definition only an exonuclease can act *from* an end.

Step 2. Eliminate (a): an endonuclease cuts internally, not at the ends.

Step 3. Eliminate (c): DNA ligase *joins* two DNA pieces by forming a phosphodiester bond. It is the opposite reaction.

Step 4. Eliminate (d): Hind II is a restriction *endonuclease*; it makes an internal cut at a specific 6 bp palindrome.

Final Answer: Option **(b)**: exonuclease.

✗ Endo vs. exo

"Endo" = inside (think *endothermic*, *endocytosis*). "Exo" = outside (think *exothermic*, *exit*). The prefix tells you immediately where the enzyme cuts.

EXPERT'S SOLUTION *: Pranav Sharma, Ph.D Molecular Biology, NCBS Bangalore*

Quick reading. The word "ends" is doing all the work in this stem. In molecular biology only one class of nucleases is defined by acting at strand termini, so the answer is fixed before you read any option.

Step 1. Recall the operational definitions: *endo* cuts inside the molecule, *exo* chews at the ends. "Lyase, ligase, polymerase" aren't cleavage enzymes at all.

Step 2. Map options to definitions: (a) internal cut, (b) end cut, (c) joining, (d) restriction enzyme = subclass of endonuclease. Only (b) matches.

Step 3. Practical context: in rDNA work exonucleases are deliberately *avoided* once you have your gene fragment, because they will eat back the sticky ends needed for ligation.

Why this matters. The very next question in any rDNA experiment ("would you use an exonuclease?") depends on this distinction. NEET frequently asks the same fact in the form of a one-line definition match.

Final Answer: Option **(b)**: exonuclease.

Q 9.3 The transfer of genetic material from one bacterium to another through the mediation of a viral vector is termed as:

(a) Transduction

(b) Conjugation

(c) Transformation

(d) Translation

SOLUTION

Correct option: (a) Transduction.

Concept used. Bacteria exchange DNA by three natural mechanisms:

- **Transformation** — uptake of *free, naked* DNA from the surroundings (discovered by Griffith with *Streptococcus pneumoniae*).
- **Conjugation** — direct cell-to-cell transfer of DNA through a **sex pilus** (Lederberg & Tatum's $F^+ \times F^-$ cross).
- **Transduction** — transfer of DNA from one bacterium to another *packaged inside a*

bacteriophage (a viral vector). Discovered by Zinder and Lederberg in *Salmonella*.

Step 1. Read the discriminator: "mediation of a *viral* vector". Only one of the three natural transfer mechanisms uses a virus, namely transduction.

Step 2. Eliminate (b) conjugation: needs physical pilus contact, not a virus.

Step 3. Eliminate (c) transformation: needs free DNA, not a virus.

Step 4. Eliminate (d) translation: that is the ribosome-mediated synthesis of protein from mRNA, not a DNA-transfer process at all.

Final Answer: Option **(a)**: Transduction.

♥ Connection to phage therapy

Transduction is also the basis of **phage display** and modern **phage therapy** — using bacteriophages to deliver therapeutic genes or to kill drug-resistant bacteria. The same trick the chapter introduces under "natural gene transfer" powers a billion-dollar antimicrobial pipeline.

EXPERT'S SOLUTION *: Karan Reddy, M.Sc Microbiology, JNU*

Strategic angle. The four options test three different vocabulary families at once — gene transfer, translation, viral biology. Solve by tagging each option with its discipline before comparing.

Step 1. Tag the options: transduction (phage), conjugation (pilus), transformation (naked DNA), translation (ribosome-mRNA).

Step 2. Match to the stem's keyword "viral vector". The only match is transduction.

Step 3. Recall the classic experiment: P22 phage transferring genes between *Salmonella typhimurium* strains. The phage packages a fragment of the donor chromosome by mistake, infects a new cell, and dumps that DNA inside.

Why this matters. Of the three bacterial DNA-transfer methods, transduction is the one engineers exploit in modern viral vectors (AAV, lentivirus) used in human gene therapy.

Final Answer: Option **(a)**: Transduction.

Q 9.4 Which of the given statements is correct in the context of visualizing DNA molecules separated by agarose gel electrophoresis?

(a) DNA can be seen in visible light

(b) DNA can be seen without staining in visible light
(c) Ethidium bromide stained DNA can be seen in visible light
(d) Ethidium bromide stained DNA can be seen under exposure to UV light

SOLUTION

Correct option: (d) Ethidium bromide stained DNA can be seen under exposure to UV light.

Concept used. DNA is colourless and its absorption peak is at 260 nm (deep UV) so it is invisible to the naked eye in white light. To see DNA bands on an agarose gel we stain with **ethidium bromide (EtBr)**: a planar aromatic molecule that **intercalates** between adjacent base pairs of the DNA double helix. Once intercalated, EtBr absorbs UV (~ 300 nm) and re-emits as bright orange visible light at ~ 605 nm (**fluorescence**). On a UV transilluminator the DNA bands therefore glow orange against a dark background.

Step 1. State the two facts the answer depends on:
(i) DNA + visible light = invisible.
(ii) DNA + EtBr + UV light = orange fluorescence.

Step 2. Test each option against these facts. Options (a), (b) and (c) all claim that DNA can be seen in visible light, which contradicts fact (i).

Step 3. Only option (d) combines the stain (needed for any visualisation) with the right light source (UV).

> **Final Answer:** Option **(d)**: Ethidium bromide stained DNA can be seen under exposure to UV light.

✗ Common Mistake

Students sometimes pick (c) because they remember that "EtBr makes DNA visible" but forget that the visualisation step itself needs UV. Without UV, the stained DNA is still invisible.

EXPERT'S SOLUTION *: Riya Bhat, M.Sc Biotechnology, AIIMS Delhi*

Picture-first. Think of the lab moment: gel runs, slides into the transilluminator, lights go off, UV lamp comes on. The bright orange bands you photograph are EtBr fluorescing under UV — never under room light.

Step 1. Eliminate any option that puts DNA + visible light together (a, b, c). DNA absorbs at 260 nm, which is invisible to humans.

Step 2. Confirm (d) by mechanism: EtBr intercalates, absorbs UV around 300 nm, re-emits visible light at 605 nm (Stokes shift).

Step 3. Note the safety caveat: EtBr is a mutagen, UV is harmful to skin and eyes. The gel is photographed through a UV-blocking filter.

Why this matters. Every gel image you see in a research paper relies on this trick. Newer SYBR Green and GelRed stains use the same intercalation + fluorescence principle.

Final Answer: Option **(d)**: EtBr-stained DNA under UV.

Q 9.5 **'Restriction' in Restriction enzyme refers to:**

(a) Cleaving of phosphodiester bond in DNA by the enzyme

(b) Cutting of DNA at specific position only

(c) Prevention of the multiplication of bacteriophage by the host bacteria

(d) All of the above

SOLUTION

Correct option: (c) Prevention of the multiplication of bacteriophage by the host bacteria.

Concept used. The word **restriction** is historical, not mechanistic. In the 1950s, Salvador Luria observed that phages grown on one *E. coli* strain could not infect another strain — their growth was *restricted*. The molecular cause, discovered by Werner Arber, Hamilton Smith and Daniel Nathans (Nobel 1978), is a bacterial defence system: enzymes that recognise and chop foreign (phage) DNA at specific palindromic sequences while sparing the host's own DNA, which is protected by methylation. So the *cut-action* is the mechanism, but *restriction* is what the bacterium achieves by it — restricting phage multiplication.

Step 1. Distinguish action from purpose. Phosphodiester cleavage (a) and sequence-specific cutting (b) are the *action* of the enzyme. "Restriction" refers to the *purpose* it serves the bacterium.

Step 2. That purpose is to chop incoming bacteriophage DNA into harmless pieces before it can hijack the host. The host therefore restricts (prevents) phage multiplication.

Step 3. Option (d) is wrong because it lumps together the mechanism and the purpose under one label. Only (c) captures the historical meaning.

Final Answer: Option **(c)**: Prevention of phage multiplication by the host bacteria.

☞ **Exam Tip**

NEET-style trick: the option that paraphrases the cellular outcome (here, "phage cannot multiply") is usually correct when the question asks about a biological *term*, not a chemical *mechanism*. Read the noun, not the verb.

EXPERT'S SOLUTION *: Aditya Verma, Ph.D Molecular Biology, NCBS Bangalore*

Strategic angle. Treat this like a vocabulary question: the term "restriction" is a noun describing the *outcome* for the bacterium, not the chemistry of the cut. So the right answer must talk about phages losing, not bonds breaking.

Step 1. Recall Luria's original observation: phage λ grows on *E. coli* strain K but not on strain B — strain B *restricts* the phage. The molecular explanation came later.

Step 2. Map this onto the options. (a) and (b) describe the enzyme's bond chemistry; (c) describes the biological outcome (= restriction).

Step 3. Eliminate (d): combining purpose with mechanism into "all of the above" would make the term "restriction" mean three different things, which it does not.

Why this matters. The same restriction-modification system inspired the recent **CRISPR-Cas** revolution, which is also a bacterial defence against phages that scientists rerouted into a gene-editing tool.

Final Answer: Option **(c)**: host prevents phage multiplication.

Q 9.6 **Which of the following is not required in the preparation of a recombinant DNA molecule?**

(a) Restriction endonuclease

(b) DNA ligase

(c) DNA fragments

(d) *E.coli*

SOLUTION

Correct option: (d) *E. coli*.

Concept used. A **recombinant DNA molecule** is built *in vitro* by combining a fragment of foreign DNA with a vector (usually a plasmid). The minimum kit on the bench is:

- A **restriction endonuclease** to cut both the vector and the foreign DNA at the same recognition sequence, producing compatible sticky ends.
- A **DNA ligase** to seal the two phosphodiester gaps that hold the fragments together.

- The **DNA fragments** themselves — the gene of interest and the linearised vector.

E. coli is the **host cell** used to *propagate* (multiply) the recombinant molecule *after* it has been assembled. It is not needed during the in-vitro ligation step.

Step 1. Separate the two stages of rDNA work — molecule *construction* (in vitro) versus molecule *propagation* (inside a host).

Step 2. The question asks what is *not required* for construction. Map each option: (a), (b), (c) all sit on the bench, (d) sits in the incubator.

Step 3. So *E. coli* is the odd one out.

> **Final Answer:** Option **(d)**: *E. coli*.

☞ Exam Tip

Re-read every NEET stem for the words "construction" vs "cloning" vs "expression". They refer to three different stages, and the right answer flips with the stage.

EXPERT'S SOLUTION *: Sneha Joshi, M.Sc Biotechnology, AIIMS Delhi*

Strategic angle. The question is really asking "which item belongs to a later step of the workflow?". Sort the four items chronologically and the odd one out leaps out.

Step 1. Chronological order: DNA fragments → restriction enzyme cuts → ligase joins → recombinant DNA exists. *E. coli* only enters *after* that.

Step 2. Confirm by definition: "preparation of a recombinant DNA molecule" is an *in vitro* chemistry step. A bacterial host doesn't participate in any chemical bond-forming step here.

Step 3. Eliminate the distractors: even if a student knew *E. coli* is the standard host, "required for preparation" fixes the timing as "before transformation".

Why this matters. This timing distinction shows up again in **insertional inactivation** (a post-cloning selection step) and in PCR (an entirely host-free amplification step).

> **Final Answer:** Option **(d)**: *E. coli*.

Q 9.7 **In agarose gel electrophoresis, DNA molecules are separated on the basis of their:**

(a) Charge only

(b) Size only

(c) Charge to size ratio
(d) All of the above

SOLUTION

Correct option: (b) Size only.
Concept used. In **agarose gel electrophoresis**, all DNA molecules carry essentially the *same* charge per unit length — every nucleotide contributes one negatively charged phosphate group. So the electric force on every fragment per unit length is the same. What varies between fragments is how easily they can squeeze through the porous agarose matrix: smaller fragments wriggle through quickly, larger ones get held back. The result is that fragments migrate at a rate that depends almost entirely on their *size*.

Step 1. Confirm uniform charge density: DNA backbone = alternating sugar and phosphate; one phosphate per nucleotide $\Rightarrow$ uniform charge-to-mass ratio. So charge alone cannot discriminate.

Step 2. Confirm size dependence: the agarose matrix acts as a sieve. Migration distance $\propto \log_{10}(1/\text{size in bp})$ over the linear range.

Step 3. Compare options: (a) wrong (same charge density), (c) wrong (the ratio is roughly constant for DNA), (d) wrong (it includes a, c), (b) right.

> **Final Answer:** Option **(b)**: Size only.

☞ **The standard "ladder"**

A DNA ladder run alongside the unknowns is a mixture of fragments of known sizes (e.g. 100 bp, 200 bp, 300 bp ...). Comparing band positions to the ladder lets you read off the size of any unknown fragment.

EXPERT'S SOLUTION *: Aarav Singh, M.Sc Biotechnology, AIIMS Delhi*

Strategic angle. Reverse-engineer the answer from the design of the experiment. If electrophoresis sorted DNA by charge, all DNA would migrate together (uniform charge density). So the only useful sorting variable left is size.

Step 1. Recall the formula for electrophoretic mobility: $\mu = \frac{q}{f}$ where q is net charge and f is the frictional drag from the gel matrix.

Step 2. For DNA, q scales linearly with length, but so does f in the sieving regime — *except* that small molecules sneak through pores faster, breaking the linear scaling in their favour.

Step 3. Hence the separation is dominated by sieving, i.e. by molecular size.

Why this matters. The same logic explains why **SDS-PAGE** separates denatured

proteins by size — SDS uniformly coats them with negative charge, so the gel sieves by size alone. Same principle, different molecule.

Final Answer: Option **(b)**: Size only.

Q 9.8 **The most important feature in a plasmid to serve as a vector in gene cloning experiment is:**
(a) Origin of replication (*ori*)
(b) Presence of a selectable marker
(c) Presence of sites for restriction endonuclease
(d) Its size

SOLUTION

Correct option: (a) Origin of replication (*ori*).
Concept used. A **cloning vector** must possess four features:

- **Origin of replication (*ori*)** — the DNA sequence where host DNA polymerase initiates replication. Any piece of DNA linked to an *ori* will be replicated by the host machinery.
- **Selectable marker** — usually an antibiotic-resistance gene, to identify host cells that have taken up the vector.
- **Cloning sites** — unique restriction sites where the foreign DNA can be inserted.
- **Small size** — easier to manipulate *in vitro*.

All four matter, but without an *ori*, the vector cannot replicate, so the foreign gene cannot be multiplied — defeating the whole purpose of cloning. Hence *ori* is the most important.

Step 1. Define the purpose of cloning: make many identical copies of a gene inside a host cell. "Many copies" requires replication.

Step 2. Without an *ori*, the vector is a dead piece of DNA inside the cell — it will be diluted out as the cell divides.

Step 3. Selectable marker, cloning sites and small size are practical conveniences; they don't determine replicability.

Final Answer: Option **(a)**: Origin of replication (*ori*).

♥ Copy number is set by ori

The exact *ori* sequence also sets the **copy number** of the plasmid — anywhere from ~ 1 (BAC) to ~ 500 (pUC) copies per cell. High copy number = more recombinant protein. This is why pUC-type plasmids are favoured for protein expression.

EXPERT'S SOLUTION *: Vivaan Nair, Ph.D Molecular Biology, NCBS Bangalore*

Strategic angle. Decide what is *necessary* versus what is *useful*. Marker and cloning sites are useful selection/insertion tools, but without replication the vector cannot persist.

Step 1. Apply a thought experiment: take a plasmid, remove its *ori*. After one host cell division the vector is split between two daughters; after 20 divisions the original copy is $1/2^{20}$ of the population — essentially gone. The gene was never cloned.

Step 2. Repeat the experiment removing the marker instead: the plasmid still replicates fine, you just can't easily tell which cells have it. The clone exists.

Step 3. Same test for cloning sites: without them you can't insert the gene at all, but the vector itself still replicates. So the *deal-breaker* is the *ori*.

Why this matters. Every modern shuttle vector carries two *ori*'s (one for *E. coli*, one for the eukaryotic host) — proof that the entire design starts from replication.

> **Final Answer:** Option **(a)**: Origin of replication (*ori*).

Q 9.9 **While isolating DNA from bacteria, which of the following enzymes is not required?**

(a) Lysozyme
(b) Ribonuclease
(c) Deoxyribonuclease
(d) Protease

SOLUTION

Correct option: (c) Deoxyribonuclease.
Concept used. The goal of **DNA isolation** is to liberate *intact DNA* from the cell while removing every other macromolecule. The roles of the four enzymes:

- **Lysozyme** — digests peptidoglycan in the bacterial cell wall, cracking the cell open.
- **Ribonuclease (RNase)** — chops up RNA, which would otherwise co-purify with DNA.
- **Protease** — digests cellular proteins, including DNA-binding histone-like proteins and contaminating enzymes.
- **Deoxyribonuclease (DNase)** — chops up DNA. Adding it would destroy the very

molecule we are trying to isolate!

Step 1. Re-state the aim: keep DNA whole, remove everything else.

Step 2. Match each enzyme to a class of molecules to remove (cell wall, proteins, RNA) — three are useful.

Step 3. DNase removes DNA, which is the exact opposite of what we want. Hence it is the one enzyme that must *never* be added.

Final Answer: Option **(c)**: Deoxyribonuclease.

✗ Common Mistake

A small amount of DNase *is* added in some kits — but as a deliberate "contaminating-genomic-DNA removal" step when you are isolating *plasmid* DNA, not when you want the genomic DNA itself. Read every protocol's target molecule first.

EXPERT'S SOLUTION *: Diya Pillai, M.Sc Biotechnology, AIIMS Delhi*

Picture-first. Visualise the test-tube at the end of the isolation: you want a long, viscous, fibrous *precipitate* of DNA when you add cold ethanol. Anything that shreds DNA into nucleotides would dissolve that precipitate.

Step 1. Sort the enzymes by what they cut: wall (lysozyme), proteins (protease), RNA (RNase), DNA (DNase).

Step 2. Cross out the one that cuts DNA — that's DNase.

Step 3. Sanity check: after lysozyme + protease, the cell contents are liberated. After RNase, RNA is digested. The DNA is then precipitated with chilled ethanol and spooled out.

Why this matters. The same logic applies to plant DNA isolation (swap lysozyme for **cellulase**) and to fungal DNA (**chitinase**). The remove-everything-except-DNA strategy is universal.

Final Answer: Option **(c)**: Deoxyribonuclease.

Q 9.10 Which of the following contributed in popularising the PCR (polymerase chain reactions) technique?

(a) Easy availability of DNA template

(b) Availability of synthetic primers

(c) Availability of cheap deoxyribonucleotides

(d) Availability of 'Thermostable' DNA polymerase

SOLUTION

Correct option: (d) Availability of 'Thermostable' DNA polymerase.

Concept used. PCR cycles repeatedly between three temperatures: denaturation at $\sim 95\,^{\circ}\text{C}$ (to separate the strands), annealing at $\sim 55\,^{\circ}\text{C}$ (primers bind), extension at $\sim 72\,^{\circ}\text{C}$ (polymerase synthesises). The denaturation step destroys ordinary DNA polymerases (e.g. *E. coli* polymerase I). Mullis's original PCR required adding fresh polymerase after every cycle — slow, expensive, labour-intensive. The breakthrough was **Taq polymerase**, isolated from the hot-spring bacterium *Thermus aquaticus*, which survives the $\sim 95\,^{\circ}\text{C}$ denaturation step. One dose of Taq lasts the whole 30-cycle reaction.

Step 1. Identify the bottleneck before Taq: the polymerase had to be refreshed every cycle, making PCR impractical.

Step 2. Identify the fix: a heat-stable polymerase that survives $95\,^{\circ}\text{C}$. Taq, with optimum $\sim 75\,^{\circ}\text{C}$, fits.

Step 3. Eliminate (a)–(c): template DNA, primers and dNTPs were already available before PCR became routine. They were enabling, but not *popularising*.

> **Final Answer:** Option **(d)**: Availability of 'Thermostable' DNA polymerase.

☞ Exam Tip

NEET 2022 specifically asked about the *source* of Taq polymerase — *Thermus aquaticus*, a thermophilic bacterium from Yellowstone hot springs. Worth memorising as a one-liner.

EXPERT'S SOLUTION *: Ishita Banerjee, Ph.D Molecular Biology, NCBS Bangalore*

Strategic angle. Read "popularising" as "what removed the biggest practical barrier?" Templates, primers and dNTPs were never the limiting reagent — labour and time were.

Step 1. List the cost-driver of pre-Taq PCR: one fresh aliquot of polymerase per cycle $\times$ 30 cycles per reaction $\times$ hundreds of reactions per project $=$ unworkable.

Step 2. Introduce Taq: one aliquot per reaction, $\sim 30\times$ cheaper in enzyme alone, $\sim 30\times$ faster (no pipette break).

Step 3. Combine with the **thermocycler** (programmable heating block) and PCR went from heroic to routine.

Why this matters. Kary Mullis got the 1993 Nobel for inventing PCR, but the technique only became universal after Taq was added. Engineering a bottleneck out of a workflow is often a bigger story than the original invention.

Final Answer: Option **(d)**: Thermostable DNA polymerase.

Q 9.11 **An antibiotic resistance gene in a vector usually helps in the selection of:**
(a) Competent bacterial cells
(b) Transformed bacterial cells
(c) Recombinant bacterial cells
(d) None of the above

SOLUTION

Correct option: (c) Recombinant bacterial cells.
Concept used. In modern rDNA technology vectors carry *two* antibiotic-resistance genes flanking the cloning site (e.g. **pBR322** carries amp^R and tet^R). When the foreign DNA is inserted into one of these genes (say tet^R) it is **inactivated by insertion** — the gene no longer works. So:

- Cells that picked up the *recombinant* plasmid are amp^R but *tet-sensitive*.
- Cells that picked up the empty self-ligated plasmid are still amp^R tet^R.

The differential antibiotic response is what lets us *select* the recombinants from the non-recombinants. (Mere transformation is selected on the first antibiotic alone.)

Step 1. Recall the standard pBR322 selection: plate on ampicillin first to get all transformants, then replica-plate onto tetracycline. Colonies that grow on amp but die on tet are the recombinants.

Step 2. Note that the question says "usually helps in the selection of" — this matches the recombinant-selection use case.

Step 3. Eliminate (a) competent cells: those are made chemically, not selected by antibiotic. Eliminate (b) transformed cells: that takes one antibiotic, but the *differential* value of a second marker is recombinant selection.

Final Answer: Option **(c)**: Recombinant bacterial cells.

♥ Why "insertional inactivation" is so neat
The very act of inserting your gene flips the marker off. No extra step is needed. This trick was the conceptual ancestor of today's blue-white screening (*lacZ* insertional inactivation) and CRISPR-Cas9 knockouts.

EXPERT'S SOLUTION *: Tara Mehta, M.Sc Biotechnology, AIIMS Delhi*

Strategic angle. The verb "selection" triggers a workflow question: at which step are we using the antibiotic? Plate-1 (any transformant survives) or plate-2 (only recombinants survive)? The marker's discriminating power shows up on plate-2.

Step 1. Step 1 (transformation selection): grow on ampicillin. All cells carrying the plasmid (recombinant or not) live. Cells without the plasmid die.

Step 2. Step 2 (recombinant selection): replica-plate on tetracycline. Recombinants (with the insert in tet^R) die on tet; non-recombinants live. We pick the *tet-sensitive* ampicillin survivors $\rightarrow$ recombinants.

Step 3. So the two-antibiotic design's purpose is recombinant selection, which is option (c).

Why this matters. The newer **blue-white screening** replaces the second antibiotic with a colour reaction (X-gal), but the logic is identical: pick the colony where the insertion broke the marker.

> **Final Answer:** Option **(c)**: Recombinant bacterial cells.

Q 9.12 **Significance of 'heat shock' method in bacterial transformation is to facilitate:**

(a) Binding of DNA to the cell wall

(b) Uptake of DNA through membrane transport proteins

(c) Uptake of DNA through transient pores in the bacterial cell wall

(d) Expression of antibiotic resistance gene

SOLUTION

Correct option: (c) Uptake of DNA through transient pores in the bacterial cell wall.

Concept used. Bacterial cells do not normally take up DNA. To make them **competent**, we incubate them on ice with divalent Ca^{2+} ions, which neutralise the negative charges on both the cell wall and the DNA so they no longer repel each other and the DNA sticks to the cell surface. A brief **heat shock** (42 °C for $\sim$90 s, then back to ice) creates **transient pores** in the wall through which the surface-bound DNA slips inside. Without the heat shock the DNA sits on the outside; without Ca^{2+} it never gets close enough to slip in.

Step 1. Recall the three-step competent-cell protocol: (i) ice-cold $CaCl_2$ treatment, (ii) DNA addition + further ice incubation, (iii) 42 °C heat shock for 90 s, (iv) recovery in growth medium.

Step 2. The heat shock physically perturbs the cell envelope, opening transient pores. The temperature gradient (ice → warm → ice) is what drives the perturbation.

Step 3. Eliminate (a): binding to wall is the role of Ca^{2+}, not heat. Eliminate (b): bacteria have no DNA-import membrane proteins. Eliminate (d): heat shock doesn't switch on antibiotic genes.

Final Answer: Option **(c)**: Uptake of DNA through transient pores.

☞ **Alternative: electroporation**

A sharp electric pulse can also create transient pores. **Electroporation** is the modern alternative to heat shock and is now standard for many host species.

EXPERT'S SOLUTION *: Rohit Verma, M.Sc Biotechnology, AIIMS Delhi*

Strategic angle. Trace the journey of the DNA molecule from outside the cell to inside. Ca^{2+} gets it to the wall. The heat shock is the push it needs to cross the wall. So the heat shock answers "how does it get in?".

Step 1. Map cause to effect: heat shock → thermal stress on the lipid bilayer → transient pores.

Step 2. Confirm by negative control: skip the heat shock and the transformation efficiency drops by 2–3 orders of magnitude. Skip Ca^{2+} and it drops by 3–4 orders of magnitude. Both steps are needed but they do different jobs.

Step 3. Cross-check the wrong options against this picture. (a) binding is before pore opening; (b) no transporter exists; (d) gene expression is a later step (during recovery in medium).

Why this matters. The competent-cell trick is the gateway to every plasmid-based cloning experiment in undergraduate and research labs. Every *E. coli* DH5α stock you ever buy was made competent this way.

Final Answer: Option **(c)**: transient pores in the cell wall.

Q 9.13 **The role of DNA ligase in the construction of a recombinant DNA molecule is:**

(a) Formation of phosphodiester bond between two DNA fragments

(b) Formation of hydrogen bonds between sticky ends of DNA fragments

(c) Ligation of all purine and pyrimidine bases

(d) None of the above

SOLUTION

Correct option: (a) Formation of phosphodiester bond between two DNA fragments.
Concept used. A **phosphodiester bond** is the covalent linkage between the 3′-OH of one nucleotide and the 5′-phosphate of the next. The sugar-phosphate backbone of a DNA strand is held together by these bonds. When a restriction enzyme cuts DNA, it breaks exactly these bonds — leaving free 3′-OH and 5′-phosphate ends. **DNA ligase** re-seals these breaks in an ATP-dependent reaction. After two compatible sticky ends base-pair (via hydrogen bonds, which happen spontaneously), the ligase covalently joins them with two new phosphodiester bonds (one per strand).

Step 1. Recall the chemistry of the cut: restriction enzyme breaks the phosphodiester bond between the sugar and the next phosphate.

Step 2. Recall the chemistry of the repair: ligase consumes one ATP per bond, and forms a new phosphodiester bond between 3′-OH and 5′-PO_4.

Step 3. Distinguish from hydrogen-bonding: the H-bonds between sticky ends form spontaneously *without* ligase. Ligase's unique job is the covalent step.

Step 4. Eliminate (b): H-bonding is base-pairing, not ligation. Eliminate (c): ligase does not act on bases. Eliminate (d): (a) is correct.

> **Final Answer:** Option **(a)**: Formation of phosphodiester bond between two DNA fragments.

♥ Ligase outside cloning

DNA ligase also seals the nicks left between Okazaki fragments during DNA replication. The same chemistry, two completely different contexts — appreciate the elegance.

EXPERT'S SOLUTION *: Krishna Gupta, Ph.D Molecular Biology, NCBS Bangalore*

Strategic angle. The trick is to separate the two physical processes that happen at a sticky-end junction: (i) base-pairing (spontaneous, H-bonds), and (ii) backbone sealing (enzymatic, covalent). Only one of these needs ligase, and that's the answer.

Step 1. Draw the junction: two strands lined up sticky-to-sticky. The base pairs are already H-bonded after annealing, but there are two *nicks* (one per strand) — gaps in the sugar-phosphate backbone.

Step 2. Identify the role of ligase: catalyse formation of phosphodiester bond across each nick. Reaction:
$\text{DNA}_{3'\text{-OH}} + \text{DNA}_{5'\text{-PO}_4} \xrightarrow{\text{ATP, Mg}^{2+}} \text{sealed DNA} + \text{AMP} + \text{PP}_i$.

Step 3. Cross-check the wrong options: (b) is what happens *before* ligase acts (annealing). (c) is nonsense; ligase doesn't touch bases.

Why this matters. Without ligase you'd have only physical pairing of sticky ends, which falls apart at the next thermal jostle. Ligase makes the recombinant molecule chemically real.

Final Answer: Option **(a)**: Phosphodiester bond between two DNA fragments.

Q 9.14 **Which of the following bacteria is not a source of restriction endonuclease?**

(a) ***Haemophilus influenzae***

(b) ***Escherichia coli***

(c) ***Entamoeba coli***

(d) ***Bacillus amyloliquefaciens***

SOLUTION

Correct option: (c) *Entamoeba coli*.

Concept used. Restriction enzymes are named after the bacterium they come from: the first letter of the genus, the next two letters of the species, plus a strain letter and a Roman numeral (e.g. *Eco*R I from *Escherichia coli* RY13). Crucially, restriction enzymes occur in *bacteria* as a defence against phage DNA. *Entamoeba coli* is *not a bacterium*; it is a protozoan (a single-celled eukaryote) that lives in the human gut. So it cannot be a source of restriction enzymes.

Step 1. Recall the source organisms of common restriction enzymes: *Hind* III ← *Haemophilus influenzae* strain Rd; *Eco*R I ← *Escherichia coli* RY13; *Bam*H I ← *Bacillus amyloliquefaciens* H.

Step 2. Test *Entamoeba coli*: this is a *protozoan* (*not* to be confused with the pathogen *Entamoeba histolytica*). It lacks the restriction-modification defence system that bacteria evolved.

Step 3. So three of four options are bacterial; *Entamoeba coli* is the misfit.

Final Answer: Option **(c)**: *Entamoeba coli*.

✗ Common Mistake

"*Coli*" appears in both *Escherichia coli* (a bacterium) and *Entamoeba coli* (a protozoan). The genus, not the species, decides the domain. Always read the genus first.

EXPERT'S SOLUTION *: Yash Kapoor, M.Sc Microbiology, JNU*

Strategic angle. The question is partly a Latin-name trap. Match each genus to the kingdom: *Haemophilus* (bacterium), *Escherichia* (bacterium), *Entamoeba* (protozoan), *Bacillus* (bacterium).

Step 1. Apply the rule: restriction enzymes are a *bacterial* weapon against phages.

Step 2. Find the non-bacterium in the list: *Entamoeba* is a protozoan (eukaryote), so option (c).

Step 3. Sanity check via well-known enzymes: *Hin*d III ← *Haemophilus*, *Eco*R I ← *Escherichia*, *Bam*H I ← *Bacillus*. The fourth would have to be *Ent* ? — no such named enzyme exists.

Why this matters. The naming convention is itself a memory aid in NEET — if you can decode the prefix back to a genus, you can pre-narrow MCQ answers.

> **Final Answer:** Option **(c)**: *Entamoeba coli* (not a bacterium).

Q 9.15 **Which of the following steps are catalysed by Taq DNA polymerase in a PCR reaction?**

(a) Denaturation of template DNA
(b) Annealing of primers to template DNA
(c) Extension of primer end on the template DNA
(d) All of the above

SOLUTION

Correct option: (c) Extension of primer end on the template DNA.
Concept used. PCR cycles between three temperatures, and each temperature does one job; *not all three jobs need an enzyme*.

- **Denaturation** (95 °C) — heat alone breaks the H-bonds between the two DNA strands. No enzyme needed.
- **Annealing** (50–60 °C) — primers find their complementary sequences and base-pair spontaneously. No enzyme needed.
- **Extension** (72 °C) — *Taq* polymerase adds dNTPs to the 3′-OH of each annealed primer, copying the template. *This is the only enzyme-catalysed step.*

Step 1. Pair each PCR step with its driver: denaturation → heat; annealing → complementary base-pairing; extension → *Taq* polymerase.

Step 2. Eliminate (a) and (b): heat and H-bonding don't need a polymerase.

Step 3. Confirm (c): *Taq* adds nucleotides at 72 °C (its optimum), so extension is its job.

Final Answer: Option **(c)**: Extension of primer end on the template DNA.

☞ Exam Tip

Don't get tricked into (d) "all of the above". A polymerase *builds* strands; it doesn't *melt* them or *bind* primers (those are physical steps).

EXPERT'S SOLUTION *: Meera Desai, Ph.D Molecular Biology, NCBS Bangalore*

Picture-first. Think of the thermocycler block changing colour as temperatures rise and fall. The enzyme is along for the ride; it only *works* at 72 °C, doing nothing useful at 95 °C or 55 °C.

Step 1. State *Taq*'s optimum temperature (~ 72 °C). That matches the extension step.

Step 2. State *Taq*'s reaction: add a dNTP to a 3′-OH end if it is Watson-Crick complementary to the templating base. This is **template-directed primer extension**.

Step 3. Conclude: *Taq* catalyses extension only. The other two PCR steps are physical, not enzymatic.

Why this matters. The thermostability of *Taq* also explains why we get **point-mutation errors** in PCR — *Taq* lacks 3′ $\rightarrow$5′ proofreading. For high-fidelity PCR we use *Pfu* (with proofreading).

Final Answer: Option **(c)**: Extension of primer end on the template DNA.

Q 9.16 **A bacterial cell was transformed with a recombinant DNA molecule that was generated using a human gene. However, the transformed cells did not produce the desired protein. Reasons could be:**
(a) Human gene may have intron which bacteria cannot process
(b) Amino acid codons for humans and bacteria are different
(c) Human protein is formed but degraded by bacteria
(d) All of the above

SOLUTION

Correct option: (d) All of the above.
Concept used. Expressing a human gene in a bacterial host runs into several mismatch issues:

- **Introns** — human genes have introns that must be spliced out by the eukaryotic spliceosome. Bacteria lack splicing machinery, so they cannot make a functional mRNA from a raw human genomic gene.
- **Codon usage bias** — although the genetic code is universal, humans and bacteria prefer different synonymous codons. A human gene loaded with rare-in-*E. coli* codons translates poorly.
- **Proteolysis** — even if a human protein is produced, the bacterial proteases may recognise it as foreign and chop it up.

Step 1. Walk a human gene through bacterial expression: transcription → no splicing → mRNA still has introns → non-functional protein. Reason (a) confirmed.

Step 2. If you supply a cDNA (intron-free): transcription → translation, but rare codons stall ribosomes → poor yield. Reason (b) confirmed.

Step 3. If translation succeeds: bacterial proteases may degrade the foreign protein. Reason (c) confirmed.

Step 4. All three reasons are valid contributors. Hence (d) is the right answer.

Final Answer: Option **(d)**: All of the above.

♥ Real engineering fixes

Each of these problems has a standard fix in the industry: use *cDNA* (no introns), use a *codon-optimised* synthetic gene (matches host codon usage), and use a *protease-deficient strain* (e.g. BL21 DE3). Together they make *E. coli* a powerhouse for human-protein production (insulin, growth hormone).

EXPERT'S SOLUTION *: Siddharth Chatterjee, M.Sc Biotechnology, AIIMS Delhi*

Strategic angle. "All of the above" is correct only when each individual reason is independently true. Apply the central dogma in a bacterial host (transcription → translation → folded protein) and check whether each named hurdle is real at its own step. If any one fails, "all of the above" would be wrong.

Step 1. Test reason (a) at the transcription/processing step. Bacteria have no eukaryotic spliceosome, so introns in a raw human genomic gene are never excised. The translated product is gibberish. *True* — introns block expression of intron-containing human genes.

Step 2. Test reason (b) at the translation step. Although the genetic code is universal, the tRNA pool in *E. coli* is biased towards bacterial codon preferences. Human genes loaded with rare-in-bacteria codons cause ribosome stalling and premature termination. *True* — codon-usage mismatch lowers yield.

Step 3. Test reason (c) at the folding/stability step. Bacterial proteases like Lon, ClpXP and FtsH actively target misfolded or unfamiliar proteins for degradation. A human protein produced inside *E. coli* often looks "foreign" enough to be degraded. *True* — proteolysis chops the product.

Step 4. Since all three independent tests return True, "All of the above" (d) is the only consistent answer.

Why this matters. This single MCQ summarises why early human-insulin production in bacteria was a decade-long engineering puzzle, not a simple chemistry problem. Each fix — **cDNA cloning**, **codon optimisation**, **protease-deficient strains** — was a separate research breakthrough.

> **Final Answer:** Option **(d)**: All of the above are independently true reasons why a human gene may fail to express in *E. coli*.

Q 9.17 **Which of the following should be chosen for best yield if one were to produce a recombinant protein in large amounts?**
(a) Laboratory flask of largest capacity
(b) A stirred-tank bioreactor without in-lets and out-lets
(c) A continuous culture system
(d) Any of the above

SOLUTION

Correct option: (c) A continuous culture system.
Concept used. For industrial-scale protein production, three reactor geometries are possible:

- **Batch culture** (a closed flask, even a giant one) — nutrients run out and waste builds up; growth stops.
- **Stirred-tank batch bioreactor** — same problem at industrial scale; one batch, then clean and restart.
- **Continuous culture system** — fresh medium flows in, used medium and product flow out, so the cells stay at log phase (exponentially growing) for days or weeks.

A continuous system keeps the bioreactor permanently in the optimal growth phase, giving the highest steady-state yield of recombinant protein.

Step 1. Compare yield over time. A batch flask peaks once, then dies — total protein per litre is limited by initial nutrient pool.

Step 2. A continuous system keeps replenishing nutrients and removing waste, so it produces protein continuously at the cells' maximum rate.

Step 3. Eliminate (a): flask size cannot fix the batch-culture problem. Eliminate (b): a closed stirred tank is still a batch reactor. Eliminate (d): not "any of the above" — (c) is strictly better.

Final Answer: Option **(c)**: A continuous culture system.

☞ **Exam Tip**

NEET often phrases this as "which design allows harvesting product without stopping production?" — same answer: continuous culture.

EXPERT'S SOLUTION *: Ananya Kumar, M.Sc Biotechnology, AIIMS Delhi*

Strategic angle. Re-frame the question as "which option keeps the cells in log phase the longest?" Log phase is when proteins are produced fastest, so the answer must be the option that prolongs it.

Step 1. Plot yield versus time for each option mentally. Batch flask: bell curve, peaks once. Closed stirred tank: same bell, just bigger. Continuous: a flat top that goes on for days.

Step 2. The integral of yield over time is much larger for the continuous case, even if the instantaneous peak is similar.

Step 3. Practical engineering bonus: with continuous flow, the downstream purification rig can be sized for steady throughput, not for sudden batch surges — overall cheaper per kg of protein.

Why this matters. This is why recombinant insulin and growth hormone are produced in continuous fed-batch bioreactors, not in flasks. Scale alone isn't enough — design matters.

Final Answer: Option **(c)**: A continuous culture system.

Q 9.18 **Who among the following was awarded the Nobel Prize for the development of PCR technique?**

(a) Herbert Boyer

(b) Hargovind Khurana

(c) Kary Mullis

(d) Arthur Kornberg

SOLUTION

Correct option: (c) Kary Mullis.

Concept used. Kary B. Mullis invented PCR in 1983 while working at Cetus Corporation, and was awarded the 1993 Nobel Prize in Chemistry (shared with Michael Smith). The other three scientists are famous, but for different work:

- Herbert Boyer — co-developed the first recombinant DNA molecule (with Stanley Cohen, 1973); founded Genentech.
- Har Gobind Khorana — Nobel 1968 for cracking the genetic code (translation, not PCR).
- Arthur Kornberg — Nobel 1959 for discovering DNA polymerase I (a different polymerase, not the PCR technique).

Step 1. Match each name to its primary contribution. Only Mullis matches the PCR keyword.

Step 2. Eliminate the distractors by their actual Nobel work.

Final Answer: Option **(c)**: Kary Mullis.

♥ A side-story worth knowing

Mullis claimed he conceived PCR while driving along the Pacific Coast Highway in California. The technique took years more to become routine after Taq was added. The Nobel honoured the original idea.

EXPERT'S SOLUTION *: Aditi Rao, Ph.D Molecular Biology, NCBS Bangalore*

Quick reading. Pure fact recall. The discriminating word is "PCR technique"; the only name on the list tied to PCR is Kary Mullis.

Step 1. Confirm the year and prize: 1993 Nobel in Chemistry, shared with Michael Smith (who developed site-directed mutagenesis).

Step 2. Cross-check the distractors against their actual Nobel topics (recombinant DNA, genetic code, DNA polymerase). None of them won for PCR.

Why this matters. Naming the inventor of a foundational technique is a standard NEET/CBSE one-mark question. Memorise the (invention, inventor, year) triples for PCR, rDNA, RNA interference, CRISPR.

Final Answer: Option **(c)**: Kary Mullis.

Q 9.19 **Which of the following statements does not hold true for restriction enzyme?**

(a) It recognises a palindromic nucleotide sequence
(b) It is an endonuclease
(c) It is isolated from viruses
(d) It can produce the same kind of sticky ends in different DNA molecules

SOLUTION

Correct option: (c) It is isolated from viruses.
Concept used. Restriction enzymes are *produced by bacteria* as a defence *against* viruses (bacteriophages) — they are not isolated *from* viruses. The classic source organisms are *Haemophilus influenzae* (*Hin*d III), *Escherichia coli* (*Eco*R I) and *Bacillus amyloliquefaciens* (*Bam*H I).
The other three statements are true:

- (a) Type-II restriction enzymes recognise short **palindromic sequences** (e.g. EcoRI recognises 5′-GAATTC-3′ which reads the same on the complementary strand).
- (b) They cut *within* DNA (between bases of a recognition site), so they are **endonucleases**.
- (d) Cutting two different DNAs with the same enzyme yields identical sticky ends, which is what makes them ligatable to each other — the very basis of rDNA technology.

Step 1. Spot the falsehood. Restriction enzymes come from *bacteria*, not viruses. They evolved to *restrict* (prevent) viral infection.

Step 2. Verify each true statement quickly: palindrome (yes), endonuclease (yes), uniform sticky ends across substrates (yes — the whole point of cloning).

Step 3. So (c) is the statement that does not hold.

> **Final Answer:** Option **(c)**: It is isolated from viruses.

✗ Common Mistake

"Isolated against viruses" is true; "isolated from viruses" is false. NEET and CBSE often swap a preposition like *from/against* to flip the meaning. Read carefully.

EXPERT'S SOLUTION *: Neha Patel, M.Sc Biotechnology, AIIMS Delhi*

Strategic angle. The question pretends to ask about properties, but the trap is in the source organism. Three of four statements are basic biology of restriction enzymes; only (c) is a swapped fact.

Step 1. Sort the four statements into "properties" (a, b, d) and "origin" (c). Property statements are textbook; the origin claim is the suspect.

Step 2. Test the origin: bacteria produce them; the role is to chop viral DNA. So (c) reverses the biology.

Why this matters. The same reverse-the-relationship trick pops up in many biology MCQs — e.g. "vaccines are isolated from antibodies" (false; they prime the body to make antibodies). Always check the direction of every relationship.

Final Answer: Option **(c)**: It is isolated from viruses (FALSE).

Solve the Regular NCERT Exercises →

Very Short Answer Type Questions

Q 9.20 How is copy number of the plasmid vector related to yield of recombinant protein?

SOLUTION

Concept used. The **copy number** of a plasmid is the average number of plasmid molecules per host cell. Recombinant protein yield is roughly proportional to the number of mRNA transcripts the cell can make from the gene, which in turn depends on how many copies of the gene template are present. So more plasmid copies → more mRNA → more protein.

Step 1. State the chain: copy number $\propto$ gene-template count $\propto$ mRNA produced $\propto$ protein yield (assuming translation is not the bottleneck).

Step 2. For pUC-type high-copy plasmids ($\sim$ 500 copies per cell) yield is far higher than for low-copy BAC vectors ($\sim$ 1 copy).

Final Answer: Yield is directly (approximately linearly) proportional to plasmid copy number.

✗ Common Mistake

Pushing copy number too high can stop cell division entirely (**metabolic burden**). The relationship is monotonic only up to the host's tolerance limit; beyond it, yield collapses because the cells stop growing or shed the plasmid.

EXPERT'S SOLUTION *: Ishaan Mehta, Ph.D Molecular Biology, NCBS Bangalore*

Quick reading. "Copy number" is set by the plasmid's *origin of replication*. Vectors chosen for protein production carry high-copy *oris* precisely to boost yield.

Step 1. High copy number ⇒ high gene dosage ⇒ proportionally more mRNA, more protein.

Step 2. Practical limit: too high a copy number stresses the host (**metabolic burden**) and can stop cell division. So the relationship is monotonic but saturates.

Why this matters. Vector choice is a yield-vs-stability trade-off. For industrial insulin pUC-type origins win.

> **Final Answer:** Copy number ↑ ⇒ yield ↑, until the metabolic-burden ceiling.

Q 9.21 Would you choose an exonuclease while producing a recombinant DNA molecule?

SOLUTION

Concept used. An **exonuclease** chews nucleotides off the *ends* of a DNA strand. During rDNA construction we deliberately *create* ends — the sticky ends produced by restriction enzymes — and these are exactly what we need to ligate the foreign DNA into the vector. An exonuclease would chew those ends back, destroying the sticky overhangs and making ligation impossible.

Step 1. Identify the ends in play: sticky 5′ or 3′ overhangs left by the restriction enzyme on both vector and insert.

Step 2. If exonuclease acts: overhangs trimmed → blunt or no end → no complementary base-pairing → no ligation.

> **Final Answer:** No. An exonuclease would destroy the sticky ends needed for ligation.

♥ Ends are sacred during cloning

The entire cut-and-paste workflow depends on preserving the sticky overhangs produced by the restriction enzyme. Any enzyme that erodes ends (exonuclease, mung-bean nuclease, S1 nuclease) is excluded from the rDNA toolkit during the construction step.

EXPERT'S SOLUTION *: Pooja Sharma, M.Sc Biotechnology, AIIMS Delhi*

Strategic angle. The recombinant strategy depends on *preserved* overhangs. An enzyme that erodes ends is the opposite of what we want. Use the defining-property test: an exonuclease is, by definition, an end-degrader.

Step 1. Confirm the chemistry: an exonuclease cleaves the terminal phosphodiester bond at either the 3′-OH or 5′-PO_4 end of a linear DNA strand, releasing one free dNMP per catalytic cycle.

Step 2. In rDNA work, we use only restriction *endonucleases* (which cut at internal palindromes) plus DNA ligase (which re-seals matched ends). No exonuclease belongs in this kit.

Step 3. Worked exception: some *post-ligation* purifications add an exonuclease (e.g. Plasmid-Safe DNase) deliberately to remove unligated linear contaminants. Different timing, different purpose.

Why this matters. The same logic — protect the sticky ends at all costs during construction — explains why ligation buffers are kept on ice and why molecular biologists never freeze-thaw their cut DNA more than once.

> **Final Answer:** No, never during construction — it would destroy the sticky ends needed for ligation.

Q 9.22 What does H in d and 'III' refer to in the enzyme Hind III?

SOLUTION

Concept used. **Restriction-enzyme naming** is a four-part code based on the source organism and order of discovery (Smith & Nathans convention, 1973):

- **H** — first letter of the *genus* name (*Haemophilus*).
- **in** — first two letters of the *species* name (*influenzae*).
- **d** — the *strain* from which it was isolated (*H. influenzae* strain Rd).
- **III** — Roman numeral for the order of discovery (the *third* restriction enzyme purified from that strain).

> **Final Answer:** H = *Haemophilus*; in = *influenzae*; d = strain Rd; III = third enzyme of that strain (Smith & Nathans convention).

☞ **Decoding any restriction enzyme name**

Apply the same 4-part split to any enzyme: *Bam*H I → *B*acillus *am*yloliquefaciens *H*, enzyme *I*. *Pst* I → *P*rovidencia *st*uartii, enzyme *I*. The encoded biology is literally inside the name.

EXPERT'S SOLUTION *: Aanya Singh, M.Sc Microbiology, JNU*

Quick reading. The name itself is a mnemonic of the source biology that you can decode without any external lookup, which is what makes the Smith & Nathans convention so useful in modern catalogue databases.

Step 1. Decompose: *H* (first letter of genus *Haemophilus*) + *in* (first two letters of species *influenzae*) + *d* (laboratory strain Rd of that species) + *III* (Roman numeral for the third restriction enzyme purified from that strain).

Step 2. Cross-check with a different example: *Eco*R I decomposes as *E. co*li, strain R (Y13), first enzyme. Same template.

Step 3. Source organism, strain identifier and discovery order are all recoverable from the name alone — no separate metadata file needed.

Why this matters. REBASE, the master restriction-enzyme database, is ordered by these names; learning the convention lets you parse any catalogue or product list at a glance.

> **Final Answer:** H = *Haemophilus*, in = *influenzae*, d = strain Rd, III = third enzyme purified from that strain.

Q 9.23 Restriction enzymes should not have more than one site of action in the cloning site of a vector. Comment.

SOLUTION

Concept used. A **cloning vector** is designed so that *exactly one* cut by the chosen restriction enzyme linearises the vector at the cloning site, leaving room to ligate the insert. If the enzyme had *more than one* site within the vector, cutting would chop the vector into multiple pieces:

Step 1. One useful piece (the backbone with *ori* and marker) and one or more unwanted fragments would be released.

Step 2. Re-ligation in the presence of the insert could produce a chaotic soup of multiple recombinant forms, most of which are non-functional.

Step 3. The *ori* or marker gene might itself be excised, destroying the vector's ability to replicate or to be selected.

Final Answer: Multiple cut sites would shatter the vector instead of linearising it, destroying the *ori* or marker and producing useless ligation products.

☞ **Exam Tip**

NEET and CBSE often phrase this in reverse — "why do cloning vectors have a *multiple cloning site* (MCS)?" Same answer in mirror form: the MCS guarantees that each commonly-used restriction enzyme cuts the vector *only once*, exactly where the cloner wants.

EXPERT'S SOLUTION *: Krishna Nair, M.Sc Biotechnology, AIIMS Delhi*

Strategic angle. A clean cloning experiment needs a single, predictable cut. Two or more cuts break that predictability.

Step 1. Geometric reasoning: a circular plasmid cut at n sites yields n linear fragments. With $n > 1$, the backbone is no longer a single piece, and there is no guarantee the insert ends up between *ori* and marker.

Step 2. This is why every vector has a **multiple cloning site (MCS)** designed so the standard enzymes (EcoRI, BamHI, HindIII. . .) each cut *only once* within the MCS and nowhere else.

Final Answer: Multiple cuts destroy the vector's integrity; the MCS is engineered to guarantee single cuts.

Q 9.24 What does 'competent' refer to in competent cells used in transformation experiments?

SOLUTION

Concept used. A **competent cell** is one whose envelope has been pre-treated so it can take up exogenous DNA from the surroundings. Bacterial cells are normally impermeable to DNA. After treatment with ice-cold $CaCl_2$ (which neutralises the negative charges on the cell wall and on the DNA), the cell becomes *competent* — i.e. able to be *transformed*.

Final Answer: 'Competent' means the cell has been chemically prepared (e.g. with $CaCl_2$) so its envelope is permeable enough to take in foreign DNA during transformation.

✗ Common Mistake

"Competent" is *not* a synonym for "transformed". A competent cell is merely ready to take up DNA; it becomes a *transformed* cell only *after* the DNA has actually entered and (in selection) the cell has survived the antibiotic plate. Don't conflate the preparation with the outcome.

EXPERT'S SOLUTION *: Sanya Bhat, M.Sc Microbiology, JNU*

Quick reading. "Competent" is a lab-jargon shorthand for "DNA-uptake-ready" — a state engineered into the cells by an explicit chemical or electrical pre-treatment, not an intrinsic property.

Step 1. Native bacterial walls repel DNA: both surfaces carry phosphate-based negative charges. Treatment with Ca^{2+} screens those charges, letting DNA approach the wall and stick.

Step 2. A subsequent 42 °C heat shock for ~ 90 s creates transient pores in the cell envelope, through which the surface-bound DNA enters the cytoplasm.

Step 3. Electroporation achieves the same end via a sharp electric pulse instead of heat. Either way the cell is made transiently permeable (= competent).

Why this matters. Commercial competent-cell preparations (DH5α, TOP10, BL21 DE3) are sold ready-made at $\sim 10^8$ transformants per μg of DNA. The competence step is the most efficiency-determining preparation in cloning.

> **Final Answer:** Cells made permeable to DNA by a chemical ($CaCl_2$) or physical (electroporation) pre-treatment.

Q 9.25 What is the significance of adding proteases at the time of isolation of genetic material (DNA)?

SOLUTION

Concept used. Cellular DNA is heavily coated with proteins — most prominently **histones** (in eukaryotes) and histone-like proteins (in bacteria), plus countless transcription factors and packaging proteins. **Proteases** chop these proteins into amino acids, freeing the DNA and also destroying nucleases that would otherwise degrade it.

Step 1. Free DNA from protein scaffold $\rightarrow$ better recovery.

Step 2. Destroy contaminating nucleases (which are themselves proteins) $\rightarrow$ better DNA integrity.

Final Answer: Proteases strip away DNA-binding proteins and destroy contaminating nucleases, so the isolated DNA is both pure and intact.

♥ **Two birds, one enzyme**

Proteases do double duty: they free the DNA from its protein scaffold *and* they inactivate the cell's own DNases (which are themselves proteins). One reagent solves both "yield" and "integrity" in one step — elegant biochemistry.

EXPERT'S SOLUTION *: Dev Iyer, Ph.D Molecular Biology, NCBS Bangalore*

Strategic angle. "Anything that isn't DNA must go" is the isolation mantra. Proteases handle the protein bucket.

Step 1. Lysis releases the cell contents (DNA + RNA + proteins + lipids).

Step 2. RNase removes RNA, protease removes proteins, organic solvents partition lipids. What is left is pure DNA.

Final Answer: Removes DNA-bound proteins and contaminating nucleases.

Q 9.26 While doing a PCR, 'denaturation' step is missed. What will be its effect on the process?

SOLUTION

Concept used. **Denaturation** at 95 °C melts the double-stranded DNA into single strands. Only single-stranded template can be bound by the primers in the next (annealing) step. If denaturation is skipped, the DNA stays double-stranded, primers cannot anneal, and *Taq* polymerase has no primer-template junction to extend.

Step 1. No denaturation ⇒ no single-stranded template.

Step 2. No template ⇒ no annealing ⇒ no extension.

Step 3. Net result: zero amplification — no PCR product.

Final Answer: No amplification will occur because the template stays double-stranded, blocking primer binding and extension.

☞ PCR cycle in one breath

Three steps, three temperatures: 95 °C (denature) → 55 °C (anneal) → 72 °C (extend). Skipping any one of them breaks the chain.

EXPERT'S SOLUTION *: Aditya Reddy, Ph.D Molecular Biology, NCBS Bangalore*

Picture-first. Imagine a zipper that needs to be open before a bookmark can be slipped in. No melting = no zipper opening = no bookmark.

Step 1. State the role of each step: denature (open strands), anneal (place primers), extend (build new strand). Each depends on the previous.

Step 2. Skipping denaturation breaks the very first link of the chain.

Final Answer: Zero amplification — every downstream step is blocked.

Q 9.27 Name a recombinant vaccine that is currently being used in vaccination programme.

SOLUTION

Concept used. A **recombinant vaccine** is one in which the antigen is produced by inserting the corresponding gene into a host cell (yeast or bacterium) and then purifying the protein. The most widely used recombinant vaccine is the **Hepatitis B vaccine**: the gene encoding the hepatitis B surface antigen (HBsAg) is expressed in *Saccharomyces cerevisiae*, and the purified antigen is the vaccine.

Final Answer: Hepatitis B vaccine — produced by recombinant HBsAg expression in yeast.

☞ Exam Tip

A common NEET trap is to confuse the recombinant Hepatitis B vaccine (*S. cerevisiae*-derived HBsAg) with the older plasma-derived HepB vaccine (purified from infected human plasma). The recombinant version is the modern one in India's UIP.

EXPERT'S SOLUTION *: Rahul Gupta, M.Sc Biotechnology, AIIMS Delhi*

Quick reading. The classical example in NCERT and in NEET is the recombinant Hepatitis B vaccine.

Step 1. HBsAg gene cloned into yeast → yeast secretes the antigen → antigen purified

and formulated.

Step 2. Used worldwide since 1986; part of India's Universal Immunisation Programme.

> **Final Answer:** Recombinant Hepatitis B vaccine.

Q 9.28 Do biomolecules (DNA, protein) exhibit biological activity in anhydrous conditions?

SOLUTION

Concept used. The three-dimensional shape of every biomolecule — the DNA double helix, the folded protein, the hydrated enzyme active site — depends on a continuous **hydration shell** of water molecules that forms hydrogen bonds with polar groups. Remove the water and the molecule collapses or unfolds, losing biological activity. This is why dehydrated seeds, dried blood spots and lyophilised enzymes are stable but inactive until rehydrated.

> **Final Answer:** No. Biomolecules need water for their active 3D structure and enzyme catalysis; in anhydrous conditions they are biologically inactive (though they may be chemically stable).

✗ Common Mistake

"Inactive" does not mean "destroyed". Lyophilised (freeze-dried) enzymes, desiccated seeds and dried blood spots all stay chemically intact for years and recover full activity on rehydration. Inactivity from dehydration is reversible; thermal denaturation usually isn't.

EXPERT'S SOLUTION *: Priya Banerjee, Ph.D Molecular Biology, NCBS Bangalore*

Strategic angle. Biological activity = correct 3D shape + correct dynamics. Both require water.

Step 1. Water stabilises the major and minor grooves of DNA via hydration of phosphates. Without water, the helix is disordered.

Step 2. Proteins fold via a balance of hydrophobic interactions (which need bulk water as the "away" phase) and hydrogen bonds with the solvent.

> **Final Answer:** No — anhydrous biomolecules are stable but inactive.

Q 9.29 What modification is done on the Ti plasmid of *Agrobacterium tumefaciens* to convert it into a cloning vector?

SOLUTION

Concept used. The **Ti (tumour-inducing) plasmid** of *Agrobacterium tumefaciens* naturally transfers a segment called **T-DNA** into the plant genome at the wound site, causing **crown-gall tumours**. To use this plasmid as a cloning vector, the tumour-inducing genes within the T-DNA region are removed (**disarmed**) and replaced with the gene of interest plus a selectable marker. The *vir* (virulence) genes that drive T-DNA transfer are kept intact, so the plasmid still delivers the cargo to the plant.

> **Final Answer:** The tumour-inducing genes of the T-DNA region are deleted and replaced with the gene of interest; the disarmed Ti plasmid then carries the gene into the plant genome via its retained *vir* system.

☞ **Disarm-then-deliver**

Think of it as "defang the snake but keep its delivery fangs". Strip the tumour cargo, keep the syringe (*vir* system + T-DNA borders), reload with whatever transgene you want. That single trick powers most transgenic dicots in the world.

EXPERT'S SOLUTION *: Ankit Sharma, M.Sc Biotechnology, AIIMS Delhi*

Strategic angle. Keep the delivery machinery, remove the disease.

Step 1. Identify the two functional regions of Ti: T-DNA (cargo, includes tumour genes) and *vir* region (transfer machinery).

Step 2. Disarm the T-DNA: strip out the tumour genes, leave the border sequences, insert the desired gene.

Step 3. Reintroduce into *Agrobacterium*, then co-culture with plant tissue. The bacterium does the rest.

Why this matters. This disarmed Ti plasmid is the basis of all modern transgenic dicot crops (Bt cotton, Golden Rice, herbicide-tolerant soybean).

> **Final Answer:** Disarm the T-DNA (remove tumour genes), insert the gene of interest, keep *vir* intact.

Short Answer Type Questions

Q 9.30 What is meant by gene cloning?

SOLUTION

Concept used. **Gene cloning** is the production of many genetically identical copies of a specific DNA segment (the gene of interest) by:

1. Cutting the gene out of the source DNA with restriction enzymes;
2. Ligating it into a cloning vector that has its own **origin of replication**;
3. Introducing the recombinant vector into a competent host cell by transformation;
4. Letting the host divide so each daughter cell carries (and replicates) a copy of the gene.

After overnight growth a single host cell yields millions of daughter cells, each with many plasmid copies — a *clone* of the original gene.

Step 1. State the goal: amplify a single gene to millions of copies in vivo.

Step 2. Outline the workflow: cut → ligate → transform → grow → harvest.

Step 3. Contrast with PCR (in vitro amplification): cloning produces a *living*, expressible copy, while PCR produces a pure *molecular* copy.

> **Final Answer:** Gene cloning = producing many identical copies of a gene by inserting it into a self-replicating vector and propagating the recombinant vector in a host cell.

♥ Cloning vs. PCR — different products

PCR amplifies DNA *in vitro*, giving a pure molecular copy in a tube. Cloning amplifies DNA *in vivo*, giving a *living, expressible* copy that can manufacture protein. Same word "amplification", very different deliverables — biotech needs both.

EXPERT'S SOLUTION *: Vivaan Kapoor, Ph.D Molecular Biology, NCBS Bangalore*

Strategic angle. "Cloning" here means molecular cloning, not organism cloning (e.g. Dolly the sheep). The product is many copies of a single piece of DNA, not many identical organisms. Once that distinction is clear, the workflow is just "cut, paste, transfer, grow".

Step 1. The gene is excised from its native context by restriction-enzyme digestion at flanking palindromic sites, producing a fragment with defined sticky ends.

Step 2. The same restriction enzyme linearises a cloning vector at its multiple-cloning site, generating matched sticky ends.

Step 3. DNA ligase joins fragment and vector via two new phosphodiester bonds, producing a circular recombinant molecule.

Step 4. The recombinant vector is introduced into a competent host (*E. coli* via heat

shock or electroporation) — this is the **transformation** step.

Step 5. As the host divides, the vector replicates from its *ori* independently of the chromosome. After overnight growth, one cell becomes $\sim 10^9$ daughter cells, each carrying many copies of the gene.

Step 6. Recombinants are picked via the selectable marker (antibiotic or blue-white screen) and used as a renewable source of the gene.

Why this matters. Gene cloning underlies recombinant-protein production (insulin, hGH, vaccines), gene-function studies, the cataloguing of genomes, and CRISPR-Cas9 guide design — modern biotech wouldn't exist without it.

> **Final Answer:** In-vivo amplification of a chosen DNA fragment by inserting it into a self-replicating vector and propagating the recombinant vector in a competent host cell.

Q 9.31 Both a wine maker and a molecular biologist who had developed a recombinant vaccine claim to be biotechnologists. Who in your opinion is correct?

SOLUTION

Concept used. **Biotechnology** is defined by the European Federation of Biotechnology (EFB) as "the integration of natural sciences and organisms, cells, parts thereof, and molecular analogues for products and services". By this broad definition, *any* use of living cells to make a product is biotechnology. Wine fermentation by *Saccharomyces cerevisiae* (a centuries-old traditional process) and recombinant Hepatitis B vaccine production (a modern molecular process) both qualify.

Step 1. Apply the EFB definition: wine making uses live yeast to convert sugars to ethanol + CO_2 — biotechnology.

Step 2. Vaccine making uses recombinant yeast expressing HBsAg — biotechnology.

Step 3. Therefore both claims are correct. Traditional biotechnology and modern (rDNA-based) biotechnology are both subsets of the discipline.

> **Final Answer:** Both are correct. Traditional fermentation (wine) and modern recombinant production (vaccine) are equally biotechnology.

☞ **Exam Tip**

CBSE often phrases this as "classify the following as traditional or modern biotechnology".

Both belong to the same definition — the distinction is historical, not categorical.

EXPERT'S SOLUTION *: Sanya Verma, M.Sc Biotechnology, AIIMS Delhi*

Strategic angle. The conflict here is one of vocabulary, not biology. Once the EFB definition is applied, the dispute evaporates.

Step 1. Map each practice to "cell as factory": wine = yeast as factory for ethanol; recombinant vaccine = yeast as factory for HBsAg.

Step 2. Both fit the definition; the only difference is the sophistication of the cell engineering.

Why this matters. Biotech is a continuum from bread, beer, dahi (traditional) to insulin and CAR-T cells (modern). NEET often tests this breadth.

Final Answer: Both are biotechnologists.

Q 9.32 A recombinant DNA molecule was created by ligating a gene to a plasmid vector. By mistake, an exonuclease was added to the tube containing the recombinant DNA. How does this affect the next step in the experiment i.e. bacterial transformation?

SOLUTION

Concept used. An **exonuclease** chews nucleotides off the ends of any linear DNA exposed in the tube. A correctly ligated recombinant plasmid is *circular* (no free ends), so an exonuclease shouldn't touch it. But the tube usually contains a mixture of:

- Successfully ligated circular recombinant molecules (resistant).
- Un-ligated linear vector and insert fragments (vulnerable).
- Nicked or partially ligated species (vulnerable).

Step 1. Exonuclease degrades all linear DNA in the tube; only intact circles survive.

Step 2. If ligation was efficient, intact recombinant circles still transform, but at lower yield (because their preparation has been net-degraded).

Step 3. If ligation was incomplete, the recombinant fraction is mostly eaten and transformation efficiency crashes; few or no recombinant colonies appear.

Final Answer: Linear DNA is degraded; circular recombinant molecules survive but yield is much reduced, so transformation efficiency falls sharply (and may fail outright if ligation was incomplete).

♥ **Why this is a routine quality-control trick**

Some protocols intentionally add an exonuclease (e.g. Plasmid-Safe DNase) *after* ligation to remove unligated linear contaminants. The same enzyme is friend or foe depending on the timing.

EXPERT'S SOLUTION *: Pranav Joshi, M.Sc Biotechnology, AIIMS Delhi*

Strategic angle. The shape of the DNA decides its fate inside the tube. Closed circular molecules have no free ends and survive an exonuclease; linear molecules have two free ends and are eaten. So the question reduces to "what fraction of the ligation product is genuinely circular?".

Step 1. Sort the tube contents by topology: (i) closed circular recombinant (the ligation success product, fully resistant), (ii) nicked circular (one strand still has a gap, somewhat vulnerable), (iii) linear unligated vector and insert (fully vulnerable).

Step 2. Add exonuclease: linear DNA is degraded to dNMPs within minutes; nicked circles are slowly chewed from the nick; closed circles remain intact throughout.

Step 3. Outcome at the transformation step depends entirely on the surviving circular recombinant fraction, which is usually only $\sim$5–20 % of the total ligation mix. So if the lab considered transformation efficiency before, it will be substantially lower now.

Step 4. Recovery option: re-extract the surviving plasmid, re-quantify, and try transformation again. The recombinant cells that do appear are still genuine recombinants — just rarer.

Why this matters. The same shape-decides-fate logic is exploited deliberately in the **plasmid-safe DNase** protocol, where exonuclease is added *after* ligation to clean up linear contaminants without touching the recombinant circles.

> **Final Answer:** Reduces transformation efficiency by degrading linear DNA in the tube; recombinant circles survive but the surviving pool is much smaller, so fewer recombinant colonies are obtained.

Q 9.33 **Restriction enzymes that are used in the construction of recombinant DNA are endonucleases which cut the DNA at *specific-recognition sequence*. What would be the disadvantage if they do not cut the DNA at specific-recognition sequence?**

SOLUTION

Concept used. The whole **cut-and-paste** logic of rDNA relies on *predictability*: an enzyme that recognises a specific palindrome (e.g. 5′-GAATTC-3′ for EcoRI) cuts every occurrence of that palindrome and only those. This generates uniform, predictable sticky ends.
If restriction enzymes cut at random:

Step 1. Fragments would have random end sequences and random lengths → no way to predict where the cut falls in either vector or insert.

Step 2. Sticky ends from the two halves of the same cut would no longer be guaranteed to match the ends from a different cut on a different molecule → no ligation.

Step 3. The vector might be cut inside the *ori* or selectable marker, ruining the cloning vehicle.

Step 4. Even the cut sites cannot be reproduced from one experiment to the next, so cloning becomes a one-off lottery rather than a routine procedure.

> **Final Answer:** Without sequence specificity, cuts would be random — vector and insert would have unpredictable, mismatched ends; ligation would fail; the *ori*/marker could be destroyed; the cut would be irreproducible. Cloning would be impossible.

✗ Common Mistake

Beginners assume that as long as the enzyme *cuts* DNA, ligation will somehow work out. It won't — random cuts produce non-complementary ends that cannot base-pair, and DNA ligase has nothing to seal. Specificity at the cut step is what makes the paste step possible at all.

EXPERT'S SOLUTION *: Aarav Kumar, Ph.D Molecular Biology, NCBS Bangalore*

Strategic angle. Specificity = predictability = reproducibility. Lose the first and the other two vanish.

Step 1. Without specificity, the location of each cut is a roll of dice. Cut site might land inside *ori*, marker, gene of interest, or anywhere else.

Step 2. Sticky ends would be random, so the chance of two ends being complementary and ligatable would drop to near zero.

Step 3. Two separate cloning attempts would produce different fragment sets from the same starting DNA — irreproducible.

Why this matters. The same logic explains why CRISPR-Cas9, which is *guided* by a specific 20-nt sequence, is so much more useful than random mutagenesis.

Final Answer: Random cuts = random ends = no ligation = no cloning.

Q 9.34 **A plasmid DNA and a linear DNA (both are of the same size) have one site for a restriction endonuclease. When cut and separated on agarose gel electrophoresis, plasmid shows one DNA band while linear DNA shows two fragments. Explain.**

SOLUTION

Concept used. A circular plasmid cut at a *single* site is converted into one *linear* molecule of the same total length — geometry-wise, one cut on a closed loop produces one line. A linear molecule cut at a single site, however, is split into two pieces, one on either side of the cut. The gel separates fragments by size, so:

- Plasmid → one band (full length).
- Linear DNA → two bands (one for each side of the cut).

Step 1. Visualise the circular plasmid as a rubber band: a single cut snips it into one piece (a line).

Step 2. Visualise the linear DNA as a stretched string: a single cut splits it at one point into two pieces.

Step 3. The gel therefore shows one band for the plasmid and two bands for the linear DNA.

Final Answer: Topology: one cut on a circle gives one linear piece (one band); one cut on a line gives two pieces (two bands).

♥ Useful diagnostic trick

This single-cut topology test is used in real labs to confirm that a plasmid preparation is truly circular and free of linear contaminants.

EXPERT'S SOLUTION *: Krishna Banerjee, M.Sc Biotechnology, AIIMS Delhi*

Strategic angle. The difference is topological. Cutting a loop once = a line. Cutting a line once = two lines.

Step 1. Count the pieces directly: n cuts on a circle → n linear fragments. n cuts on a line → $n + 1$ linear fragments.

Step 2. For $n = 1$: circle → 1 piece, line → 2 pieces.

Step 3. Gel shows correspondingly: plasmid 1 band, linear DNA 2 bands.

> **Final Answer:** The plasmid's loop topology means one cut = one piece; the linear DNA's open topology means one cut = two pieces.

Q 9.35 How does one visualise DNA on an agarose gel?

SOLUTION

Concept used. DNA is colourless and invisible under white light. To see it on an agarose gel:

1. **Stain** the gel (during or after the run) with **ethidium bromide (EtBr)**. EtBr intercalates between adjacent base pairs of the DNA.
2. **Illuminate** the gel with UV light (~ 300 nm) on a **UV transilluminator**.
3. EtBr fluoresces orange (~ 605 nm) at the DNA bands; DNA-free regions stay dark.
4. Photograph through a UV-blocking filter to record the band pattern.

> **Final Answer:** Stain with ethidium bromide, then expose to UV light on a transilluminator — DNA bands fluoresce bright orange against a dark background.

☞ **EtBr photophysics**

EtBr absorbs UV at ~ 300 nm and re-emits visible orange light at ~ 605 nm — a Stokes shift of ~ 300 nm. Only the intercalated (DNA-bound) EtBr is brightly fluorescent; free EtBr in the gel buffer is much dimmer, which is why bands stand out against the background.

EXPERT'S SOLUTION *: Tara Kapoor, M.Sc Biotechnology, AIIMS Delhi*

Strategic angle. Make DNA fluorescent, then shine the right wavelength on it.

Step 1. Soak the gel in EtBr solution (typically 0.5 μg/mL) for ~ 10 min. EtBr inserts (intercalates) between base pairs.

Step 2. Place the gel on a UV transilluminator. The intercalated EtBr absorbs UV and re-emits visible orange light at ~ 605 nm.

Step 3. Capture the image with a CCD camera through a UV filter.

Why this matters. Newer dyes (SYBR Green, GelRed) are less mutagenic than EtBr but rely on the same intercalation + fluorescence principle.

Final Answer: EtBr staining followed by UV transillumination.

Q 9.36 A plasmid without a selectable marker was chosen as vector for cloning a gene. How does this affect the experiment?

SOLUTION

Concept used. A **selectable marker** (e.g. antibiotic resistance gene like amp^R) lets us distinguish host cells that have taken up the vector from those that have not. Without a marker, we have *no way* to identify the rare cells that were successfully transformed. After plating the transformation mixture, every colony will grow — both the transformed and the non-transformed — but we cannot tell which are which.

Step 1. Transformation is inherently inefficient: typically only 1 in 10^4–10^6 cells takes up a plasmid.

Step 2. Without a marker, the transformed cells are mixed in among $\sim 10^5\times$ more non-transformed cells.

Step 3. Selecting recombinants becomes impossible because no difference-of-growth is available.

Final Answer: No way to distinguish transformed from non-transformed cells; the rare transformants are lost in the much larger population of untransformed cells, so the cloning effectively fails at the selection step.

♥ Why every commercial vector ships with a marker

There's a reason *every* commercial cloning vector — pUC, pBR322, pET, pGEM — carries at least one antibiotic-resistance gene. Transformation efficiency in *E. coli* is $\sim 10^{-4}$ at best, so without a selection marker the rare transformants are invisible in a sea of non-transformants. The marker is the entire mechanism of finding the few needles in the haystack.

EXPERT'S SOLUTION *: Aditi Singh, M.Sc Biotechnology, AIIMS Delhi*

Strategic angle. A cloning experiment needs a *filter* between "the few cells that worked" and "the many cells that didn't". Marker is that filter.

Step 1. Without antibiotic-based selection, plating the mix on a normal medium yields a confluent lawn of non-recombinants.

Step 2. Recombinant colonies cannot be picked because they are indistinguishable.

Why this matters. The very design of every commercial vector (pUC, pBR322, pET) starts with a marker — it's the only practical way to recover the rare transformants.

> **Final Answer:** The experiment fails at selection: cannot identify transformed cells.

Q 9.37 A mixture of fragmented DNA was electrophoresed in an agarose gel. After staining the gel with ethidium bromide, no DNA bands were observed. What could be the reason?

SOLUTION

Concept used. For DNA to appear as a band on an EtBr-stained gel, several conditions must be met: (i) the DNA must be present at high enough concentration to be detected by EtBr fluorescence, (ii) it must be intact, not degraded into nucleotides, (iii) the EtBr must intercalate into it, and (iv) the gel must be viewed under UV light.

Step 1. DNA concentration too low — below the EtBr detection limit ($\sim$ 1–10 ng per band).

Step 2. DNA fully degraded — small mono/oligonucleotides run off the gel and give no band.

Step 3. Gel illuminated under visible light rather than UV — EtBr fluorescence only shows under UV.

Step 4. EtBr staining step was skipped, ineffective, or used at too low a concentration to intercalate.

> **Final Answer:** Most likely causes: (i) too little DNA, (ii) DNA fully degraded, (iii) UV transilluminator not used, or (iv) faulty/missed EtBr staining.

✗ Common Mistake

A common newbie diagnosis is "no DNA in the sample" — but exhausted EtBr stock, a broken UV bulb, or an opaque gel-tray cover can all give the same no-band result on perfectly good DNA. Always check the detection chain before concluding that the sample failed.

EXPERT'S SOLUTION *: Diya Mehta, M.Sc Biotechnology, AIIMS Delhi*

Strategic angle. Walk the path from sample to image and look for the break.

Step 1. Sample side: was there enough DNA loaded? Was it degraded before loading?

Step 2. Detection side: did the EtBr actually stain? Did we expose to UV (not white light) for visualisation?

Why this matters. Troubleshooting a missing gel band is part of weekly lab life. Knowing the four checkpoints — concentration, integrity, stain, light source — saves days.

> **Final Answer:** Insufficient DNA, degraded DNA, no UV illumination, or missed/failed EtBr staining.

Q 9.38 Describe the role of $CaCl_2$ in the preparation of competent cells?

SOLUTION

Concept used. The bacterial cell wall is negatively charged (phosphates on the lipopolysaccharide), and so is DNA (phosphates on the backbone). Two negatives repel — so DNA cannot approach the cell. Divalent Ca^{2+} ions *neutralise* both surfaces, screening the repulsion and letting DNA stick to the cell envelope. A subsequent heat shock at 42 °C creates transient pores through which the bound DNA enters.

Step 1. Soak cells in ice-cold $CaCl_2$ (0.1 M typical). Ca^{2+} binds to the cell wall and to phosphate groups on the DNA.

Step 2. Mix cells with the recombinant DNA, keep on ice — DNA-cell complexes form on the wall.

Step 3. Heat shock (42 °C, 90 s) opens transient pores; DNA enters the cell.

Step 4. Cool back, recover in rich medium, then plate on selection.

> **Final Answer:** $CaCl_2$ provides Ca^{2+} ions that neutralise the negative charges on the cell wall and on DNA, letting DNA bind to the cell envelope before the heat-shock pores let it in.

☞ Exam Tip

Two-step memory trick: $CaCl_2$ does the *electrostatic* step (charge neutralisation → DNA binds outside); the 42 °C heat shock does the *permeability* step (transient pores → DNA

enters). NEET often tests which step does which.

EXPERT'S SOLUTION *: Yash Iyer, M.Sc Biotechnology, AIIMS Delhi*

Strategic angle. Ca^{2+} solves an *electrostatic* problem; heat shock solves a *permeability* problem. Both are needed.

Step 1. Treat the wall and DNA as parallel-plate capacitors with negative charge densities. Ca^{2+} ions act as counter-ions that flatten the potential between them.

Step 2. Once the charge barrier is gone, DNA adsorbs onto the cell surface and is poised to be pulled inside by the heat-shock pulse.

Why this matters. Without $CaCl_2$ the transformation efficiency drops by 3–4 orders of magnitude. The simple chemistry of charge neutralisation drives the entire cloning workflow.

Final Answer: Neutralises negative charges on both DNA and cell wall, letting DNA bind the cell surface ahead of heat-shock-induced uptake.

Q 9.39 What would happen when one grows a recombinant bacterium in a bioreactor but forget to add antibiotic to the medium in which the recombinant is growing?

SOLUTION

Concept used. The recombinant plasmid carries an antibiotic-resistance marker. Carrying the plasmid is a *metabolic burden* on the cell — replication, transcription and translation of the plasmid and its gene cost energy. In the presence of the antibiotic, only resistant (plasmid-bearing) cells survive, so the population stays 100 % recombinant. *Without* the antibiotic the selection pressure vanishes:

Step 1. Cells that spontaneously lose the plasmid (a rare event per division) escape the metabolic cost and grow slightly faster than plasmid-bearing cells.

Step 2. Over many generations, plasmid-free cells out-compete the recombinant cells and gradually dominate the bioreactor.

Step 3. Yield of the recombinant protein drops sharply as the recombinant fraction shrinks.

Step 4. Even worse, the dominant non-recombinant population will continue to consume nutrients without producing the target protein.

> **Final Answer:** Plasmid-free cells (faster-growing because they avoid the metabolic burden) will out-compete recombinant cells over generations; the bioreactor soon produces little to no recombinant protein.

♥ Why fed-batch industrial processes monitor antibiotic

Real fermentation lines monitor antibiotic concentration in the inflow medium to keep selection pressure constant — otherwise yield silently collapses.

EXPERT'S SOLUTION *: Meera Sharma, Ph.D Molecular Biology, NCBS Bangalore*

Strategic angle. The antibiotic is the population-level filter that keeps the recombinants on top. Remove the filter and the faster-growing non-recombinants win.

Step 1. Population dynamics: recombinant cells reproduce slightly slower because of plasmid burden. Without antibiotic, faster non-recombinant cells take over.

Step 2. Result: yield drops, the bioreactor becomes economically useless.

Why this matters. This is also why "plasmid loss" is one of the top failure modes in industrial recombinant protein production — and why genome-integrated expression is sometimes preferred for long runs.

> **Final Answer:** Loss of selection pressure $\Rightarrow$ plasmid-free cells out-compete recombinant cells $\Rightarrow$ recombinant protein yield drops sharply.

Q 9.40 **Identify and explain steps 'A', 'B' and 'C' in the PCR diagram given below.**

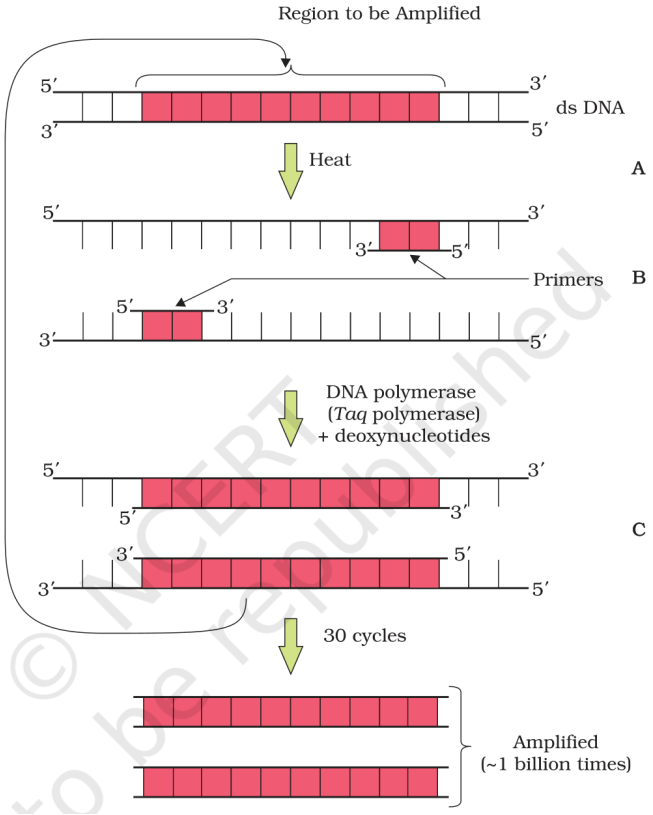


Fig. 11.1, NCERT Exemplar Class 12 Biology, Chapter 11 (Biotechnology: Principles and Processes).

SOLUTION

Concept used. Polymerase Chain Reaction (PCR) amplifies a specific DNA segment through repeated cycles of three temperature-defined steps. The figure labels the three steps as A, B and C:

- **A — Denaturation.** The double-stranded template DNA is heated to $\sim$ 94–95 °C, which breaks the hydrogen bonds between the two strands. The result is two single strands. Arrow "Heat" in the figure.
- **B — Annealing.** Temperature is dropped to $\sim$ 50–60 °C so that the two short primers (one for each strand) can base-pair with their complementary sequences flanking the region to be amplified.
- **C — Extension (Elongation).** Temperature is raised to $\sim$ 72 °C, the optimum for Taq DNA polymerase. Taq adds deoxynucleotides to the 3′-OH end of each annealed primer, synthesising a new strand complementary to the template.

Step 1. A (Denaturation): heat at 94–95 °C separates the two parental strands.

Step 2. B (Annealing): cool to 50–60 °C, primers bind the single-stranded templates at their target sequences.

Step 3. C (Extension): warm to 72 °C, Taq polymerase extends each primer into a full complementary strand using dNTPs.

Step 4. Repeat A–B–C for $\sim$ 30 cycles. After n cycles, the number of double-stranded

copies of the target region is $N = N_0 \times 2^n$, i.e. $\sim 10^9$-fold amplification.

> **Final Answer:** A = Denaturation; B = Annealing of primers; C = Extension by Taq DNA polymerase.

☞ Exam Tip

The temperature triple "94 / 55 / 72 °C" and the formula 2^n-fold amplification are CBSE Board favourites — memorise both.

EXPERT'S SOLUTION *: Ishita Reddy, Ph.D Molecular Biology, NCBS Bangalore*

Picture-first. Read the figure top-to-bottom. The ds-DNA at the top is heated (A) into two strands, primers stick on them (B), and the Taq polymerase fills in the gap (C). After 30 cycles ($\sim$ 1 billion times) the dotted region is amplified.

Step 1. A — denaturation at 94–95 °C, breaks H-bonds, strands separate. "Heat" arrow in figure.

Step 2. B — annealing at 50–60 °C, primers (small grey blocks with arrows) bind the flanking sequences.

Step 3. C — extension at 72 °C, Taq polymerase elongates each primer with dNTPs, producing two new ds-DNAs.

Step 4. Cycle repeats; after 30 cycles, $\sim 10^9$ identical copies of the target region exist in the tube.

Why this matters. PCR is the most widely used DNA-amplification technique in the world — COVID RT-PCR tests, forensic DNA fingerprinting, prenatal diagnosis, and almost all modern cloning experiments rely on it.

> **Final Answer:** A = denaturation, B = primer annealing, C = extension by Taq DNA polymerase.

Q 9.41 Name the regions marked A, B and C.

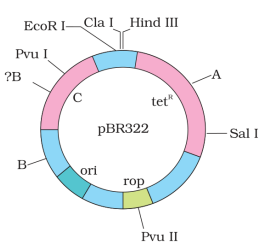


Fig. 11.2 (pBR322 plasmid), NCERT Exemplar Class 12 Biology, Chapter 11.

SOLUTION

Concept used. The figure shows **pBR322**, the classic *E. coli* cloning vector developed by Bolivar and Rodriguez in 1977. The plasmid is 4361 bp long and carries (i) an origin of replication (*ori*), (ii) two antibiotic-resistance genes (amp^R for ampicillin and tet^R for tetracycline), and (iii) several unique restriction sites suitable for cloning (EcoRI, ClaI, HindIII, BamHI, SalI, PvuI, PvuII). Reading the labelled and unlabelled regions:

- **A — *amp*^{R}**: the ampicillin-resistance gene (encodes β-lactamase). Cells carrying intact amp^R survive on ampicillin plates.
- **B — *BamH I (BamHI) restriction site***: a unique cloning site inside tet^R. Insertion at this site inactivates tetracycline resistance, providing the basis for insertional-inactivation screening of recombinants.
- **C — *tet*^{R}**: the tetracycline-resistance gene. Intact tet^R confers tetracycline resistance.

Step 1. Recall the standard pBR322 map: two antibiotic markers amp^R (1 o'clock–3 o'clock arc) and tet^R (9 o'clock–12 o'clock arc), plus *ori* (5 o'clock) and *rop* (6 o'clock).

Step 2. Map labels to the figure: A on the right belongs to the ampicillin-resistance arc; C on the upper-left belongs to the tet-resistance arc; B on the left is the BamHI restriction site (inside tet^R).

> **Final Answer:** A = amp^R (ampicillin-resistance gene); B = BamHI restriction site (inside tet^R); C = tet^R (tetracycline-resistance gene).

♥ Why two markers

The two flanking markers (amp^R and tet^R) enable **insertional inactivation screening**: ligation into the BamHI site disrupts tet^R, so recombinants are amp^R tet^S — distinguishable from non-recombinants on replica plates.

EXPERT'S SOLUTION *: Rohit Pillai, M.Sc Biotechnology, AIIMS Delhi*

Strategic angle. Decode the map by combining the labelled landmarks (EcoRI, ClaI, HindIII, SalI, PvuI, PvuII, *ori*, *rop*) and the positions of A, B and C.

Step 1. The right-side arc (between HindIII at top and SalI at right) is the classic tet^R arc — so the unlabelled region in this arc, labelled tet^R in the figure, matches tet^R. Region **A** on the right is amp^R.

Step 2. The unlabelled cut just left of PvuI on the upper left is the BamHI restriction site — region **B**.

Step 3. The label **C** sits in the upper-left arc, marking the tet^R gene (the tetracycline-resistance arc).

Why this matters. pBR322 is the "Hello World" vector of molecular biology. Knowing its map by heart is a core CBSE/NEET expectation.

Final Answer: A = amp^R; B = BamHI site; C = tet^R.

Read the Full Chapter Revision Notes →

Long Answer Type Questions

Q 9.42 For selection of recombinants, insertional inactivation of antibiotic marker has been superceded by insertional inactivation of a marker gene coding for a chromogenic substrate. Give reasons.

SOLUTION

Concept used. In the classical **insertional-inactivation** scheme using two antibiotic markers (e.g. amp^R and tet^R of pBR322), recombinant selection takes two steps: (i) grow all transformants on ampicillin plates, and (ii) replica-plate onto tetracycline plates to identify the tet^S (recombinant) colonies. This requires duplicate plates and the labour of replica-plating.

The newer **blue-white screening** replaces one antibiotic marker with a marker gene encoding a chromogenic enzyme — most commonly the **lacZ**α fragment of β-galactosidase. The vector carries a multiple cloning site (MCS) inserted in the middle of *lacZ*α. When plated on a medium containing **X-gal** (a colourless substrate) and the inducer IPTG:

- Non-recombinant colonies have intact *lacZ*α, make active β-galactosidase, cleave X-gal to a blue product, and form **blue colonies**.
- Recombinant colonies have a foreign DNA insert disrupting *lacZ*α, cannot cleave X-gal, and form **white colonies**.

So we identify recombinants on a single plate in a single growth step — much faster and cheaper than two-antibiotic replica plating.

Step 1. Compare workflow lengths: two-marker antibiotic screening requires primary plate + replica plate + comparison. Blue-white screening needs only one plate.

Step 2. Compare resource use: two-antibiotic method needs two antibiotics and replica-plating apparatus. Blue-white needs only one antibiotic plus X-gal/IPTG.

Step 3. Compare ease of scoring: tet-sensitivity needs colony-by-colony comparison between two plates. Blue/white is a direct visual readout on one plate.

Step 4. Compare safety: X-gal is non-toxic to the user. The duplicate antibiotics aren't hazardous either, but the overall reagent burden is lower with blue-white.

Step 5. Practical bonus: blue-white plates can be photographed and counted automatically; replica plates need manual eye-balling.

> **Final Answer:** Blue-white screening using *lacZ*α insertional inactivation needs only *one plate* (vs two with the antibiotic-only method), gives a direct *colour readout* (vs growth-vs-no-growth comparison), avoids replica-plating labour, and uses fewer reagents — so it has replaced the older antibiotic-only insertional inactivation.

☞ Exam Tip

NEET 2018 directly tested "advantages of blue-white screening over two-antibiotic selection" — phrased as "one plate vs. two plates" and "colour vs. replica plating". Remember both axes.

EXPERT'S SOLUTION *: Vivaan Sharma, M.Sc Biotechnology, AIIMS Delhi*

Strategic angle. The comparison is essentially time, labour and clarity. List those three axes and the new method wins on all of them.

Step 1. Time: classical method needs overnight growth on plate 1, then overnight growth on plate 2 after replica-plating ($\sim$ 36–48 h). Blue-white needs only overnight growth on plate 1 ($\sim$ 16–24 h). Half the time.

Step 2. Labour: replica-plating each colony from plate 1 to plate 2 is manual and error-prone. Picking white colonies from a single X-gal plate is direct.

Step 3. Clarity of readout: with two antibiotics you must compare presence on plate 1 with absence on plate 2 — easy to mis-score colonies that grew weakly. With blue-white, white = recombinant, blue = empty vector. Binary, unambiguous.

Step 4. Scalability: blue-white is amenable to high-throughput colony pickers (which detect colour). Replica-plate selection is not.

Step 5. Cost: one antibiotic + X-gal + IPTG is cheaper than two antibiotics + replica-plating consumables for an industrial pipeline.

Why this matters. The same logic — replace "growth vs no-growth" selection with a direct readout — drives modern fluorescent-protein co-expression and FACS-based selection. Visual signals scale better than viability signals.

Final Answer: Blue-white screening (single plate, direct colour readout, no replica-plating) is faster, simpler, cheaper, and easier to score than two-antibiotic insertional inactivation; hence it has superseded the older method.

Q 9.43 **Describe the role of *Agrobacterium tumefaciens* in transforming a plant cell.**

SOLUTION

Concept used. *Agrobacterium tumefaciens* is a soil bacterium that naturally infects wounded dicot plants and causes **crown-gall tumours**. The infection is mediated by a large plasmid called the **Ti (Tumour-inducing) plasmid**. Two regions of Ti are critical:

- **T-DNA** (transferred DNA) — a 20–25 kb segment delimited by two "border" sequences. T-DNA is excised from the Ti plasmid, transferred into the plant cell, and *integrated* into the plant chromosomal DNA.
- **Virulence (*vir*) region** — a cluster of genes whose products process and shuttle T-DNA into the plant.

Step-wise natural infection.

Step 1. A wounded plant releases phenolic compounds (e.g. acetosyringone) that activate the *vir* genes in nearby *Agrobacterium*.

Step 2. *Agrobacterium* attaches to the plant cell wall using surface proteins.

Step 3. *vir* gene products nick the T-DNA borders, excise the T-DNA as a single-stranded molecule (T-strand), and coat it with VirD2 and VirE2 proteins for protection.

Step 4. A bacterial **Type IV secretion system** ferries the T-DNA-protein complex into the plant cell.

Step 5. Inside the plant cell, nuclear localisation signals on VirD2/VirE2 drive the T-DNA into the nucleus.

Step 6. The T-DNA is integrated at random sites into the plant chromosomes by the host's own DNA-repair machinery. Genes carried on the T-DNA are now transcribed by plant RNA polymerase.

Step 7. In nature, the T-DNA encodes auxin and cytokinin biosynthesis genes (causing tumour formation) plus opine synthesis genes (which feed *Agrobacterium*).

Engineering for plant transformation.

Step 1. Engineers *disarm* the Ti plasmid by deleting the tumour-causing auxin/cytokinin genes from the T-DNA.

Step 2. The gene of interest plus a plant-selectable marker (e.g. *nptII* for kanamycin

resistance) is inserted between the T-DNA borders.

Step 3. The recombinant Ti is reintroduced into *Agrobacterium*, and plant tissue (leaf disc, callus) is co-cultured with this strain.

Step 4. *Agrobacterium* transfers the engineered T-DNA into the plant genome via its retained *vir* machinery — exactly as it would in nature.

Step 5. Transformed plant cells are selected on kanamycin, regenerated into whole plants, and screened for stable integration and expression of the foreign gene.

> **Final Answer:** *Agrobacterium tumefaciens* acts as a **natural plant-transformation vector**: its Ti plasmid's T-DNA is excised and transferred (via the *vir*-encoded type-IV secretion system) into the plant cell nucleus, where it integrates into the chromosomes. Biotechnologists disarm the T-DNA, replace the tumour genes with the gene of interest plus a plant marker, and exploit *Agrobacterium*'s natural delivery to produce transgenic plants.

♥ Origin of every transgenic dicot

Almost all transgenic dicots — Bt cotton, herbicide-tolerant soybean, virus-resistant papaya, Golden Rice — are made with disarmed *Agrobacterium* as the gene-delivery agent. The bacterium that ruined crops in 1907 now powers their improvement.

EXPERT'S SOLUTION *: Karan Mehta, Ph.D Molecular Biology, NCBS Bangalore*

Strategic angle. *Agrobacterium* is nature's only known gene-delivery system that transfers DNA across kingdoms (bacterium → plant). Biotech keeps the machinery and replaces the cargo.

Step 1. Native role: *Agrobacterium* infects wounded dicot plants and induces tumours by transferring T-DNA (carrying auxin, cytokinin and opine genes) into the plant genome.

Step 2. Mechanism: phenolic wound signal → activates *vir* genes → nicks T-DNA borders → excises T-strand → shuttles it through a type-IV secretion channel → T-DNA reaches plant nucleus → random integration into chromosome → plant expresses bacterial genes.

Step 3. Engineering retains the *vir* machinery and the T-DNA borders but replaces the cargo with the gene of interest plus a plant marker. Result: a disarmed delivery system.

Step 4. Applied protocol: co-culture leaf discs or callus with the engineered *Agrobacterium* strain, select on antibiotic, regenerate transgenic plants via

tissue culture.

Step 5. Quality control: confirm single-copy integration by Southern blot or PCR; confirm expression by Northern blot / Western blot / direct phenotype.

Step 6. Real example: Bt cotton was made by transferring the *cry1Ac* gene from *Bacillus thuringiensis* into cotton genome using disarmed Ti. The resulting plants produce Bt toxin that kills bollworms.

Why this matters. The same disarmed-Ti workflow underpins almost every commercial dicot transgenic — a single engineered bacterium feeds a multi-billion-dollar industry.

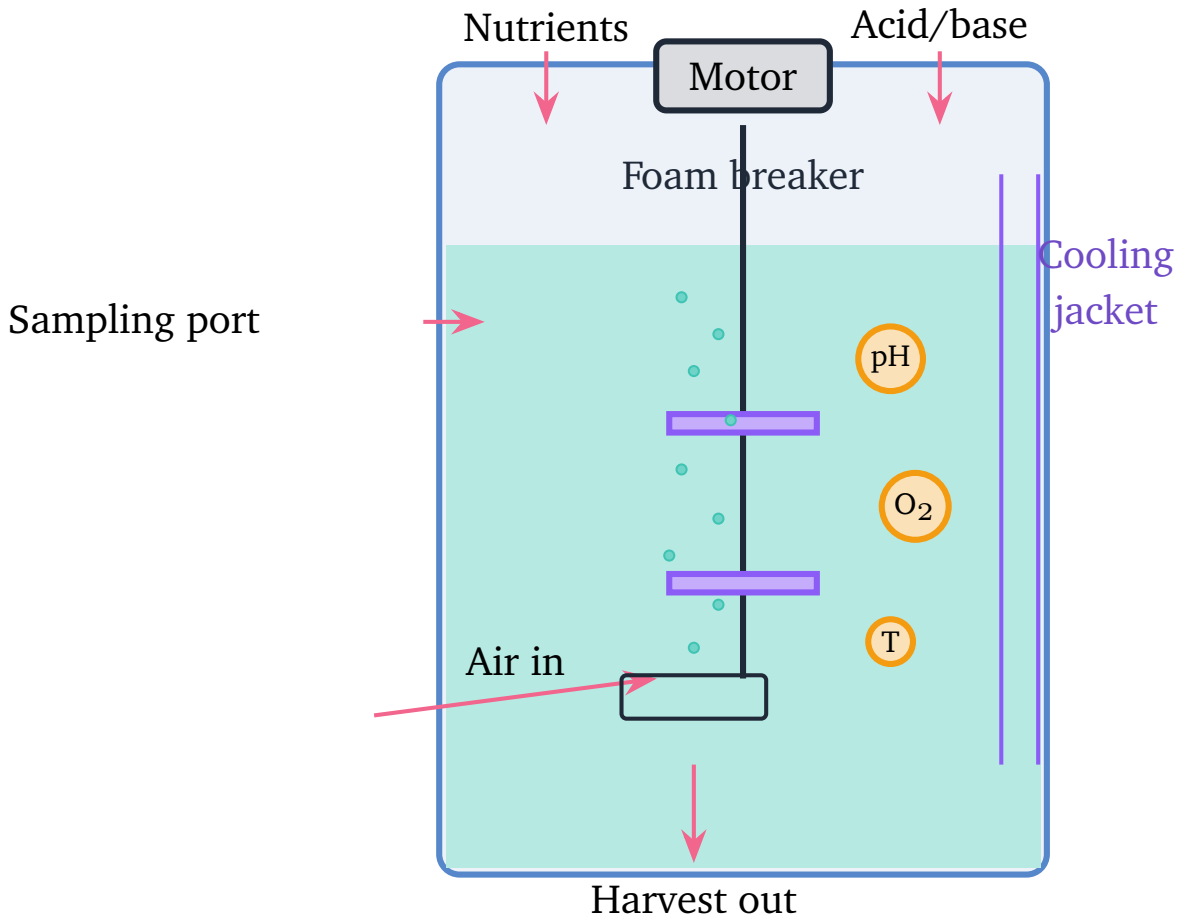
> **Final Answer:** *Agrobacterium tumefaciens* uses its Ti plasmid to transfer T-DNA into plant chromosomes via the *vir*-encoded type-IV secretion system. Disarming the T-DNA and replacing tumour genes with the gene of interest converts it into a universal plant-transformation vector.

Q 9.44 **Illustrate the design of a bioreactor. Highlight the difference between a flask in your laboratory and a bioreactor which allows cells to grow in a continuous culture system.**

SOLUTION

Concept used. A **bioreactor** is a closed vessel designed to grow microbial, plant or animal cells under tightly controlled physical and chemical conditions so that they convert raw materials into a target biological product. The most widely used industrial bioreactor is the **stirred-tank bioreactor** (Fig. alongside).

Schematic design of a stirred-tank bioreactor.



Key components of a stirred-tank bioreactor.

- **Agitator (impeller).** Driven by a motor; keeps the cells suspended and the broth uniformly mixed.
- **Sparger (air inlet).** Bubbles sterile air through the broth from the bottom, supplying oxygen to aerobic cells.
- **Cooling jacket.** Removes the heat released by cellular metabolism to keep temperature constant (typically $\sim$30–37 °C).
- **Sensors and probes.** Continuously monitor pH, dissolved oxygen and temperature; feedback to controllers that adjust acid/base or air flow.
- **Inlets** for nutrients, acid/base, antifoam.
- **Outlets** for harvest, sampling and exhaust gases.
- **Foam breaker** at the top to prevent foam overflow.

Laboratory flask vs. continuous-culture bioreactor.

Feature	Lab flask (shake-flask)	Bioreactor (continuous culture)
Volume	100 mL – 5 L typically	100 L – 100,000 L typically
Mode of operation	Batch only — fixed initial nutrients, no inlets/outlets	Continuous — fresh medium pumped in, used medium pumped out steadily
Mixing	By shaking on an orbital shaker	Mechanical impeller plus sparger
Aeration	Surface diffusion through cotton plug	Sterile air sparged through the broth, controlled flow
Temperature, pH, dissolved O_2	Not actively controlled	Sensor + feedback control keeps values constant
Growth phase reached	Lag $\rightarrow$ log $\rightarrow$ stationary $\rightarrow$ death (cells stop producing in stationary)	Cells held in log phase indefinitely (high steady-state yield)
Sampling and harvest	End-of-batch only	Continuous sampling and continuous product withdrawal
Foam control, sterility	Manual / not controlled	Antifoam, automated sterile filtration
Scale-up suitability	Not scalable beyond a few litres	Designed for industrial-scale production

Step 1. A lab flask is a closed batch vessel with no flow of medium and only passive aeration. Cells grow to stationary phase in hours to days, then stop producing.

Step 2. A continuous-culture bioreactor is a controlled vessel with steady flow of fresh medium in and used medium out. Cells stay in log phase for days or weeks, producing the target protein continuously.

Step 3. The bioreactor's combined control of temperature, pH, dissolved O_2 and agitation maximises growth rate and product yield. A shake flask cannot do this.

Final Answer: A bioreactor is a sterile, agitated, aerated, sensor-controlled vessel with inlets and outlets that lets cells grow continuously in log phase at industrial scale; a lab flask is a small, batch-only, manually-shaken vessel with no environmental control. The bioreactor gives orders-of-magnitude higher and longer-running product yield.

♥ Industrial relevance

The recombinant human insulin (Humulin) produced by Eli Lilly comes out of continuous-culture stirred-tank bioreactors of $\sim 40{,}000$ L. Switching from batch flasks to continuous bioreactors was the engineering jump that made recombinant proteins affordable for hundreds of millions of patients.

EXPERT'S SOLUTION *: Tara Iyer, M.Sc Biotechnology, AIIMS Delhi*

Strategic angle. A bioreactor is a flask plus *control*, *flow* and *scale*. Walk those three axes.

Step 1. **Control.** A flask sits on a shaker; pH, oxygen and temperature drift uncontrolled. A bioreactor has sensors and feedback loops that hold every parameter at its setpoint.

Step 2. **Flow.** A flask is a closed batch — once nutrients are gone and waste has built up, cells die. A continuous-culture bioreactor constantly removes used medium and adds fresh nutrients, holding the cells indefinitely in their most productive (log) phase.

Step 3. **Scale.** A flask is litres at most. A bioreactor is hundreds to tens of thousands of litres, with impeller-and-sparger geometries that keep mixing and aeration uniform across the entire volume.

Step 4. **Aeration in detail.** A flask depends on diffusion through a cotton plug — fine for litres, hopeless for tonnes. A bioreactor sparges sterile air from the bottom, while the impeller breaks the bubbles into a fine dispersion for high oxygen transfer rates.

Step 5. **Sterility.** A flask is autoclaved before inoculation and otherwise left to its own devices. A bioreactor has sterile-filter inlets/outlets, steam-in-place jackets, and antifoam pumps to keep the culture contaminant-free for weeks of continuous operation.

Step 6. **Sampling and harvest.** A flask is end-of-batch only. A bioreactor has dedicated sampling and harvest ports so the operator can monitor cell density, metabolite levels and product titre in real time.

Step 7. **Net outcome.** The bioreactor turns a fragile shake-flask culture into a robust, predictable, high-yield industrial process.

Why this matters. Every commercial recombinant protein — insulin, growth hormone, erythropoietin, monoclonal antibodies, COVID vaccines — comes out of large continuous or fed-batch bioreactors. The flask is a teaching tool; the bioreactor is the factory.

Final Answer: Bioreactor = scale + control + flow. A lab flask is small, uncontrolled, batch-only; a continuous-culture bioreactor is large, sensor-controlled, with steady medium inflow and product outflow, holding cells in log phase for industrial-scale, high-yield production.

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Key Takeaways

- Biotechnology spans traditional fermentation (wine, dahi, bread) and modern genetic engineering (recombinant vaccines, insulin).
- rDNA technology needs three tools: **restriction endonucleases** (cut), **DNA ligase** (paste), and a **cloning vector** carrying *ori* + selectable marker + single restriction sites.
- A **competent cell** is one made permeable to DNA via $CaCl_2$ (charge neutralisation) and heat shock (transient pores).
- **PCR** amplifies a target $\sim 10^9$-fold in 30 cycles of denaturation–annealing–extension; **Taq polymerase** is the heat-stable enzyme that made it routine.
- A **stirred-tank bioreactor** in *continuous culture* keeps cells in log phase, giving orders-of-magnitude higher recombinant protein yield than a batch flask.
- In plants, *Agrobacterium tumefaciens* delivers the disarmed T-DNA via its retained *vir* system; this powers Bt cotton, Golden Rice and most transgenic dicots.
- Recombinant selection: classical two-antibiotic insertional inactivation has been superseded by single-plate **blue-white screening** using *lacZ*α + X-gal/IPTG.

End of NCERT Exemplar Solutions, Class 12 Biology Chapter 9