NEET BIOLOGY · CLASS 12 · CHAPTER 9

Biotechnology: Principles and Processes

Cut, join, copy, grow: how a gene is moved

Genetic engineering is a recipe with six steps. Learn why each step exists, and every question becomes a step you can name.

14 diagrams | 17 worked questions | 22 quick-review cards | Fact sheet

Logic Bloom
logicbloom.co.in

The simplest way to learn science.

Contents

0	How to Read This Set	*and what NEET 2026 asks*
01	What Biotechnology Is	*two techniques, one first experiment*
02	Restriction Enzymes: The Molecular Scissors	*names, palindromes, sticky ends*
03	Gel Electrophoresis: Separating DNA Pieces	*smaller runs farther*
04	Cloning Vectors	*ori, markers, pBR322*
05	Competent Host: Getting DNA Inside	*ice, 42°C, ice*
06	Process Step 1: Isolating the Genetic Material	*break, clean, precipitate*
07	Process Step 2: Cutting DNA at Specific Locations	*one enzyme, both DNAs*
08	Process Step 3: Amplifying the Gene by PCR	*each cycle doubles*
09	Process Step 4: Inserting the Recombinant DNA	*the marker picks transformants*
10	Process Step 5: Obtaining the Foreign Gene Product	*bioreactors*
11	Process Step 6: Downstream Processing	*from product to medicine*
12	Quick Review: What NEET Repeats	*22 cards for the last week*
★	Fact Sheet	*one page for the night before*

In short

- Modern biotechnology rests on two techniques: genetic engineering and bioprocess engineering.
- Restriction enzymes cut DNA at palindromes and leave sticky ends. DNA ligase joins the ends.
- Gel electrophoresis sorts DNA pieces by size. Smaller pieces travel farther.
- A cloning vector needs an ori, a selectable marker and few cloning sites. pBR322 is NCERT's example.
- The process runs: isolate DNA, cut it, amplify by PCR, insert it, grow the host, then purify the product.

0 How to Read This Set

- This chapter is a process. Read Sections 01 to 05 as the toolkit. Read Sections 06 to 11 as the recipe that uses it.
- After each tool, ask: "What problem does this solve?" Questions are built on exactly that.
- Orange boxes are traps. Read every one.
- Questions after each section are NEET level. Try each one before reading the answer.
- The pBR322 map (FIG 5) is the most important figure here. Redraw it from memory once.

WHAT NEET 2026 ASKS FROM THIS CHAPTER

The NMC syllabus line reads: "Principles and process of Biotechnology: Genetic engineering (Recombinant DNA technology)." That is this whole chapter. These notes cover NCERT Chapter 9 in full, and nothing outside it. Uses such as insulin, Bt crops and gene therapy belong to Chapter 10, which gets its own notes.

PYQ ANALYSIS **See how NEET tested this chapter from 2015 to 2025** →

We analysed 44 NEET questions from this chapter. Restriction enzymes, PCR, gel electrophoresis and cloning vectors gave the most. The question formats have also changed. Since 2022, multi-statement, diagram and ordering questions have replaced many plain MCQs.

01 What Biotechnology Is

IN NEET | SEEN SEVERAL TIMES *genetic engineering steps and history: 2 questions each*

Definitions

- Biotechnology uses live organisms, or enzymes from organisms, to make products and processes useful to humans.
- The European Federation of Biotechnology (EFB) gives a wider definition.
- It calls biotechnology "the integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services".

The two core techniques

- **Genetic engineering:** techniques to change the chemistry of genetic material (DNA and RNA).
  - The changed material is put into a host organism.
  - This changes the host's phenotype.
- **Bioprocess engineering:** keeping a sterile ambience in chemical engineering processes.
  - Sterile means free of microbial contamination.
  - Then only the desired microbe or eukaryotic cell grows, in large quantities.
  - It is used to make antibiotics, vaccines, enzymes and other products.

Why genetic engineering beats hybridisation

- Asexual reproduction preserves genetic information.
- Sexual reproduction permits variation.
- Traditional hybridisation in plant and animal breeding often brings in undesirable genes along with the desired ones.
- Genetic engineering lets us add just one gene, or a set of desirable genes.
- Undesirable genes are not carried along.

Why a gene needs an origin of replication

- A piece of alien DNA put into an organism cannot multiply in the progeny cells on its own.
- It multiplies only if it becomes part of a chromosome.
- A chromosome can replicate because it has a specific sequence called the **origin of replication**.
- Replication starts at this sequence.

- So the alien DNA is linked with an origin of replication. Then it can replicate and multiply in the host.
- Making many identical copies of a template DNA like this is called **cloning**.

The first recombinant DNA (1972)

- **Stanley Cohen and Herbert Boyer** made the first recombinant DNA in 1972.
- They cut out a gene for antibiotic resistance from a plasmid.
- A **plasmid** is an autonomously replicating, circular, extra-chromosomal DNA.
- They used restriction enzymes, the "molecular scissors", to cut DNA at specific places.
- They linked the gene to a native plasmid of ***Salmonella typhimurium***.
- The enzyme **DNA ligase** joined the cut ends.
- The result was a new circular DNA, made in vitro, that could replicate on its own. This is **recombinant DNA**.
- It was transferred into ***Escherichia coli***, a bacterium closely related to *Salmonella*.
- There it replicated using the new host's DNA polymerase and made many copies.
- This was called cloning of the antibiotic resistance gene in *E. coli*.
- Here, the plasmid acted as a **vector**. It carried the attached DNA into the host.

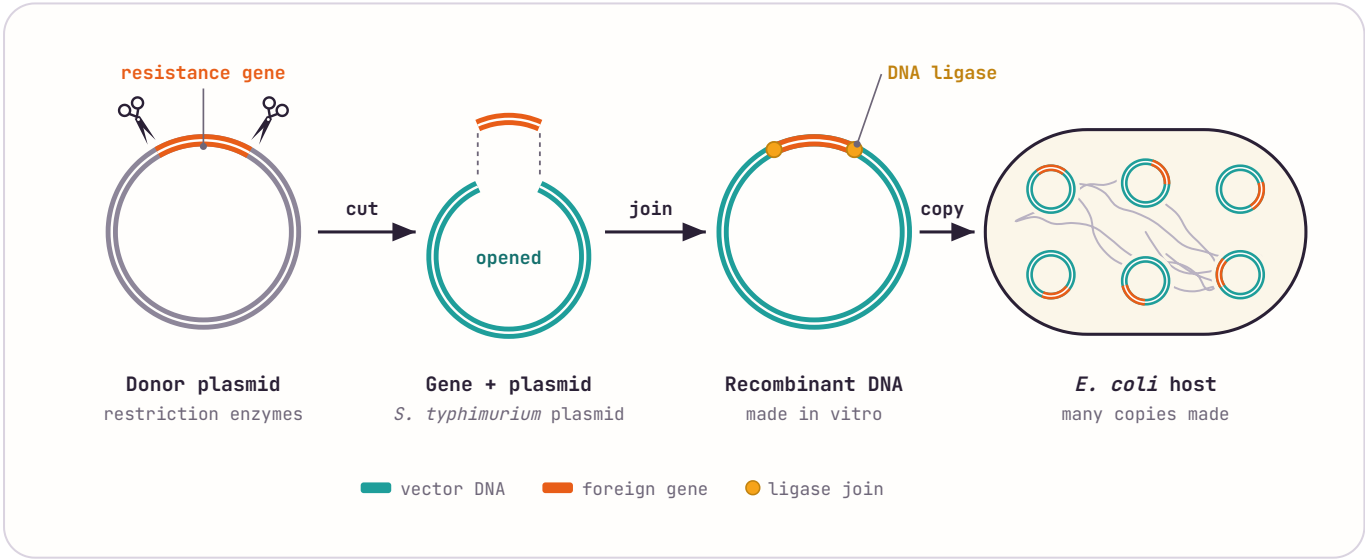


Fig 1 · The first recombinant DNA. Cut with restriction enzymes, joined with ligase, copied in E. coli.



TRAP ALERT

TRAP ALERT

- Restriction enzymes **cut**. DNA ligase **joins**. NEET options swap these two roles on purpose.

Three basic steps in genetically modifying an organism

1. Identify the DNA with the desirable genes.
2. Introduce that DNA into the host.
3. Keep the introduced DNA in the host and transfer it to the host's progeny.

Q1 Assertion-Reason

LEVEL 2

Assertion (A): To add one useful trait to a crop, genetic engineering is preferred over traditional hybridisation.

Reason (R): Traditional hybridisation often brings in and multiplies undesirable genes along with the desired ones.

1. Both A and R are true and R is the correct explanation of A
2. Both A and R are true but R is not the correct explanation of A
3. A is true but R is false
4. A is false but R is true

Answer: 1

CORE PRINCIPLE Genetic engineering moves only the chosen gene. Hybridisation mixes whole genomes, so unwanted genes come along.

TRAP Option 2 is for students who see two true lines and stop checking. Here R is exactly why A is true.

Q2 Multi-statement

LEVEL 2

Read these statements about the first recombinant DNA.

(a) It was made by Stanley Cohen and Herbert Boyer in 1972.

(b) An antibiotic resistance gene was linked to a native plasmid of *Salmonella typhimurium*.

(c) DNA ligase cut the antibiotic resistance gene out of its plasmid.

(d) In *E. coli*, it replicated using the host's DNA polymerase.

(e) The plasmid acted as a vector for the attached gene.

Choose the correct answer from the options given below.

1. (a), (b) and (c) only
2. (a), (b), (d) and (e) only
3. (b), (c), (d) and (e) only
4. (a), (c) and (d) only

Answer: 2

CORE PRINCIPLE Restriction enzymes cut the gene out; ligase joined it to the plasmid. The plasmid then carried it into *E. coli*.

TRAP (c) gives ligase the cutting job. Options 1, 3 and 4 all include it.

READ NEXT **Molecular Basis of Inheritance notes: how DNA replicates in the cell** →

02 Restriction Enzymes: The Molecular Scissors

IN NEET REPEATS OFTEN *restriction enzymes 8 questions; enzymes used in rDNA technology 5 more*

Discovery

- In 1963, two enzymes were isolated. They restricted the growth of bacteriophage in *E. coli*.
  - One added methyl groups to DNA.
  - The other cut DNA. It was called a **restriction endonuclease**.
- Five years later, the first restriction endonuclease, **Hind II**, was isolated and characterised.
- Its working depended on a specific DNA nucleotide sequence.
- Hind II always cut DNA at a particular point. It did this by recognising a specific sequence of **six base pairs**.
- This specific base sequence is called the **recognition sequence**.

- Today, more than **900** restriction enzymes are known. They come from over **230 strains** of bacteria.

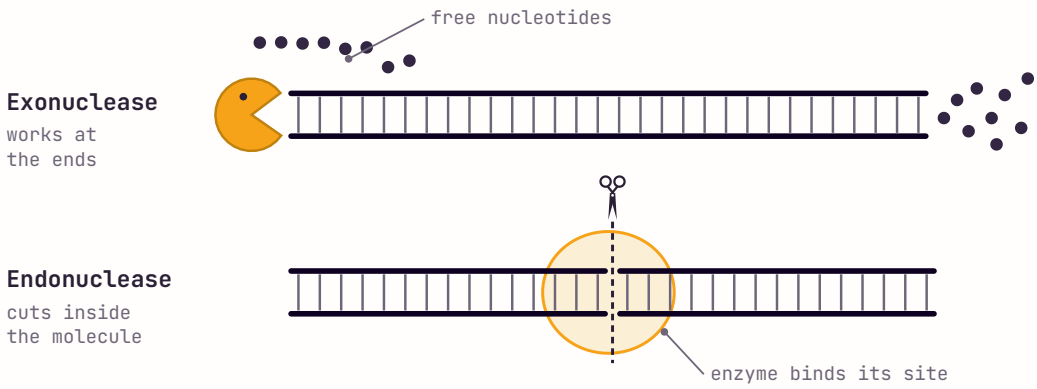
How the names work

Take **EcoRI** as the example.

PART	COMES FROM	IN ECORI
First letter	Genus	**E** = *Escherichia*
Next two letters	Species	**co** = *coli*
Next letter	Strain	**R** = strain RY 13
Roman numeral	Order in which enzymes were isolated from that strain	**I** = the first one

Exonucleases vs endonucleases

- Restriction enzymes belong to a larger class of enzymes called **nucleases**.
- **Exonucleases** remove nucleotides from the **ends** of the DNA.
- **Endonucleases** make cuts at specific positions **within** the DNA.



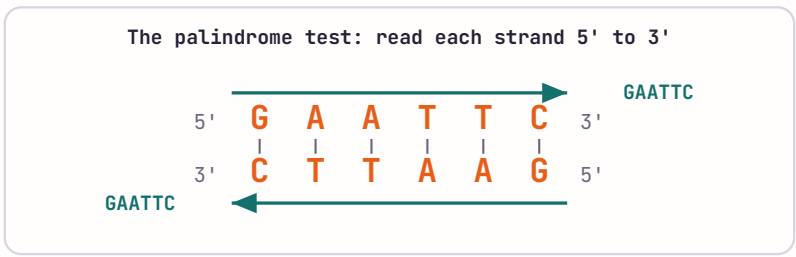


Fig 3 · Exonuclease vs endonuclease. Exo nibbles from the ends; endo cuts inside.

Palindromes

- Each restriction endonuclease inspects the length of a DNA sequence.
- When it finds its recognition sequence, it binds to the DNA.

- Then it cuts each of the two strands at specific points in their sugar-phosphate backbones.
- Each restriction endonuclease recognises a specific **palindromic** nucleotide sequence.
- A DNA palindrome reads the same on both strands when the reading direction is kept the same (5' to 3').

```
5' GAATTC 3'
3' CTTAAG 5'
```

- Read the top strand 5' to 3': GAATTC. Read the bottom strand 5' to 3': GAATTC. They match, so it is a palindrome.



WAIT, REALLY?

MYTH

A DNA palindrome reads the same backwards along one strand, like the word "level".

SCIENCE

It reads the same on the **two** strands, each read 5' to 3'. GATTAG looks like a word palindrome, but its partner strand reads CTAATC. So it is not a DNA palindrome.

Sticky ends

- Restriction enzymes cut a little away from the centre of the palindrome.
- But they cut between the **same two bases** on the opposite strands.
- This leaves single-stranded overhangs at the ends. These are **sticky ends**.
- They are called sticky because they form hydrogen bonds with their complementary cut partners.
- This stickiness helps the enzyme **DNA ligase** do its work.
- EcoRI cuts between G and A on each strand.
- When two DNAs are cut with the **same** restriction enzyme, the pieces get the same kind of sticky ends.
- DNA ligase can then join them end to end.

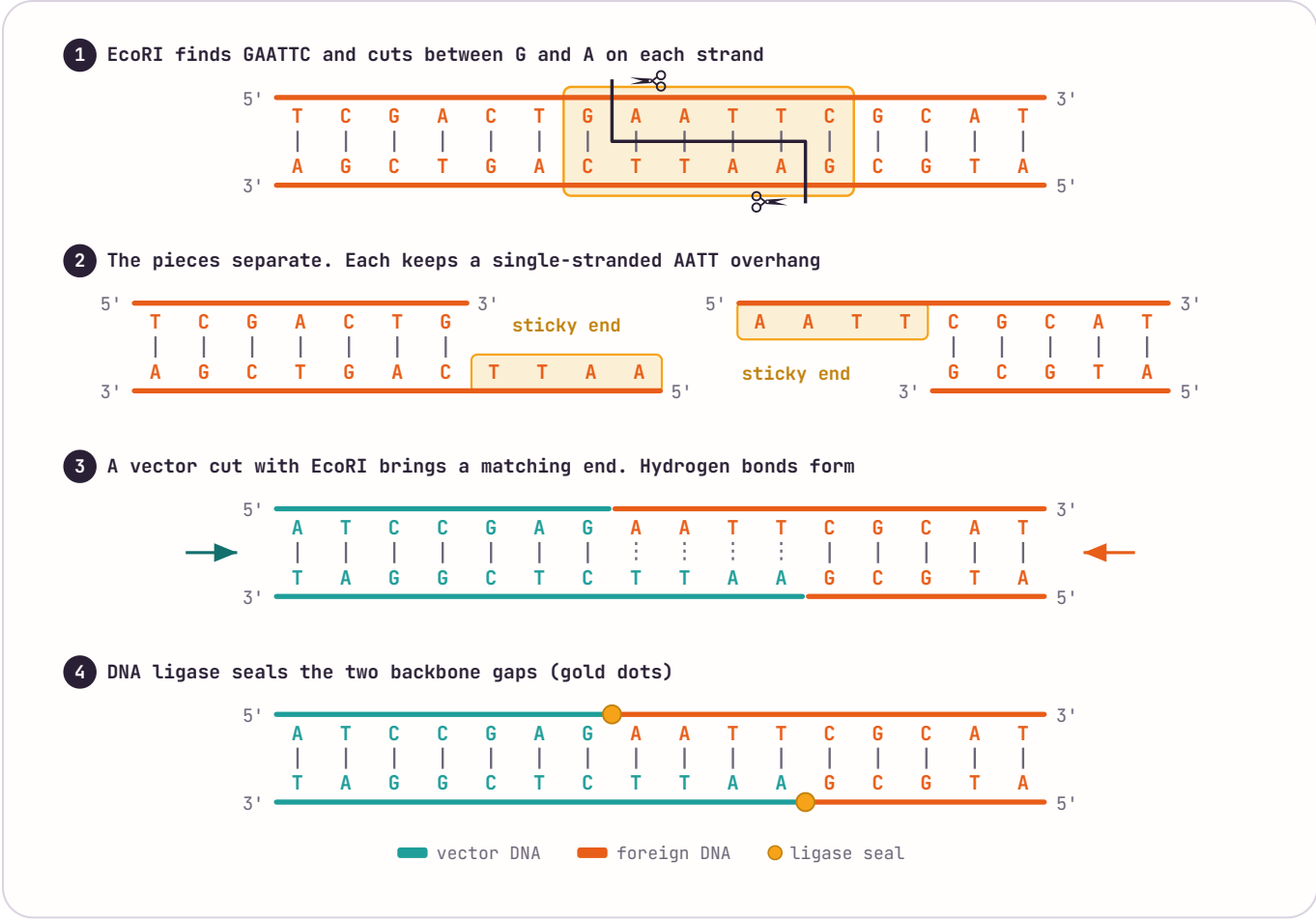


Fig 2 · EcoRI at work. Same enzyme, same sticky ends, so the pieces pair and ligase seals them.



TRAP ALERT

TRAP ALERT

- Vector DNA and source DNA must be cut with the **same** restriction enzyme. Only then do their sticky ends match.

"Restriction enzymes cut at palindromes and make sticky ends" was tested 4 times in our data. "DNA ligase joins cut DNA" was tested 4 times.

Q3 Concept application

LEVEL 2

Which of these double-stranded sequences is a palindrome in the sense used for restriction sites? (Top strand shown 5' to 3'.)

1. 5' GATTAG 3'
2. 5' GAATTG 3'
3. 5' GACGTA 3'
4. 5' GGATCC 3'

Answer: 4

CORE PRINCIPLE Write the partner strand. Then read it 5' to 3'. A palindrome gives the same sequence twice.

MATH GGATCC pairs with CCTAGG. Read 5' to 3', that strand is GGATCC. They match.

TRAP Option 1 reads the same backwards on one strand, but its partner reads CTAATC. Option 2's partner reads CAATTC. Option 3's partner reads TACGTC.

Q4 Concept application

LEVEL 2

EcoRI recognises 5' GAATTC 3' and cuts between G and A on each strand. What single-stranded overhang does each cut end carry?

1. 5' GAATTC 3'
2. 5' TTAA 3'
3. 5' AATT 3'
4. 5' G 3'

Answer: 3

CORE PRINCIPLE A cut between G and A on both strands leaves four unpaired bases at each end.

MATH Top strand: G | AATTC. Bottom strand, read 5' to 3', is also G | AATTC. So each end has a 5' AATT overhang.

TRAP Option 2 reads the overhang in the wrong direction. Option 1 is the whole site, not the overhang. Option 4 takes only the base before the cut.

Q5 Concept application

LEVEL 2

A restriction enzyme is named **BamHI**. Using NCERT's naming rule, which statement is correct?

1. "I" means it cuts the DNA at only one site.
2. "Bam" is from genus and species, "H" from the strain; "I" means the first enzyme from that strain.
3. "B" stands for bacterium and "am" for ampicillin resistance.
4. "H" means it produces the same ends as Hind II.

Answer: 2

CORE PRINCIPLE First letter = genus. Next two = species. Next letter = strain. Roman numeral = order of isolation from that strain.

TRAP Option 1 reads "I" as a count of cut sites. Option 3 invents a meaning from a word students know. Option 4 links "H" to Hind II.

Q6 Concept application

LEVEL 2

A **circular** plasmid has two EcoRI sites and one BamHI site, all at different places. A **linear** DNA has three EcoRI sites. How many fragments form in each case below?

(i) The plasmid cut with EcoRI alone

(ii) The plasmid cut with EcoRI and BamHI together

(iii) The linear DNA cut with EcoRI

1. 3, 4, 3
2. 2, 3, 3
3. 3, 4, 4
4. 2, 3, 4

Answer: 4

CORE PRINCIPLE A circle cut at n places gives n pieces. A line cut at n places gives n + 1 pieces.

MATH (i) 2 cuts in a circle = 2. (ii) 3 cuts in a circle = 3. (iii) 3 cuts in a line = 4.

TRAP Options 1 and 3 treat the circle like a line. Option 2 treats the line like a circle.



THINK IT THROUGH

SANITY CHECK

- This is why a vector should have only one site for the chosen enzyme. More sites mean more fragments, and cloning gets complicated.

03 Gel Electrophoresis: Separating DNA Pieces

IN NEET REPEATS OFTEN *6 questions*

- Restriction enzymes cut DNA into fragments.
- **Gel electrophoresis** separates these fragments.
- DNA fragments are **negatively charged**.
- So an electric field forces them to move towards the **anode** (positive end) through a medium.
- The most common medium today is **agarose**. It is a natural polymer extracted from **sea weeds**.
- The fragments separate by **size**, through the sieving effect of the agarose gel.
- **The smaller the fragment, the farther it moves.**
- Pure DNA cannot be seen in visible light without staining.
- So DNA is stained with **ethidium bromide**, then exposed to **UV** radiation.
- The DNA then shows as **bright orange bands**.
- The separated bands are cut out of the gel and the DNA is extracted from the gel piece.
- This step is called **elution**.
- DNA purified this way is joined with cloning vectors to build recombinant DNA.

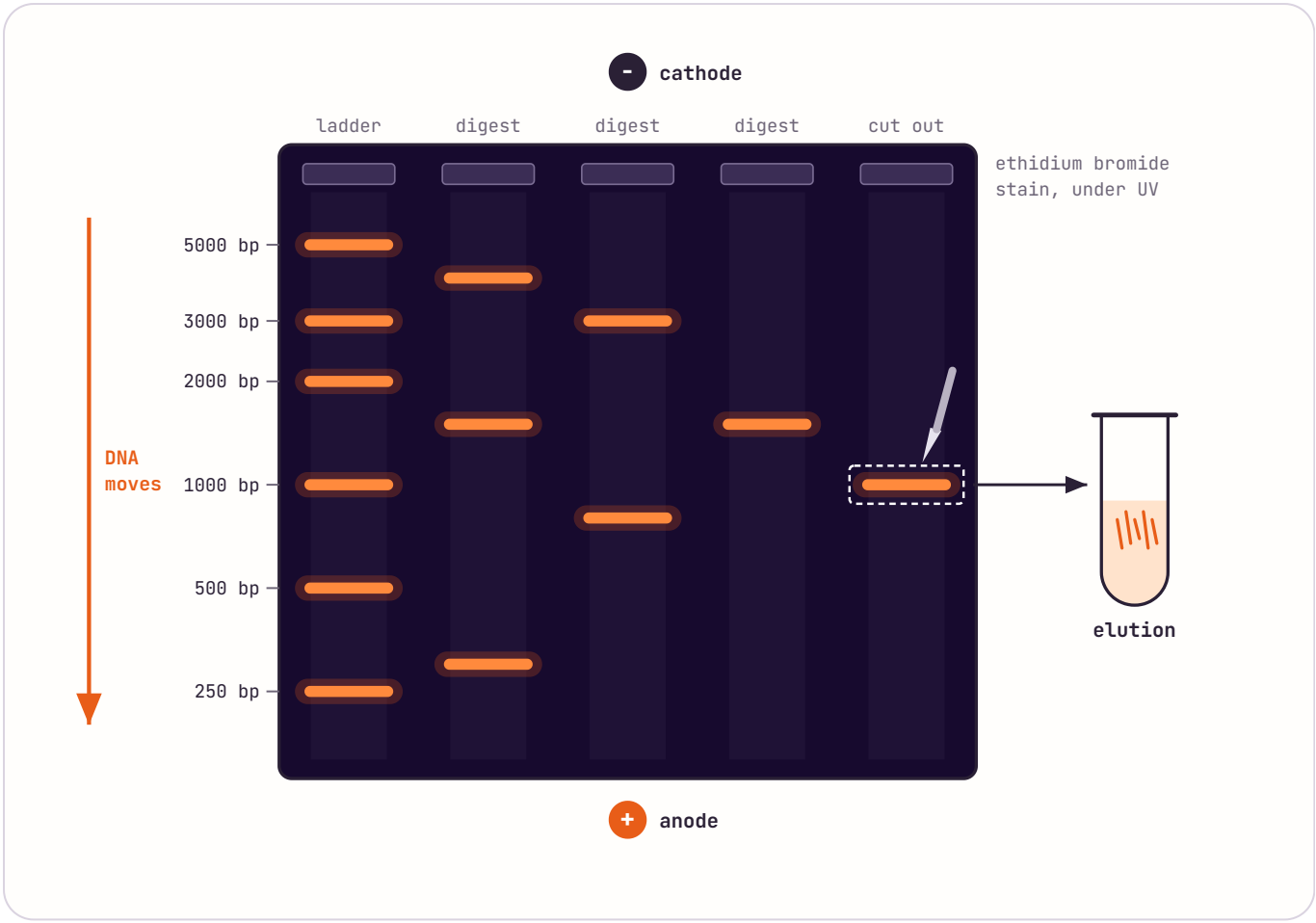


Fig 4 · Agarose gel. Negative DNA runs to the anode; small pieces travel farthest.



HAVING DIFFICULTY PICTURING WHICH BAND RUNS FARTHEST?

Play it.

This chapter is a set of short games in the Logic Bloom app.
logicbloom.co.in/download · Free to start.

SCAN



TRAP ALERT

TRAP ALERT

- DNA runs to the **anode**, the positive end, because DNA is negative. Opposite charges attract. Ethidium bromide with UV was tested 4 times in our data. No single fact in this chapter was tested more.

Q7 Multi-statement

LEVEL 2

Three DNA fragments of 300 bp, 1500 bp and 4000 bp are run on an agarose gel. Which statements are correct?

(a) The 300 bp band ends up farthest from the well.

(b) The fragments move towards the cathode.

(c) After ethidium bromide staining, the bands glow bright orange under UV light.

(d) The bands can be seen in visible light without any stain.

(e) Cutting a band out and extracting its DNA is called elution.

Choose the correct answer from the options given below.

1. (a), (c) and (e) only
2. (a), (b) and (c) only
3. (b), (d) and (e) only
4. (a), (c), (d) and (e) only

Answer: 1

CORE PRINCIPLE Negative DNA moves to the anode. Small pieces slip through the gel faster. Staining and UV make bands visible.

TRAP (b) flips the electrode. (d) forgets that pure DNA is invisible. Option 4 is for students who assume DNA is visible like a coloured dye.

04 Cloning Vectors

IN NEET REPEATS OFTEN *cloning vectors 6 questions. Insertional inactivation: seen several times (4 questions).*

Why plasmids and bacteriophages

- Plasmids and bacteriophages can replicate inside bacterial cells, independent of the chromosomal DNA.
- Bacteriophages are present in high numbers per cell. So they have very high copy numbers of their genome.
- Some plasmids have only one or two copies per cell. Others have 15 to 100 copies per cell.
- Their numbers can go even higher.
- If we link alien DNA to a bacteriophage or plasmid DNA, it multiplies to their copy number.

Feature 1: Origin of replication (ori)

- This is the sequence where replication starts.
- Any DNA linked to it can replicate within the host cells.

- It also **controls the copy number** of the linked DNA.
- Want many copies of a gene? Clone it in a vector whose ori supports a **high copy number**.

Feature 2: Selectable marker

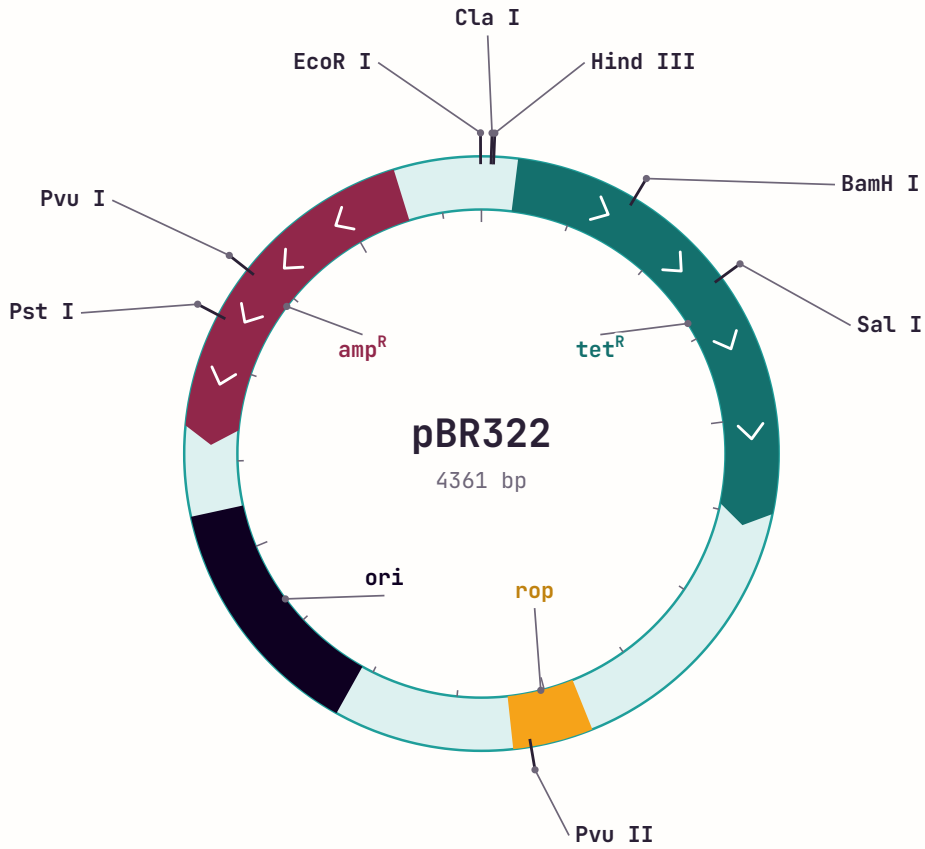
- Besides the ori, a vector needs a **selectable marker**.
- It helps identify and remove **non-transformants**.
- It lets **transformants** grow selectively.
- **Transformation** is the process of putting a piece of DNA into a host bacterium.
- For *E. coli*, useful selectable markers are genes for resistance to **ampicillin, chloramphenicol, tetracycline or kanamycin**.
- Normal *E. coli* cells carry no resistance to any of these.

Feature 3: Cloning sites

- To link alien DNA, the vector needs **very few, preferably single,** recognition sites for the common restriction enzymes.
- More than one site would cut the vector into several fragments. That complicates gene cloning.

pBR322: NCERT's example vector

- pBR322 is an *E. coli* cloning vector. It is a circular plasmid of **4361 bp**.
- It carries two antibiotic resistance genes: **ampR** (ampicillin) and **tetR** (tetracycline).
- It carries an **ori**.
- **rop** codes for proteins involved in replication of the plasmid.
- Its restriction sites in the NCERT figure: **Hind III, EcoR I, BamH I, Sal I, Pvu II, Pst I, Cla I**, and Pvu I.
- **BamH I and Sal I lie inside tetR.**
- **Pst I and Pvu I lie inside ampR.**



Inside tet^R: BamH I, Sal I
Inside amp^R: Pst I, Pvu I

Fig 5 · pBR322 plasmid map. BamH I and Sal I sit inside tetR; Pst I and Pvu I sit inside ampR.

READ NEXT **pBR322 NCERT diagram: every site and the NEET traps** →



TRAP ALERT

TRAP ALERT

- Hind III and Cla I sit just **before** tetR, in the region that switches it on, not inside the gene. EcoR I sits between ampR and tetR. Pvu II sits near rop. Only a cut **inside** a gene inactivates it.



THINK IT THROUGH

SANITY CHECK

- Neither ori nor rop is a selectable marker. The markers are ampR and tetR.

Insertional inactivation: selecting recombinants with antibiotics

- Alien DNA is ligated at a site inside one of the two resistance genes.
- Example: ligate it at the **BamH I site in tetR** of pBR322.

- The recombinant plasmid loses tetracycline resistance, because the insert breaks the gene.
- The cells are first plated on an **ampicillin** medium.
- Only **transformants** grow there. Untransformed cells die.
- These colonies are then transferred onto a **tetracycline** medium.
- **Recombinants** grow on ampicillin but **not** on tetracycline.
- **Non-recombinants** (vector without insert) grow on **both**.
- So one resistance gene selects the transformants.
- The other gets "inactivated due to insertion" of alien DNA, and selects the recombinants.
- Drawback: plating on two plates at once is cumbersome.

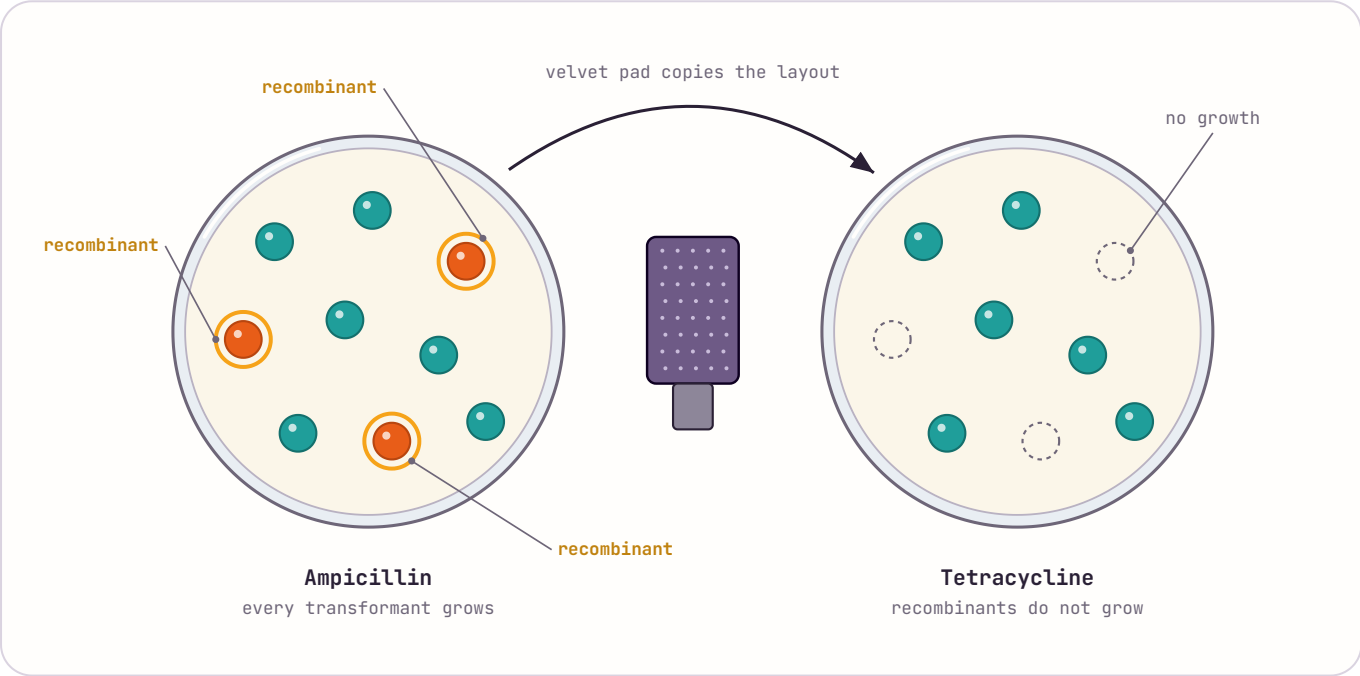


Fig 6 · Replica plating. Recombinants grow on ampicillin but vanish on tetracycline.

Insertional inactivation with colour (blue vs colourless)

- A simpler marker tells recombinants from non-recombinants by colour.
- The recombinant DNA is inserted inside the coding sequence of the enzyme **β-galactosidase**.
- This inactivates the gene. This is **insertional inactivation**.
- The medium contains a **chromogenic substrate**.
- If the plasmid has **no insert**, β-galactosidase works and the colony turns **blue**.
- If the plasmid **has an insert**, the gene is inactive. The colony gives **no colour**.
- So the **colourless colonies are the recombinants**.
- This needs only one plate.

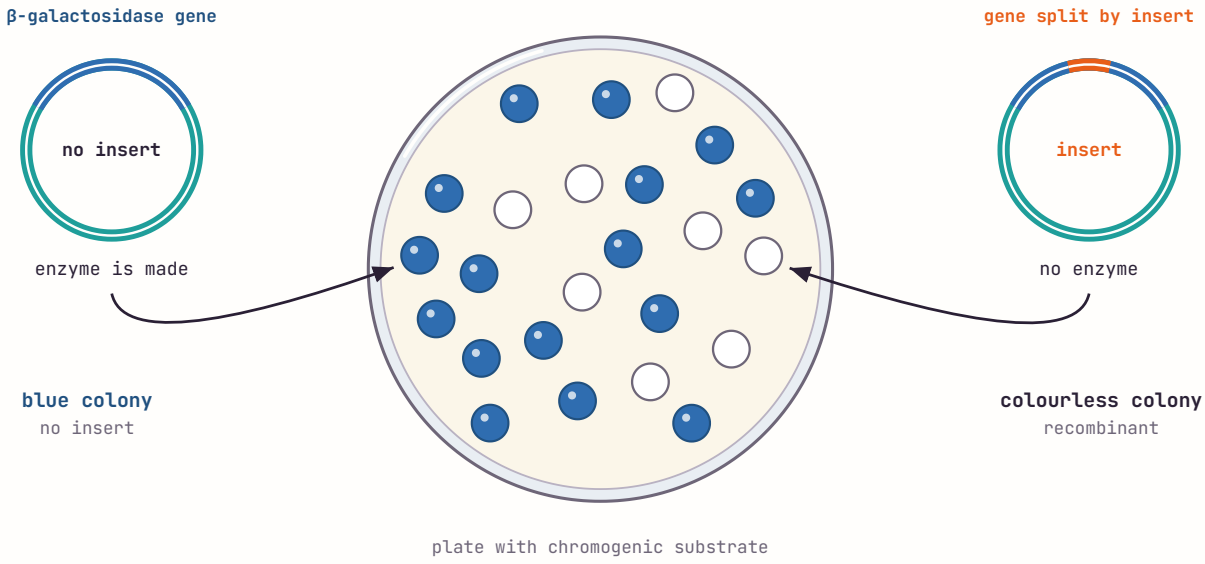
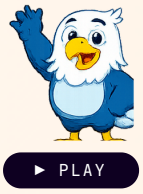


Fig 7 · Blue vs colourless colonies. A broken gene makes no colour: colourless means recombinant.



HAVING DIFFICULTY FOLLOWING WHICH COLONY IS THE RECOMBINANT?

Play it.

This chapter is a set of short games in the Logic Bloom app.
logicbloom.co.in/download · Free to start.

SCAN



TRAP ALERT

TRAP ALERT

- The colour comes from a **working** gene. The recombinant has a **broken** gene, so it stays colourless. Students who think "the insert adds colour" pick blue. Blue-white selection was tested 3 times in our data.

Vectors for plants and animals

- *Agrobacterium tumefaciens* is a pathogen of several **dicot** plants.
- It can deliver a piece of DNA called **T-DNA** into plant cells.
- The T-DNA turns normal plant cells into a tumour.
- It makes these tumour cells produce chemicals the pathogen needs.
- Similarly, **retroviruses** in animals can turn normal cells into cancerous cells.
- Scientists turned these tools to our use.
- The **tumour inducing (Ti) plasmid** of *A. tumefaciens* is now modified into a cloning vector.
- It no longer causes disease in plants. But it still delivers genes of our interest into many plants.
- Retroviruses have also been **disarmed**. Now they deliver desirable genes into animal cells.

- Once a gene is ligated into a suitable vector, it is transferred into a bacterial, plant or animal host. It multiplies there.

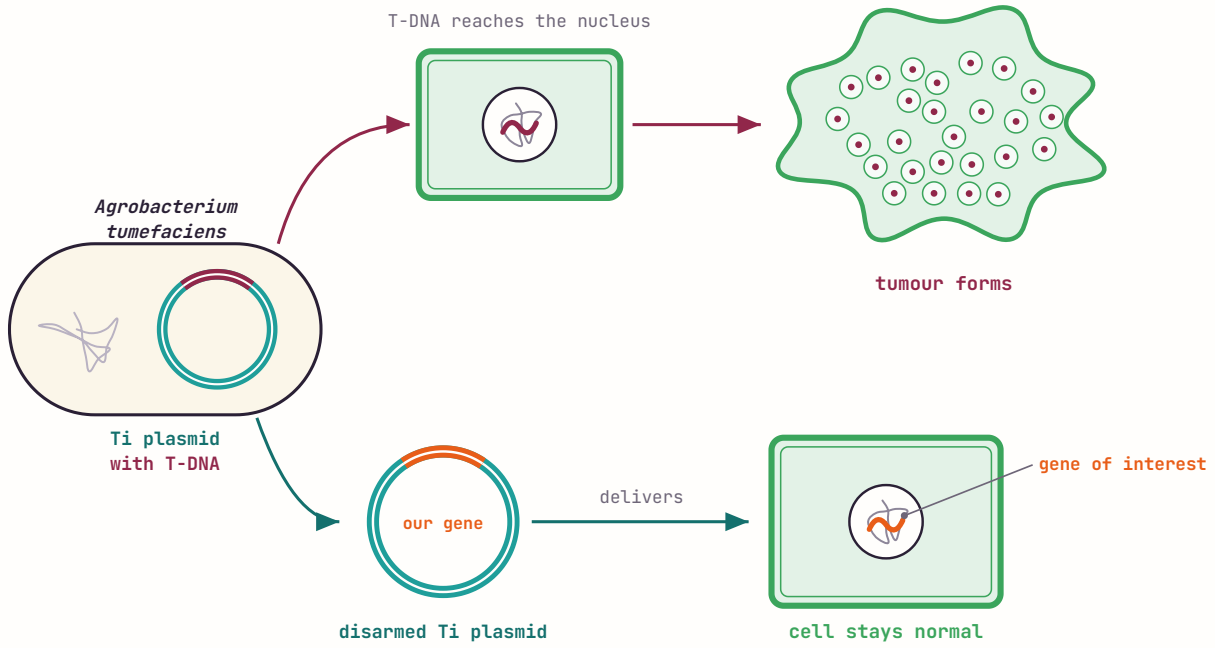


Fig 8 · Ti plasmid. Disarmed, the Ti plasmid delivers our gene instead of a tumour.

Q8 Statement I / Statement II

LEVEL 2

Statement I: Any piece of DNA linked to the origin of replication can replicate within the host cell.

Statement II: For many copies of a gene, use a vector whose ori supports a low copy number.

1. Both Statement I and Statement II are true
2. Both Statement I and Statement II are false
3. Statement I is true but Statement II is false
4. Statement I is false but Statement II is true

Answer: 3

CORE PRINCIPLE The ori starts replication and controls copy number. More copies need a high-copy-number ori.

TRAP Option 1 is for students who know the ori matters but miss the word "low".

Q9 Data interpretation

LEVEL 2

Foreign DNA was inserted at the BamH I site of pBR322. The cells were plated on ampicillin. Each colony was then copied onto a tetracycline plate.

COLONY	GROWS ON AMPICILLIN	GROWS ON TETRACYCLINE
P	Yes	Yes
Q	No	No
R	Yes	No

Which colony carries the recombinant plasmid?

1. P only
2. R only
3. P and R
4. Q only

Answer: 2

CORE PRINCIPLE Growth on ampicillin shows the cell took up a plasmid. The insert at BamH I breaks tetR. So a recombinant cannot grow on tetracycline.

MATH P has a plasmid with tetR still working: non-recombinant. Q took up no plasmid. R has a plasmid with broken tetR: recombinant.

TRAP Option 3 counts every transformant as a recombinant. Option 4 confuses "no growth" with "insert present".

Q10 Concept application

LEVEL 3

This time, foreign DNA is inserted at the **Pst I** site of pBR322. How will recombinant colonies behave?

1. Grow on both ampicillin and tetracycline
2. Grow on ampicillin but not on tetracycline
3. Grow on tetracycline but not on ampicillin
4. Grow on neither antibiotic

Answer: 3

CORE PRINCIPLE Pst I lies inside ampR. An insert there breaks ampicillin resistance. tetR stays intact.

MATH So transformants must be selected on tetracycline first. Recombinants are the ones that then fail on ampicillin.

TRAP Option 2 is the textbook BamH I answer, copied without checking which gene the site lies in. Option 1 describes a non-recombinant.

Q11 Assertion-Reason

LEVEL 2

Assertion (A): Some colonies carry an insert in the β-galactosidase gene. On a chromogenic substrate, they give no colour.

Reason (R): The insert inactivates the β-galactosidase gene, so the enzyme is not made.

1. Both A and R are true and R is the correct explanation of A
2. Both A and R are true but R is not the correct explanation of A
3. A is true but R is false
4. A is false but R is true

Answer: 1

CORE PRINCIPLE Colour needs the enzyme. The insert breaks the gene, so no enzyme and no colour.

TRAP Option 4 is for students who think recombinants turn blue.

READ NEXT **Cell notes: plasmids and prokaryotic cells** →

05 Competent Host: Getting DNA Inside

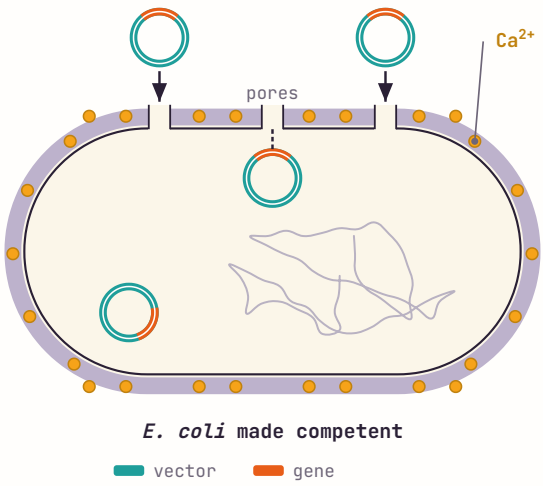
IN NEET WATCH LIST *competent host transformation is the #2 prediction in our NEET 2026 list*

Making bacteria competent

- DNA is a **hydrophilic** molecule. So it cannot pass through cell membranes.
- Bacterial cells must first be made **competent** to take up DNA.
- They are treated with a specific concentration of a **divalent cation**, such as **calcium**.
- This makes DNA enter the bacterium more efficiently, through pores in its cell wall.
- Then recombinant DNA is forced in by these steps:

  1. Incubate the cells with recombinant DNA **on ice**.
  2. Place them briefly at **42°C**. This is the **heat shock**.
  3. Put them back **on ice**.

- This lets the bacteria take up the recombinant DNA.



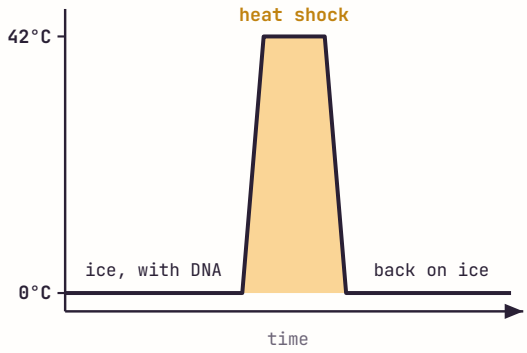


Fig 9 · Making cells competent. Calcium opens the way; the ice-heat-ice shock pushes DNA in.

Other ways to insert DNA

- **Micro-injection:** recombinant DNA is injected directly into the **nucleus of an animal cell**.
- **Biolistics (gene gun):** suitable for **plants**. Cells are bombarded with high-velocity micro-particles of **gold or tungsten** coated with DNA.
- **Disarmed pathogen vectors:** they are allowed to infect the cell. They transfer the recombinant DNA into the host.

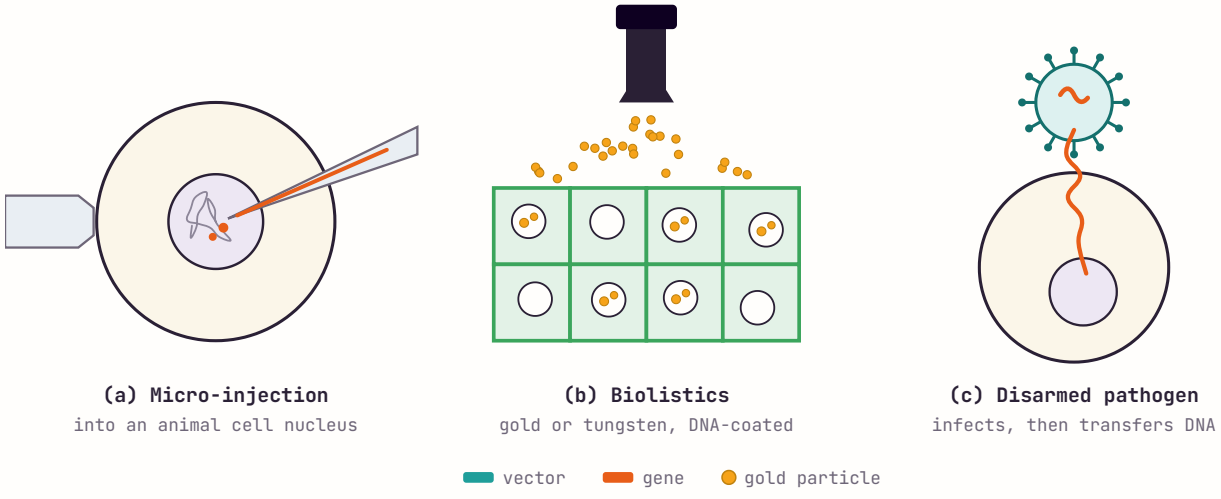


Fig 10 · Three delivery methods. Animal nucleus, plant cells, or a tamed infection.

Q12 Process ordering

LEVEL 2

Arrange these steps in the correct order to make *E. coli* take up recombinant DNA.

(A) Put the cells back on ice

(B) Treat the cells with a divalent cation such as calcium

(C) Incubate the cells with recombinant DNA on ice

(D) Place the cells briefly at 42°C

1. (C), (B), (D), (A)
2. (B), (D), (C), (A)
3. (B), (C), (A), (D)
4. (B), (C), (D), (A)

Answer: 4

CORE PRINCIPLE First make the cells competent. Then cool with DNA, heat shock, and cool again.

TRAP Option 1 adds DNA before the cells are competent. Option 2 heat-shocks before the DNA is added. Option 3 ends on the heat shock, so the "back on ice" step comes too early.

Q13 Match the Column

LEVEL 2

COLUMN I	COLUMN II
A. Micro-injection	I. Plant cells hit by DNA-coated gold or tungsten particles
B. Biolistics	II. Bacteria treated with calcium, then heat-shocked at 42°C
C. Disarmed pathogen vector	III. DNA put straight into the nucleus of an animal cell
D. Competent host	IV. Modified Ti plasmid or retrovirus infects the cell

Choose the correct answer from the options given below.

1. A-I, B-III, C-IV, D-II
2. A-III, B-I, C-IV, D-II
3. A-III, B-I, C-II, D-IV
4. A-IV, B-I, C-III, D-II

Answer: 2

CORE PRINCIPLE Each method fits a host: animal nucleus, plant cells, infection, bacteria.

TRAP Option 1 swaps micro-injection and biolistics. Option 3 swaps the pathogen and the competent host. Option 4 makes micro-injection an infection.

06 Process Step 1: Isolating the Genetic Material

IN NEET WATCH LIST *chilled ethanol last tested 2021; lysozyme, cellulase and chitinase last tested 2022*

- In most organisms, the nucleic acid is DNA.
- DNA is enclosed within membranes. So the cell must be broken open to release it.
- The cell or tissue is treated with enzymes:

SOURCE	ENZYME THAT BREAKS IT OPEN
Bacteria	**Lysozyme**
Plant cells	**Cellulase**
Fungus	**Chitinase**

- Genes lie on long DNA molecules intertwined with proteins such as **histones**.
- **RNA** is removed with **ribonuclease**.
- **Proteins** are removed with **protease**.
- Other molecules are removed by suitable treatments.
- Finally, purified DNA precipitates out after **chilled ethanol** is added.
- It shows as a collection of **fine threads** in the suspension.

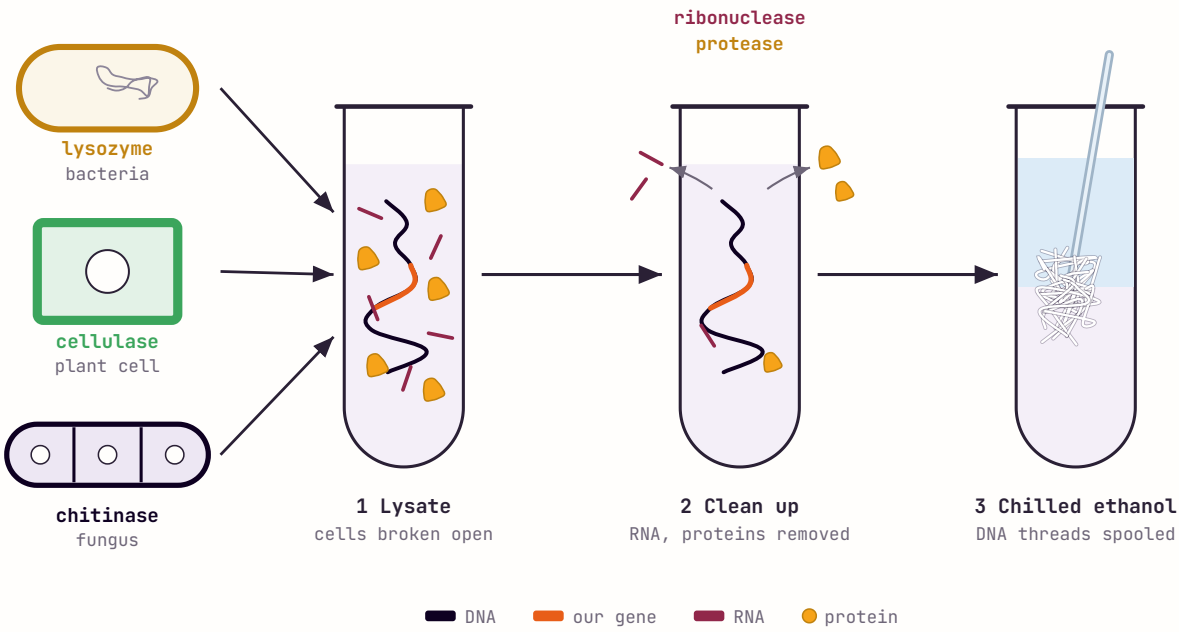


Fig 11 · DNA isolation. Break the wall, clear RNA and protein, then chilled ethanol brings DNA out as threads.



TRAP ALERT

TRAP ALERT

- Ribonuclease removes RNA. Protease removes proteins, including histones. Ribonuclease does not remove histones.

Q14 Concept application

LEVEL 2

A student needs DNA from a fungus. Which sequence of treatments is correct?

1. Lysozyme, then ribonuclease and protease, then chilled ethanol
2. Cellulase, then ribonuclease and protease, then chilled ethanol
3. Chitinase, then ribonuclease and protease, then chilled ethanol
4. Chitinase, then ribonuclease and protease, then warm ethanol

Answer: 3

CORE PRINCIPLE Match the wall-breaking enzyme to the source. Clean up RNA and proteins. Precipitate DNA with chilled ethanol.

TRAP Option 1 uses the bacterial enzyme. Option 2 uses the plant enzyme. Option 4 gets the source right but the last step wrong.

07 Process Step 2: Cutting DNA at Specific Locations

IN NEET REPEATS OFTEN *restriction enzymes, 8 questions*

- Purified DNA is incubated with a restriction enzyme under its best conditions.
- This is **restriction enzyme digestion**.
- **Agarose gel electrophoresis** checks how far the digestion has gone.
- The vector DNA is cut with restriction enzymes too.
- The source DNA is cut with the **same** enzyme.
- The cut "gene of interest" from the source and the cut vector are mixed.
- **Ligase** is added. This makes the **recombinant DNA**.



THINK IT THROUGH

SANITY CHECK

- One enzyme, both DNAs, matching sticky ends, then ligase. If the sticky ends do not match, ligase has nothing to join.

08 Process Step 3: Amplifying the Gene by PCR

IN NEET | REPEATS OFTEN | *7 questions*

- **PCR** stands for **Polymerase Chain Reaction**.
- It makes many copies of the gene (or DNA) of interest **in vitro**.
- It uses **two sets of primers** and the enzyme **DNA polymerase**.
- Primers are small, chemically synthesised oligonucleotides. They are complementary to regions of the DNA.
- The enzyme extends the primers. It uses the nucleotides provided and the genomic DNA as the template.
- Each cycle has three steps: **denaturation, annealing and extension**.
- If replication is repeated many times, the DNA segment is amplified about a **billion times**.
- That means about 1 billion copies are made.
- Repeated amplification needs a **thermostable DNA polymerase**.
- It is isolated from the bacterium ***Thermus aquaticus***.
- It stays active during the high temperature used to separate (denature) the two DNA strands.
- The amplified fragment can then be ligated with a vector for cloning.

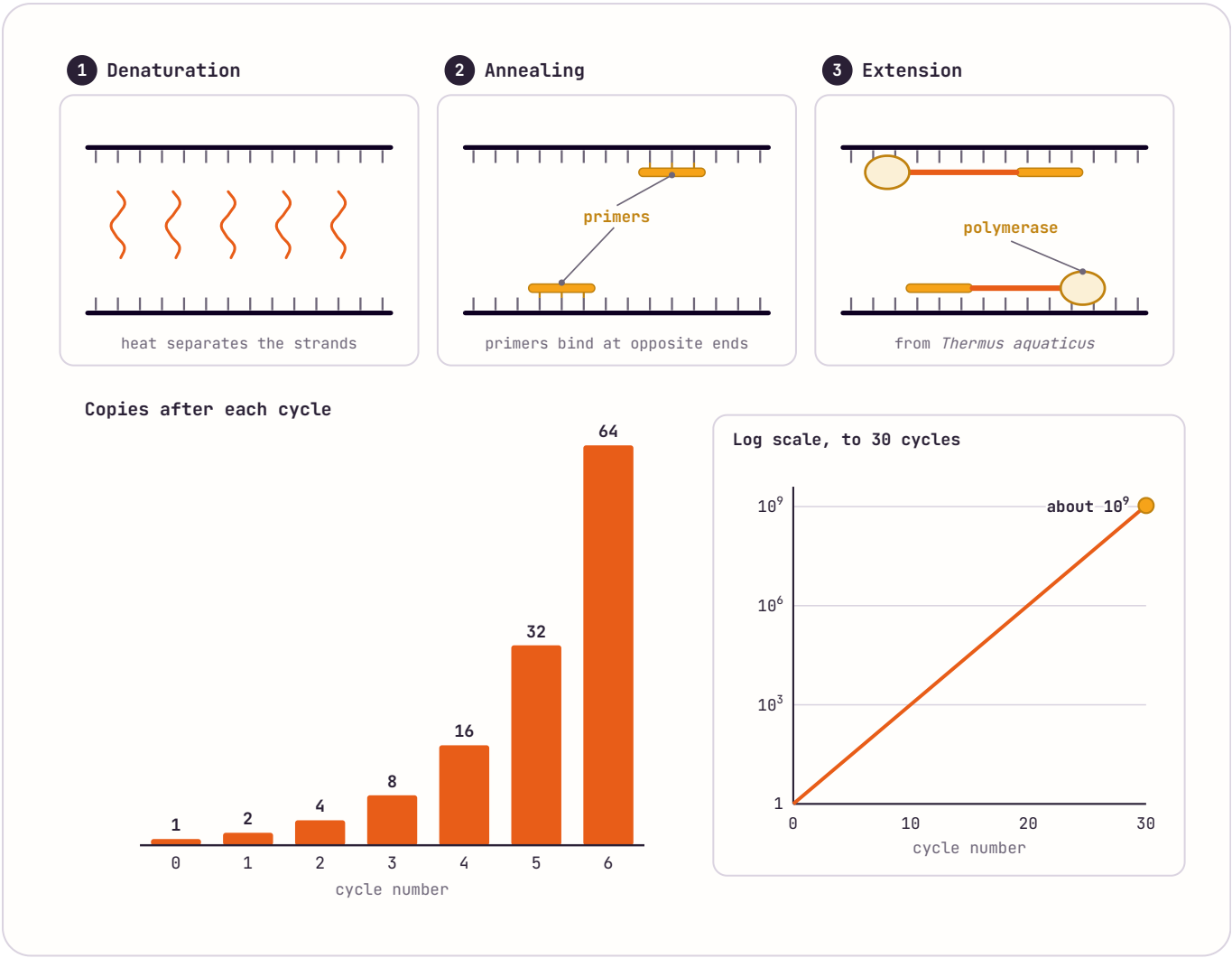


Fig 12 · PCR. Each cycle doubles the target; 30 cycles make about a billion copies.



THINK IT THROUGH

SANITY CHECK

- Each cycle doubles the copies. From one copy, 30 cycles give 2^{30} copies. That is about 1.07 billion, which matches NCERT's "approximately billion times".



TRAP ALERT

TRAP ALERT

- The polymerase is needed in every cycle. A normal polymerase would be destroyed by the heat of denaturation. That is why a thermostable one from *Thermus aquaticus* is used. Taq polymerase was tested 3 times in our data.

Q15 Numerical

LEVEL 2

A PCR starts with 5 copies of a gene. Each cycle doubles the number of copies. How many copies are there after 10 cycles?

1. 5,120
2. 50
3. 1,024
4. 10,240

Answer: 1

CORE PRINCIPLE Copies after n cycles = starting copies × 2^n.

MATH $5 \times 2^{10} = 5 \times 1{,}024 = 5{,}120$.

TRAP Option 2 adds 5 copies per cycle instead of doubling. Option 3 forgets the 5 starting copies. Option 4 counts one cycle too many.

09 Process Step 4: Inserting the Recombinant DNA

IN NEET REPEATS OFTEN *cloning vectors and markers*

- There are several ways to put the ligated DNA into recipient cells (Section 05).
- Recipient cells take up DNA from their surroundings after being made competent.
- Suppose the recombinant DNA carries a gene for **ampicillin** resistance.
- After transfer into *E. coli*, the host cells become **ampicillin-resistant**.
- Spread them on agar plates containing ampicillin.
- Only **transformants** grow. Untransformed cells **die**.
- The ampicillin resistance gene here is the **selectable marker**.

10 Process Step 5: Obtaining the Foreign Gene Product

IN NEET SEEN SEVERAL TIMES *bioreactors, 2 questions*

Why grow the host at all

- Inserted alien DNA multiplies along with the host cell.
- In most cases, the real aim is to make a **desirable protein**.

- A protein-coding gene expressed in a different (heterologous) host makes a **recombinant protein**.
- Cells with the cloned gene can be grown on a small scale in the laboratory.
- The desired protein is then extracted and purified by separation techniques.

Continuous culture

- In a **continuous culture** system, used medium drains out from one side. Fresh medium enters from the other.
- This keeps the cells in their most active **log (exponential) phase**.
- This gives a larger biomass, so a higher yield of the protein.

Bioreactors

- Small cultures cannot give large amounts of product.
- **Bioreactors** process large volumes of culture: **100 to 1000 litres**.
- A bioreactor is a vessel in which raw materials are biologically converted into specific products or enzymes.
- It uses microbial, plant, animal or human cells.
- It gives the best growth conditions: temperature, pH, substrate, salts, vitamins and oxygen.
- The most common bioreactors are of the **stirring type**.
- A **stirred-tank** reactor is usually cylindrical or has a curved base. This helps mix the contents.
- The **stirrer** gives even mixing and oxygen supply throughout the reactor.
- Alternatively, air can be **bubbled** through the reactor.
- A bioreactor has these systems:
  - an agitator system
  - an oxygen delivery system
  - a foam control system
  - a temperature control system
  - a pH control system
  - sampling ports, to withdraw small volumes of culture from time to time
- Two types in NCERT:
  - **Simple stirred-tank bioreactor**
  - **Sparged stirred-tank bioreactor**, in which sterile air bubbles are sparged through the culture

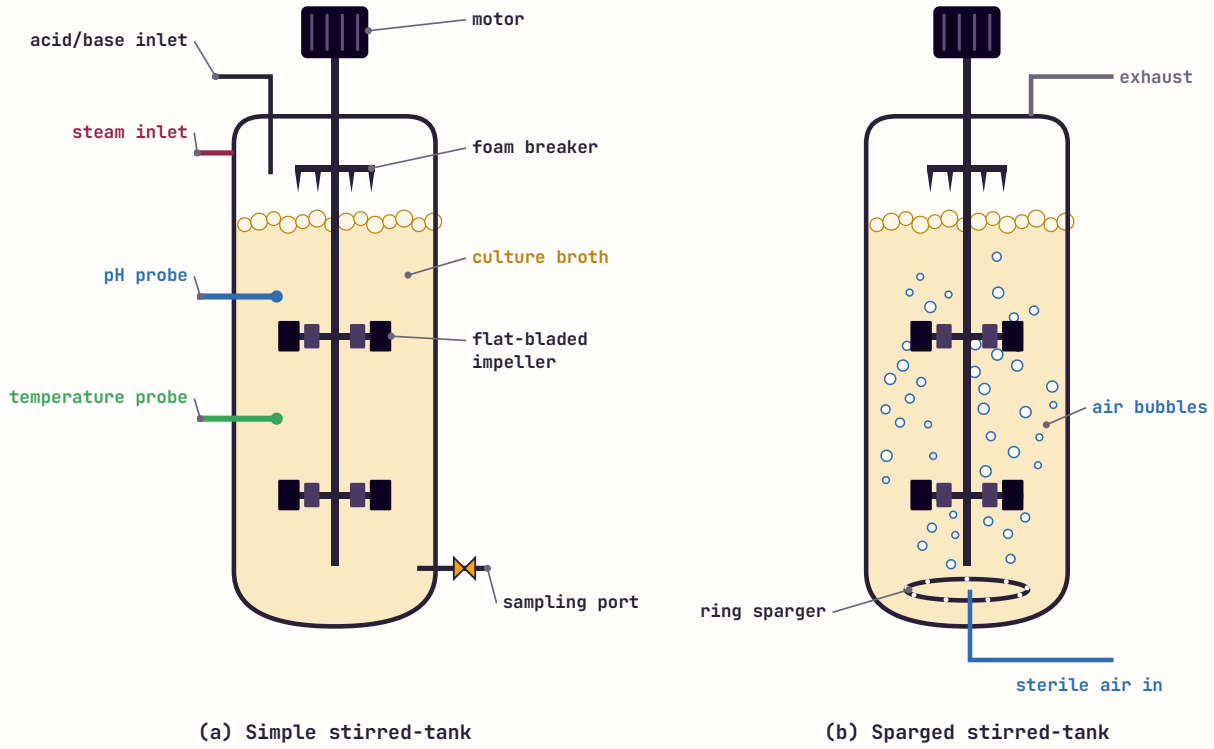


Fig 13 · Stirred-tank bioreactors. The sparged design bubbles sterile air through the culture for more oxygen.



TRAP ALERT

TRAP ALERT

- The foam breaker and the flat-bladed impeller are both in the NCERT figure. Each part was named in our data's bioreactor questions. Learn the figure, not just the list.

Q16 Counting

LEVEL 2

How many of the following are systems or parts of a stirred-tank bioreactor described in NCERT?

Agitator system, oxygen delivery system, foam control system, temperature control system, pH control system, sampling ports, gel electrophoresis unit

1. Six
2. Five
3. Seven
4. Four

Answer: 1

CORE PRINCIPLE NCERT lists six: agitator, oxygen delivery, foam control, temperature control, pH control and sampling ports.

TRAP Option 3 counts the gel unit, which belongs to the lab, not the bioreactor. Option 2 drops sampling ports, which students forget are part of the design.

11 Process Step 6: Downstream Processing

IN NEET WATCH LIST *downstream processing sequence is the #4 prediction in our NEET 2026 list*

- The biosynthetic stage ends when the product is made.
- The product then goes through a series of processes before it can be sold.
- These include **separation and purification**. Together they are called **downstream processing**.
- The product is then formulated with suitable **preservatives**.
- For drugs, the formulation goes through thorough **clinical trials**.
- Each product also needs strict **quality control** testing.
- Downstream processing and quality control vary from product to product.

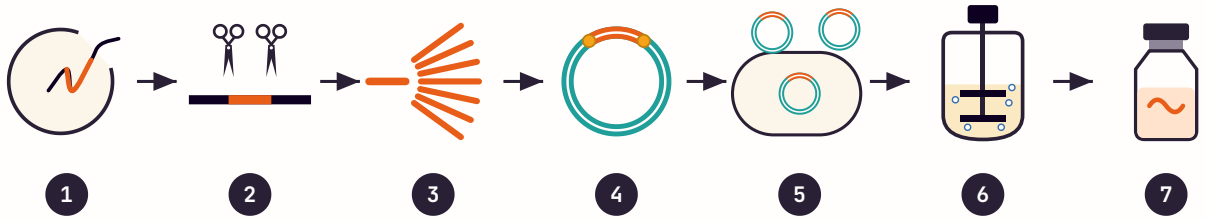


Fig 14 · The whole recipe on one strip. Isolate, cut, amplify, join, insert, grow, purify.

Q17 Statement I / Statement II

LEVEL 2

Statement I: Once a drug is separated and purified after the biosynthetic stage, it needs no clinical trials.

Statement II: Separation and purification of the product are together called downstream processing.

1. Both Statement I and Statement II are true
2. Both Statement I and Statement II are false
3. Statement I is true but Statement II is false
4. Statement I is false but Statement II is true

Answer: 4

CORE PRINCIPLE Purification is not the finish line. Drug formulations still need clinical trials and quality control.

TRAP Option 1 treats a pure product as a ready product.

READ NEXT **Biotechnology Applications NEET PYQ analysis** →

12 Quick Review: What NEET Repeats

Use this in the last week. Each card shows how often NEET repeats it, the topic and the question. The answer and the reason it recurs come next.

REPEATS OFTEN — GEL ELECTROPHORESIS

How are DNA bands made visible?

Stain with ethidium bromide, then expose to UV. Bands glow bright orange.

Why it recurs: The most repeated fact: 4 questions.

REPEATS OFTEN — RESTRICTION ENZYMES

What do restriction enzymes recognise, and what ends do they leave?

Palindromic sequences; sticky ends.

Why it recurs: Tested 4 times.

REPEATS OFTEN — ENZYMES IN RDNA

Which enzyme joins cut DNA fragments?

DNA ligase.

Why it recurs: Tested 4 times, often swapped with restriction enzymes.

REPEATS OFTEN — PCR

Where does the thermostable DNA polymerase come from?

The bacterium *Thermus aquaticus*.

Why it recurs: Tested 3 times.

SEEN SEVERAL TIMES — INSERTIONAL INACTIVATION

On a chromogenic substrate, which colonies are recombinant?

The colourless ones. Blue colonies have no insert.

Why it recurs: Tested 3 times.

REPEATS OFTEN — CLONING VECTORS

Which vector feature controls copy number of linked DNA?

The origin of replication (ori).

Why it recurs: Tested 3 times.

SEEN SEVERAL TIMES — INSERTIONAL INACTIVATION

In pBR322, which sites lie in tetR, and which in ampR?

BamH I and Sal I in tetR; Pst I and Pvu I in ampR.

Why it recurs: Tested 2 times; an A-R trap on Pvu I is predicted.

SEEN SEVERAL TIMES — BIOREACTORS

Name two parts of a stirred-tank bioreactor seen in questions.

Foam breaker; flat-bladed impeller.

Why it recurs: Tested 2 times, from the NCERT figure.

REPEATS OFTEN — PCR

Name the three steps of a PCR cycle.

Denaturation, annealing, extension.

Why it recurs: Tested 2 times; ordering questions are rising.

WATCH LIST — DNA ISOLATION

What makes purified DNA come out of solution?

Chilled ethanol. DNA shows as fine threads.

Why it recurs: Tested 2 times; due for a return.

REPEATS OFTEN — RESTRICTION ENZYMES

What was the first restriction endonuclease, and what did it recognise?

Hind II; a specific six base pair sequence.

Why it recurs: A favourite history line.

REPEATS OFTEN — RESTRICTION ENZYMES

What does each part of "EcoRI" mean?

E = genus, co = species, R = strain, I = first enzyme from that strain.

Why it recurs: Naming rules turn into match questions.

REPEATS OFTEN — RESTRICTION ENZYMES

Exonuclease vs endonuclease?

Exo removes nucleotides from ends; endo cuts at positions within DNA.

Why it recurs: A clean statement-check item.

REPEATS OFTEN — GEL ELECTROPHORESIS

Which fragment moves farthest, and what is the gel made of?

The smallest; agarose, from sea weeds.

Why it recurs: Migration and source come up together.

REPEATS OFTEN — CLONING VECTORS

Name three features every cloning vector needs.

Origin of replication, selectable marker, cloning sites.

Why it recurs: The backbone of this section.

REPEATS OFTEN — CLONING VECTORS

Name four selectable markers used for *E. coli*.

Resistance to ampicillin, chloramphenicol, tetracycline, kanamycin.

Why it recurs: Asked as "which is NOT a marker".

SEEN SEVERAL TIMES — HISTORY

What did Cohen and Boyer join in 1972?

An antibiotic resistance gene with a native plasmid of *Salmonella typhimurium*.

Why it recurs: The opening fact of the chapter.

WATCH LIST — COMPETENT HOST

How is *E. coli* made to take up rDNA?

Calcium treatment; then ice, 42°C heat shock, ice.

Why it recurs: Our #2 prediction for NEET 2026.

WATCH LIST — GENE TRANSFER

Which method suits plants, and which animal nuclei?

Biolistics (gene gun) for plants; micro-injection for animal nuclei.

Why it recurs: Pairs well with Q18 in matches.

WATCH LIST — PLANT VECTORS

Which plasmid was turned into a plant vector?

The Ti plasmid of *Agrobacterium tumefaciens*.

Why it recurs: Last tested in 2024, by name only.

WATCH LIST — DNA ISOLATION

Lysozyme, cellulase, chitinase: which cell does each open?

Bacteria, plant cells, fungus.

Why it recurs: Last tested 2022; due for a return.

WATCH LIST — DOWNSTREAM PROCESSING

What comes after the biosynthetic stage?

Separation and purification, formulation with preservatives, clinical trials (drugs), quality control.

Why it recurs: Our #4 prediction for NEET 2026.

★ Biotechnology: Principles and Processes · Fact Sheet

One page for the night before the exam.

PEOPLE AND DATES

Cohen and Boyer (1972): first recombinant DNA, *S. typhimurium* plasmid

1963: two enzymes found that restrict phage growth in *E. coli*
Five years later: Hind II, first restriction endonuclease (6 bp recognition sequence)

NUMBERS

pBR322: 4361 bp

900+ restriction enzymes from 230+ bacterial strains
Plasmid copy numbers: 1 to 2, up to 15 to 100 per cell
Heat shock: 42°C, between two spells on ice
PCR: about 1 billion copies ($2^{30} \approx 1.07$ billion)
Bioreactors: 100 to 1000 litres

WHO DOES WHAT

Restriction endonuclease: cuts at palindromes, leaves sticky ends

DNA ligase: joins ends
Taq polymerase (*Thermus aquaticus*): extends primers, survives heat
Lysozyme / cellulase / chitinase: open bacteria / plant cells / fungi
Ribonuclease: removes RNA · Protease: removes proteins
Chilled ethanol: precipitates DNA

GEL ELECTROPHORESIS

DNA is negative, runs to the anode

Agarose from sea weeds; smaller runs farther
Ethidium bromide + UV = orange bands · Cutting bands out = elution

PBR322

BamH I, Sal I in tetR · Pst I, Pvu I in ampR

Hind III, Cla I just before tetR · rop = replication proteins · ori = start of replication

SELECTION

Colour: recombinant colony is colourless; no insert = blue

Antibiotic: recombinant grows on ampicillin, not tetracycline (insert at BamH I)

GENE TRANSFER

Bacteria: Ca^{2+}, ice, 42°C, ice

Animal nucleus: micro-injection
Plants: biolistics (gold or tungsten) · Disarmed vectors: Ti plasmid, retroviruses

THE RECIPE

Isolate → cut → amplify (PCR)

→ ligate → insert → grow (bioreactor) → downstream processing

Logic Bloom

The simplest way to learn science.

logicbloom.co.in

★ STILL MIXING UP THE STEPS?

Play it. Then practice it.

Open Biotechnology: Principles and Processes in the Logic Bloom app. Play the chapter as short games, then practise the questions NEET repeats. Free to start.

SCAN → DO · READ · VERIFY