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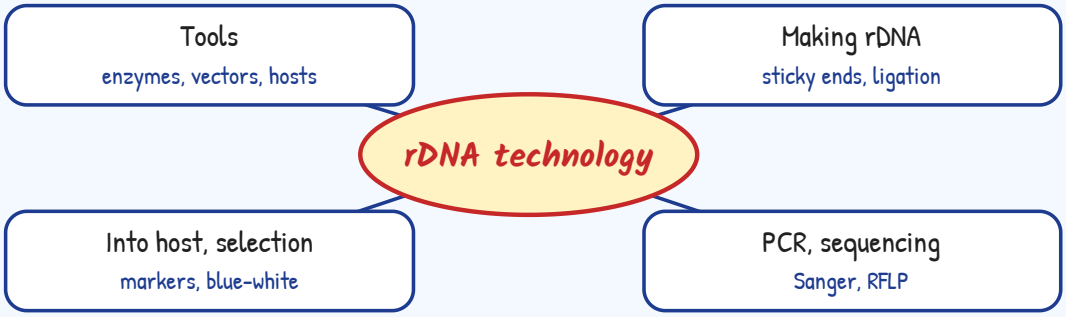
Chapter 1 Recombinant DNA Technology

plasmid

cut · join · clone · select

CBSE Biotechnology (045) syllabus 2026-27 · board 2027 · Unit V Protein and Gene Manipulation = 40 marks of 70

Chapter map



What is inside

1. Concept notes with labelled diagrams
2. Important textbook questions, fully solved
3. Every CBSE board question of 2024, 2025, 2026 from this chapter, solved to the marking scheme
4. Practice, 20 MCQs, 6 A-R, answers, summary

red = key term green = definition

purple = sub-heading black = explanation

★ = high-frequency board topic

This chapter in the board paper

year	1 mark	2–5 marks
2024	M13	rDNA steps (2); PCR (3); markers (3); Sanger (5)
2025	T4 ligase; PCR; M13	vectors / blue-white (3); Sanger (4); rDNA / RFLP (5)
2026	restriction; M13	BAC / EcoRI (2); selection (3); transfer / blue-white (3); PCR / RFLP (5)

X Don't do this: The most important chapter of the paper: **13–16 marks** every year. PCR, Sanger sequencing and blue-white selection come almost every year.

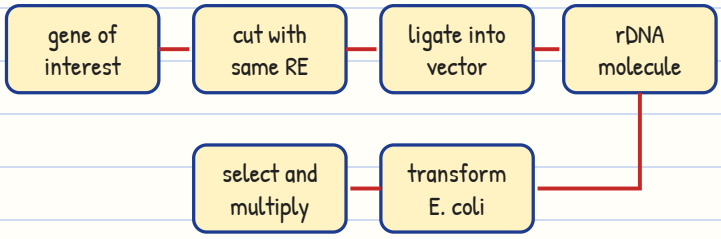
Based on the CBSE Biotechnology syllabus 2026-27 and the CBSE textbook. All answers written by us.

1.1 Introduction ★★★

Recombinant DNA (rDNA) technology is the joining of DNA from two different sources, generally a gene of interest and a vector, and its introduction into a host where it can multiply. It is also called **gene cloning** or genetic engineering. First rDNA: **Paul Berg** (1972); first cloning in E. coli: **Cohen and Boyer** (1973).

Basic steps

1. **isolation** of the DNA fragment with the gene of interest (the **insert**)
2. **insertion** into a **vector** (e.g. plasmid) to make rDNA
3. **transfer** of rDNA into a host such as E. coli (**transformation**)
4. **selection** of host cells carrying the rDNA
5. **multiplication** of recombinant cells, which multiplies the rDNA (cloning)



1.2 Restriction enzymes ★★★

Restriction endonucleases (RE) are "molecular scissors" that cut DNA at specific recognition sequences. In bacteria they are a **natural defence**: they **destroy invading viral (phage) DNA**, while the bacterium's own DNA is protected by **methylation** (restriction–modification system).

- recognition sites are usually **palindromes** of 4–8 bp (read the same 5'→3' on both strands)
- type II REs cut within the site; used in cloning
- naming: **EcoRI** = **E**scherichia **co**li, strain **R**Y13, first (I) enzyme
- **sticky (cohesive) ends**: staggered cuts leave single-stranded overhangs (EcoRI: G^AATTC); **blunt ends**: cut straight (SmaI: CCC^GGG)

EcoRI cut (2026 Q17b)

```
5' ACTAG^AATTCGCCA 3'
3' TGATCTTAA^GCGGT 5'
```

Fragments: `5' ACTAG 3'` / `3' TGATCTTAA 5'` and `5' AATTCGCCA 3'` / `3' GCGGT 5'`, each with a 4-base **AATT** sticky end.

1.3 Other enzymes ★★

DNA ligase	joins DNA fragments by forming **phosphodiester bonds**; the one commonly used is from **T4 bacteriophage**
DNA polymerase	synthesises DNA; **Taq polymerase** (Thermus aquaticus) is heat-stable, used in PCR
alkaline phosphatase	removes 5' phosphate; prevents self-ligation of vector
polynucleotide kinase	adds phosphate to 5' end (labelling)
reverse transcriptase	makes **cDNA** from mRNA

terminal transferase	adds nucleotides to 3' ends (homopolymer tails)
DNase I	creates nicks (used in nick translation)

1.4 Vectors ★★★

A vector is a DNA molecule that carries the insert into a host and replicates there. Features: **origin of replication (ori)**, a **selectable marker**, unique **restriction sites** (multiple cloning site, MCS), small size.

plasmids	extrachromosomal circular DNA; pBR322 (amp^R, tet^R), pUC series (lacZ, high copy)
bacteriophage lambda	larger inserts (up to ~20 kb)
M13	filamentous phage with **single-stranded circular** genome; infects E. coli with **F plasmid** (pili); replicates by **rolling circle** via a double-stranded **replicative form** (about 100 copies); gives **ssDNA** for **sequencing**
cosmids	plasmid + **cos sites** of lambda; ori, marker, RE sites; large inserts (~45 kb)
BAC	based on the **F (fertility) plasmid** of E. coli; replication and maintenance genes, selectable marker, cloning sites; inserts up to 300 kb
YAC, YEp	yeast vectors; YAC for very large inserts
shuttle vectors	two ori and two markers: one set for **E. coli**, one for **eukaryotic** cells
expression vectors	carry **transcription and translation signals** (promoter, RBS) to express the foreign protein
Ti plasmid	Agrobacterium tumefaciens, for plants

1.5 Making the rDNA molecule ★★★

1. cut the source DNA and the vector with the **same restriction enzyme**, giving complementary sticky ends
2. mix; sticky ends **base-pair** (anneal)
3. **DNA ligase** seals the nicks (phosphodiester bonds): rDNA formed

Blunt ends can be joined by ligase less efficiently, or by adding **linkers/adaptors** or homopolymer tails (terminal transferase). **cDNA** made from mRNA by reverse transcriptase is used to clone eukaryotic genes without introns (for expression in bacteria).

1.6 Introducing rDNA into host cells ★★★

transformation	bacterial cells made **competent** with cold $CaCl_2$, then **heat shock** (42 °C) so DNA enters
transfection	DNA mixed with a **charged substance** (calcium phosphate, cationic liposomes, DEAE-dextran) and overlaid on recipient animal cells
electroporation	an **electric pulse** creates transient microscopic **pores** in the membrane for DNA to enter
biolistics (gene gun)	**gold or tungsten** microparticles coated with DNA are shot into cells (plants)
microinjection	DNA injected directly into the nucleus (animal eggs)
Agrobacterium	natural plant transformation by Ti plasmid
viral transduction	phages / viruses deliver DNA

1.7 Identification of recombinants ★★★

Selection is based on **expression or non-expression** of traits:

- **antibiotic resistance** (ampicillin, tetracycline, kanamycin)
- enzymes like **beta-galactosidase** (colour reaction)
- fluorescent protein: **GFP**
- **nutritional** dependence or independence (e.g. **Leu2** gene for leucine synthesis in yeast)

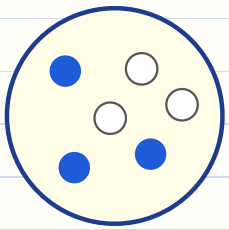
selectable marker gene	*trait / protein*
amp^R (bla)	ampicillin resistance (beta-lactamase)
tet^R	tetracycline resistance
GFP	green fluorescence
lacZ	beta-galactosidase (blue colour with X-gal)
Leu2	enzyme for leucine synthesis

1.8 Insertional inactivation and blue-white selection ★★★

Inserting a gene **inside** a marker gene **inactivates** it. In **pBR322**, an insert in tet^R makes recombinants ampicillin-resistant but tetracycline-sensitive (replica plating).

Blue-white selection (pUC19):

1. the vector carries amp^R and the **lacZ** gene (MCS inside lacZ)
2. lacZ makes **beta-galactosidase**, which cleaves colourless **X-gal** into a **blue** product
3. an insert in lacZ **inactivates** it, so no enzyme is made
4. after transformation, E. coli is plated on medium with **ampicillin + X-gal** (+ IPTG inducer)
5. **blue colonies**: transformed (amp-resistant) but **no insert**
6. **white colonies**: amp-resistant **with the insert** (recombinants); untransformed cells do not grow



blue = vector only (lacZ active)

white = recombinant (lacZ broken)

plate: ampicillin + X-gal

1.9 Applications of rDNA ★

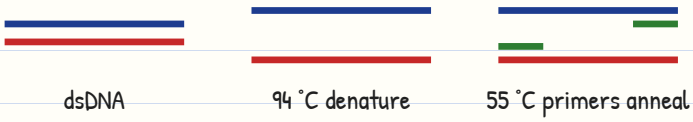
Recombinant proteins (insulin, growth hormone, vaccines), transgenic crops and animals, gene therapy (first for **ADA deficiency / SCID**, 1990), diagnostics, DNA fingerprinting.

1.10 Polymerase chain reaction (PCR) ★★★

PCR is an in vitro method to make millions of copies of a specific DNA segment. Invented by **Kary Mullis** (1985). Needs: template DNA, two **primers**, four **dNTPs**, **Taq DNA polymerase**, buffer with Mg^{2+}, a thermal cycler.

1. denaturation	**~94–95 °C**: double-stranded DNA separates into single-stranded templates
2. annealing	**~50–60 °C**: two primers bind to the 3′ ends of the complementary template strands
3. extension	**~72 °C**: Taq polymerase extends each primer in the **5′→3′** direction using dNTPs

Each cycle **doubles** the DNA: after n cycles, copies = $N \times 2^n$. One cycle turns 2 molecules into **4**; 3 molecules after 10 cycles give $3 \times 2^{10} = 3072$. Uses: diagnosis, forensics, cloning, sequencing, mutation detection.



then 72 °C: Taq extends 5′ to 3′; cycle repeats

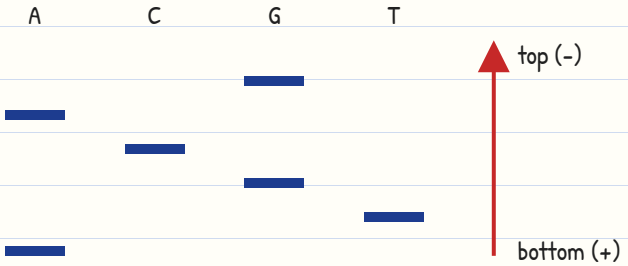
1.11 DNA sequencing: Sanger's method ★★★

Chain-termination method (Frederick Sanger, 1977). **Principle:** **ddNTPs** (2′,3′-dideoxynucleotides) lack the **3′-OH** group, so once added, the next nucleotide's 5′ phosphate cannot form a phosphodiester bond and the chain **terminates**.

1. DNA to be sequenced is cloned in **single-stranded** form (**M13** vector)
2. **four tubes**, each with ssDNA template, **primer** (radiolabelled), four dNTPs and **DNA polymerase**
3. a small amount of **one ddNTP** (ddATP, ddGTP, ddCTP or ddTTP) is added to each tube
4. chains end wherever that ddNTP is incorporated: fragments of every length
5. fragments separated on a polyacrylamide **sequencing gel** (resolves 1-nucleotide differences); shorter fragments move faster towards the anode
6. **autoradiography**; the gel is read from **bottom to top** to give the sequence **5′→3′** of the newly synthesised strand (complementary to the template)

Primers are needed because DNA polymerase can only **extend** an existing 3′-OH; it cannot start a new chain.

Reading a sequencing gel



Read bottom to top: **5′ A T G C A G 3′** (new strand). Template is the complement: 3′ TACGTC 5′.

Why bottom to top? The shortest fragment (nearest the primer, the 5′ end) runs fastest to the bottom; DNA is synthesised 5′→3′, so reading from the bottom gives 5′→3′.

Automated (single-tube) sequencing

Each ddNTP is tagged with a **different fluorescent dye**; one reaction tube and **one gel lane** (capillary); a **laser** scans and a computer reads the colour; **safer** (no radioisotopes), **faster**, results stored digitally.

1.12 RFLP ★★★

Restriction Fragment Length Polymorphism: **variation in the length of DNA fragments** produced by the same restriction enzyme in different individuals of a species (due to differences in RE sites).

1. isolate test DNA samples
2. digest each with the **same restriction enzyme**
3. separate fragments by **agarose gel electrophoresis** (Southern blot with probe)
4. analyse and compare band patterns

Uses: forensics (identify criminals), relate individuals (paternity tests), DNA fingerprinting, gene mapping, disease diagnosis.

CBSE board questions 2024–2026, fully solved

CBSE 2024 · 045 The vector that infects E. coli with F-plasmid and has a single-stranded circular genome: (A) A. tumefaciens (B) YEp (C) pBR322 (D) M13 [1]

> **Ans.** (D) M13

CBSE 2025 · 045 DNA ligase commonly used to make rDNA is isolated from: (A) T2 (B) T4 bacteriophage (C) lambda (D) M13 [1]

> **Ans.** (B) T4 bacteriophage

CBSE 2025 · 045 At the end of one PCR cycle, two DNA molecules become: (A) eight (B) sixteen (C) four (D) two [1]

> **Ans.** (C) Four (each cycle doubles).

CBSE 2025 · 045 To obtain a gene sequence in single-stranded form, choose: (A) M13-based vector (B) lambda vector (C) pUC plasmid [1]

> **Ans.** (A) M13-based vector

CBSE 2026 · 045 The natural defence mechanism of bacteria (restriction enzymes) is for: [1]

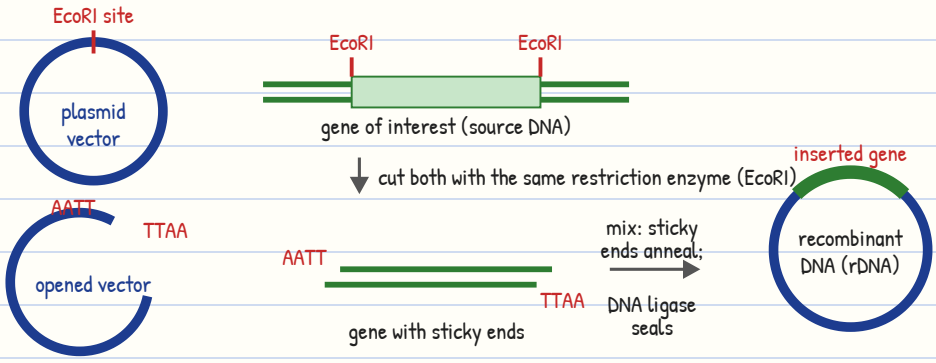
> **Ans.** (C) Destruction of invading viral DNA.

CBSE 2026 · 045 A: M13 uses rolling circle replication. R: A double-stranded replicative form is made, which replicates to about a hundred copies per cell. [1]

> **Ans.** (A) Both true; R explains A.

CBSE 2024 · 045 Illustrate the steps in the construction of a recombinant DNA molecule. [2]

> **Ans.** Draw as below: (1) the gene of interest and the plasmid vector are cut with the same restriction enzyme, producing complementary sticky ends; (2) the fragments are mixed, ends anneal, and DNA ligase joins them, giving the rDNA molecule. [2]



Construction of a recombinant DNA molecule: vector and source DNA cut with the same restriction enzyme, sticky ends annealed, joined by DNA ligase

CBSE 2026 · 045 (A) On which plasmid are BAC vectors based? List the important sequences. OR (B) Which enzyme cleaves 5′ ACTAGAATTCGCCA 3′ (double-stranded)? Show the fragments. [2]

> **Ans.** (A) The fertility (F) plasmid of E. coli. [1] Sequences: replication and maintenance genes, selectable markers, cloning sites (any two). [½ + ½]

(B) **EcoRI** (site GAATTC). [1] Fragments as drawn in the box under 1.2: 5′ACTAG3′/3′TGATCTTAA5′ and 5′AATTCGCCA3′/3′GCGGT5′. [½ + ½]

CBSE 2024 · 045 Explain the steps involved in PCR amplification. [3]

Ans. **Step 1, denaturation**: at high temperature (~94 °C) dsDNA separates into single-stranded templates. **Step 2, annealing**: at 50–60 °C the two primers bind to the 3′ ends of the templates. **Step 3, extension**: at ~72 °C each primer is extended by **Taq DNA polymerase** in the 5′→3′ direction using dNTPs. The cycle is repeated; the DNA doubles each cycle. [1 × 3]

CBSE 2024 · 045 Name three genes used as selectable markers in rDNA technology and the trait/protein they specify. [3]

Ans. Any three: **ampicillin resistance gene**: resistance to ampicillin; **tetracycline resistance gene**: resistance to tetracycline; **GFP gene**: green fluorescent protein; **Leu2**: enzyme needed to synthesise the amino acid leucine (also lacZ: beta-galactosidase). [1 × 3]

CBSE 2026 · 045 Selection methods use expression or non-expression of traits in recombinant cells. List any three such traits. [3]

Ans. (1) **Antibiotic resistance**; (2) **beta-galactosidase** activity; (3) a protein such as **GFP**; (4) **dependence or independence of a nutritional requirement**, e.g. leucine (any three). [1 × 3]

CBSE 2025 · 045 (A) Features incorporated in (i) cosmids (ii) shuttle vectors (iii) expression vectors. OR (B) Explain blue-white selection. [3]

Ans. (A) (i) **Cosmids**: **cos sites** of phage lambda plus plasmid features (ori, selectable marker, suitable RE sites). (ii) **Shuttle vectors**: **two origins of replication and two sets of markers**, one functioning in eukaryotic cells and one in E. coli. (iii) **Expression vectors**: signals for **transcription and translation** of the insert to express the foreign protein. [1 × 3]

(B) Based on **insertional inactivation** of **lacZ**, which makes beta-galactosidase that cleaves colourless **X-gal** into a **blue** product. An insert in lacZ stops enzyme expression. Transformed E. coli are plated on **ampicillin + X-gal**: **blue** colonies are transformed but have no insert; **white** colonies carry the recombinant DNA. [3]

CBSE 2026 · 045 (A) Explain (i) transfection (ii) electroporation (iii) biolistics. OR (B) Describe blue-white selection. [3]

Ans. (A) (i) **Transfection**: foreign DNA is mixed with a charged substance (calcium phosphate, cationic liposomes, DEAE-dextran) and overlaid on recipient cells. (ii) **Electroporation**: an electric current creates transient microscopic pores in the host cell membrane through which rDNA enters. (iii) **Biolistics**: a gene gun bombards cells with microscopic **gold or tungsten** particles coated with DNA. [1 × 3]

(B) Insertional inactivation of lacZ (beta-galactosidase) in **pUC19**; plating of transformed E. coli on solid medium with ampicillin and **X-gal**; blue = non-recombinant, white = recombinant. [1 × 3]

CBSE 2025 · 045 Case (Sanger's method). (i) Why are primers required? (ii) Difference between dNTPs and ddNTPs; function of ddNTPs. (iii) Advantage of fluorescent ddNTPs in automated sequencing. OR (iii) Enzyme used. [1+2+1]

Ans. (i) Only primers can be **extended** by DNA polymerase using the single-stranded template as a guide. [1] (ii) dNTPs have a **3'-OH**; ddNTPs **lack the 3'-OH**. [1] ddNTPs **terminate** the growing chain where they are incorporated. [1] (iii) Gels scanned by **laser**; no radioisotope danger; single-lane electrophoresis instead of four (any one). [1] OR **DNA polymerase**. [1]

CBSE 2024 · 045 (A) (i) ddNTPs cause growing chains to terminate prematurely. Explain how. (ii) Write the steps of Sanger's method. OR (B) (i) Why is the autoradiogram read from bottom to top? (ii) Why is single-tube sequencing better and safer? (iii) Which vector for single-stranded cloning? [5]

Ans. (A) (i) ddNTPs **lack the 3'-hydroxyl**, so no phosphodiester bond can form between their 3' end and the 5' phosphate of the next nucleotide; the chain cannot extend and terminates. [2] (ii) Four tubes, each with ssDNA template, dNTPs, primer and DNA polymerase; a small amount of one ddNTP (ddATP, ddTTP, ddGTP, ddCTP) added to each; prematurely terminated strands separated on special gels that resolve one-nucleotide differences; shorter fragments move faster towards the anode; radioactive primers allow autoradiography; the gel is read from bottom to top to get the 5'→3' sequence. [3]

(B) (i) The fastest-moving, shortest fragment is at the **5'** end (towards the anode); since DNA is synthesised 5'→3', reading from the bottom gives the sequence 5'→3'. [2] (ii) **Safer**: fluorescent dyes instead of radioisotopes. **Better**: automated, faster, single-lane gel, results displayed on a computer and stored. [1 + 1] (iii) **M13-based vector**. [1]

CBSE 2025 · 045 (A) Describe the basic steps of rDNA technology. OR (B) Describe RFLP. Why is it used in forensics? [5]

Ans. (A) (1) **Isolation** of the DNA fragment with the gene of interest (insert). (2) **Generation of rDNA** by inserting it into a **vector** (plasmid) that self-replicates in the host. (3) **Transfer** of rDNA into E. coli (transformation). (4) **Selection** of host cells carrying rDNA. (5) **Multiplication** of recombinant cells, multiplying the rDNA. [1 × 5]

(B) (1) Isolate test DNA samples; (2) digest with the **same restriction enzyme**; (3) separate fragments by **agarose gel electrophoresis**; (4) analyse the gel pattern. [1 × 4] Forensic use: band patterns differ between individuals, so it can **identify and relate individuals**. [1]

CBSE 2026 · 045 (A) (i) Describe the steps of PCR. (ii) Starting with three dsDNA molecules, how many after 10 cycles? OR (B) Full form of RFLP; its principle; two applications. [5]

Ans. (A) (i) **Denaturation** at ~94 °C (strands separate); **annealing** at 50–60 °C (primers bind to complementary 3' ends); **extension** at ~72 °C (Taq polymerase adds dNTPs 5'→3'). [1 × 3] (ii) Copies = $N \times 2^n$ [1] = $3 \times 2^{10} = 3 \times 1024 =$ **3072** molecules. [1]

(B) **Restriction Fragment Length Polymorphism**. [1] Principle: **variation in the length** of restriction-enzyme-generated fragments between individuals of a species [1], seen as **different movement** of fragments on electrophoresis. [1] Applications (any two): forensics (identify criminals); relating individuals; paternity testing; DNA fingerprinting. [1 + 1]

Important textbook questions

1. Why are restriction enzymes called molecular scissors? Why do they not cut the bacterium's own DNA?

> **Ans.** They cut DNA at specific recognition sequences, like scissors. The host's own DNA is **methylated** at those sites by a methylase, which blocks cutting (restriction–modification system).

2. Distinguish sticky ends and blunt ends.

> **Ans.** Sticky ends: staggered cuts leave short single-stranded overhangs that pair with complementary ends (EcoRI); efficient ligation. Blunt ends: cut at the same point on both strands, no overhang (SmaI); ligation less efficient.

3. State the features of a good cloning vector.

> **Ans.** An origin of replication; selectable marker(s); unique restriction sites (MCS) preferably within a marker; small size; high copy number; easy isolation.

4. How are bacterial cells made competent?

> **Ans.** By treating with ice-cold $CaCl_2$, which makes the membrane permeable; rDNA is added and a brief heat shock at 42 °C helps DNA enter; then cells are returned to ice.

20 MCQs

1. The first rDNA molecule was made by:
   (A) Sanger (B) Kary Mullis (C) Paul Berg (D) Watson

2. Restriction enzymes recognise:
   (A) palindromic sequences (B) introns (C) promoters only (D) RNA

3. EcoRI recognition site:
   (A) CCCGGG (B) GGATCC (C) AAGCTT (D) GAATTC

4. Host DNA is protected from its own RE by:
   (A) ligation (B) methylation (C) phosphorylation (D) acetylation

5. DNA ligase forms:
   (A) hydrogen bonds (B) phosphodiester bonds (C) peptide bonds (D) glycosidic bonds

6. cDNA is made by:
   (A) Taq polymerase (B) ligase (C) DNase I (D) reverse transcriptase

7. BAC vectors are based on the:
   (A) F plasmid (B) Ti plasmid (C) 2-micron plasmid (D) lambda

8. Cosmids contain:
   (A) T-DNA (B) yeast centromere (C) cos sites of lambda (D) no ori

9. Shuttle vectors have:
   (A) only a promoter (B) no marker (C) no ori (D) two origins of replication

10. Competent cells are made with:
    (A) NaCl (B) $CaCl_2$ (C) EDTA (D) SDS

11. Gene gun uses particles of:
    (A) silver only (B) iron (C) gold or tungsten (D) glass

12. Electroporation uses:
    (A) electric pulses (B) heat (C) viruses (D) liposomes

13. In blue-white selection, recombinant colonies are:
    (A) blue (B) white (C) green (D) red

14. X-gal is cleaved by:
    (A) ligase (B) beta-lactamase (C) beta-galactosidase (D) kinase

15. PCR extension temperature:
    (A) about 4 °C (B) about 94 °C (C) about 37 °C (D) about 72 °C

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16. Taq polymerase comes from:
(A) Thermus aquaticus (B) E. coli (C) T4 phage (D) yeast

17. Copies after 5 cycles from 1 molecule:
(A) 32 (B) 10 (C) 25 (D) 64

18. ddNTPs lack:
(A) sugar (B) 5′-phosphate (C) base (D) 3′-OH

19. The sequencing gel is read:
(A) top to bottom (B) bottom to top (C) left to right (D) any way

20. RFLP is used for:
(A) PCR (B) making insulin (C) forensic identification (D) cloning only

1 (C) · 2 (A) · 3 (D) · 4 (B) · 5 (B) · 6 (D) · 7 (A) · 8 (C) · 9 (D) · 10 (B) · 11 (C) · 12 (A) · 13 (B) · 14 (C) · 15 (D) · 16 (A) · 17 (A) · 18 (D) · 19 (B) · 20 (C)

Assertion–Reason (6)

(A) Both true, R explains A. (B) Both true, R does not explain A. (C) A true, R false. (D) A false, R true.

1. A: ddNTPs terminate DNA synthesis. R: ddNTPs lack the 3′-OH group.
2. A: White colonies on X-gal plates carry recombinant plasmids. R: An insert in lacZ inactivates beta-galactosidase.
3. A: Restriction enzymes cut the host bacterium's own DNA. R: Restriction enzymes recognise palindromic sequences.
4. A: PCR uses ordinary E. coli DNA polymerase. R: PCR cycles go up to about 94 °C.
5. A: M13 is used for Sanger sequencing. R: M13 gives single-stranded DNA.
6. A: Taq polymerase is heat-stable. R: EcoRI produces sticky ends.

Key: 1 (A) · 2 (A) · 3 (D) · 4 (D) · 5 (A) · 6 (B)

Practice *Exam-style*

1. Why is alkaline phosphatase used on vectors? (2)
2. How many DNA molecules from 2 molecules after 6 PCR cycles? (1)
3. Name the enzyme and the vector used in the first gene therapy trial. (2)

Answers: 1. removes 5′ phosphates so the cut vector cannot re-ligate on itself; only inserts with phosphates can join 2. $2 \times 2^6 = 128$ 3. ADA (adenosine deaminase) gene delivered by a retroviral vector into lymphocytes (SCID, 1990)

Summary

- Steps: isolate insert, ligate into vector, transform, select, multiply.
- Enzymes: RE (palindromes, sticky/blunt; defence vs phage; methylation), T4 ligase, Taq, reverse transcriptase, alkaline phosphatase.
- Vectors: pBR322, pUC, lambda, M13 (ssDNA, F pili, rolling circle), cosmid (cos), BAC (F plasmid), YAC, shuttle (2 ori), expression (signals).
- Transfer: $CaCl_2$ + heat shock, transfection, electroporation, biolistics, microinjection.
- Selection: antibiotic resistance, beta-gal (blue-white), GFP, Leu2.
- PCR: 94/55/72 °C; $N \times 2^n$. Sanger: ddNTP, 4 tubes, read bottom to top; automated: dyes, laser. RFLP: forensics, paternity.