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Step-by-step coloured PDF solutions for the 2026-27 NCERT (Latest Edition), Class 12th Biology

Chapter 9: Biotechnology: Principles and Processes

About this Chapter

Chapter 9 of NCERT Class 12 Biology develops the two pillars of modern biotechnology: **genetic engineering** (the deliberate manipulation of DNA using **restriction endonucleases**, **DNA ligase** and **vectors**) and **bioprocess engineering** (large-scale culturing of cells in **bioreactors** for the maintenance of sterile conditions). By the end of the chapter you will know how a foreign gene is cut, ligated into a plasmid, transferred to a host cell, amplified by **Polymerase Chain Reaction (PCR)**, expressed as a recombinant protein, and finally purified through **downstream processing**.

Topics covered: Recombinant DNA technology • Restriction enzymes & palindromes • Vectors & selectable markers • PCR • Bioreactors • Downstream processing

Quick Formula Sheet

Recombinant DNA equation:
Foreign DNA + Vector DNA $\xrightarrow{\text{Restriction enzyme + Ligase}}$ rDNA

EcoRI recognition site (palindrome):
5′-GAATTC-3′ / 3′-CTTAAG-5′

PCR amplification:
$N_{\text{final}} \approx N_0 \cdot 2^n$ where n = number of cycles
(30 cycles $\Rightarrow \sim 10^9$ copies)

Three steps of every PCR cycle:
Denaturation (94 °C) → Annealing ($\sim$ 55 °C) → Extension (72 °C, *Taq*)

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

NCERT Solutions — Class 12 Biology Chapter 9

Q 9.1 **Can you list 10 recombinant proteins which are used in medical practice? Find out where they are used as therapeutics (use the internet).**

SOLUTION

Concept used. A **recombinant protein** is any protein produced by expressing a cloned gene in a heterologous host (typically *E. coli*, yeast, or mammalian cell lines). Because the gene is inserted into a host through **recombinant DNA technology**, the protein it codes for is identical to (or a deliberately modified version of) the human/animal protein, but can be manufactured in industrial quantities at controlled purity. Medical recombinant proteins replace a missing endogenous protein, neutralise a pathogen, or act as a biological signal.

☞ **Why recombinant, not extracted?**

Extracting insulin from cattle/pig pancreas works, but the animal protein differs in 1–3 amino acids and triggers immune reactions in $\sim 5\%$ of patients. Cloning the human insulin gene in *E. coli* gives exactly the human sequence with zero animal contamination.

Step 1. **Ten recombinant therapeutic proteins** commonly named in NCERT-aligned references:

1. **Human Insulin (Humulin)** — treats type-1 and advanced type-2 *diabetes mellitus*.
2. **Human Growth Hormone (Somatotropin / hGH)** — treats pituitary dwarfism and Turner syndrome.
3. **Erythropoietin (EPO)** — treats anaemia in chronic kidney disease and chemotherapy patients.
4. **Interferon** α, β, γ — antiviral therapy in chronic hepatitis B/C, and disease-modifying therapy in multiple sclerosis.
5. **Tissue Plasminogen Activator (tPA / Alteplase)** — clot-buster used within hours of an acute ischaemic stroke or myocardial infarction.
6. **Factor VIII** — corrects clotting deficiency in haemophilia A.
7. **Factor IX** — corrects clotting deficiency in haemophilia B (Christmas disease).
8. **Hepatitis B surface antigen (HBsAg) vaccine** — prevents hepatitis B infection; the first widely used recombinant vaccine.
9. **Granulocyte Colony Stimulating Factor (G-CSF / Filgrastim)** — boosts neutrophil counts after chemotherapy.
10. **Monoclonal antibodies** such as **Trastuzumab** (HER2-positive breast cancer) and **Rituximab** (non-Hodgkin lymphoma, rheumatoid arthritis).

Step 2. **Why this list matters.** Each protein here was first purified in milligram amounts from human/animal sources at prohibitive cost. Recombinant production in microbial or mammalian bioreactors dropped the cost per dose by 10–100$\times$ and removed the risk of carrying through donor-derived viruses (HIV, hepatitis) that haunted plasma-derived clotting factors in the 1980s.

> **Final Answer:** Ten recombinant therapeutic proteins: insulin, hGH, EPO, interferons, tPA, Factor VIII, Factor IX, HBsAg vaccine, G-CSF, monoclonal antibodies (e.g. Trastuzumab, Rituximab).

♥ Where biotechnology meets the clinic

The global market for recombinant therapeutics crossed US$ 300 billion in 2024. Behind every dose are the same three laboratory steps you learn in this chapter: cut the gene with a restriction enzyme, ligate it into a plasmid, scale up the host in a bioreactor.

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

Strategic angle (clinical-pharmacology framing). Rather than listing proteins at random, group them by the deficiency they correct or the pathway they target. This anchors each name to a clinical endpoint that examiners reward.

Step 1. **Replacement therapies** (give back a missing protein): Insulin (diabetes), hGH (dwarfism), EPO (anaemia in renal failure), Factor VIII (haemophilia A), Factor IX (haemophilia B). Each gene is cloned and expressed because the natural source is either insufficient or unsafe.

Step 2. **Signalling/immune-modulator therapies**: Interferons (α, β, γ) for chronic viral infections and multiple sclerosis; G-CSF (Filgrastim) to rescue bone marrow after chemotherapy.

Step 3. **Acute thrombolytic**: Tissue plasminogen activator (Alteplase) dissolves fibrin clots in stroke and myocardial infarction. Cloned from human cDNA and expressed in CHO cells (Chinese-Hamster-Ovary mammalian line) because the glycosylation pattern matters for activity.

Step 4. **Vaccines and antibodies**: HBsAg subunit vaccine (*Saccharomyces cerevisiae* host) was the first recombinant vaccine; monoclonals such as Trastuzumab (anti-HER2) and Rituximab (anti-CD20) target tumour-cell surface markers.

Step 5. **Why the host matters.** Bacteria are cheap but cannot glycosylate; yeast can; mammalian cells (CHO, HEK293) give human-like post-translational modifications and are chosen whenever the protein is folded, glycosylated or disulphide-rich (antibodies, EPO, Factor VIII).

Why this matters. The category-wise grouping mirrors how pharmacology textbooks classify these molecules and the host-choice logic is a frequent NEET-PG / GATE Biotechnology question.

Final Answer: Recombinant therapeutics span replacement proteins (insulin, hGH, EPO, clotting factors), immune modulators (interferons, G-CSF), thrombolytics (tPA), vaccines (HBsAg) and monoclonal antibodies (Trastuzumab, Rituximab).

Q 9.2 **Make a chart (with diagrammatic representation) showing a restriction enzyme, the substrate DNA on which it acts, the site at which it cuts DNA and the product it produces.**

SOLUTION

Concept used. A **restriction endonuclease** is a bacterial enzyme that scans double-stranded DNA, recognises a specific short *palindromic* sequence (usually 4–8 base pairs), and cleaves the sugar–phosphate backbone of *both* strands inside or adjacent to that recognition site. The most studied member is **EcoRI** from *Escherichia coli*, which recognises the hexamer $5'$-GAATTC-$3'$ and cuts between G and A on each strand, leaving 4-nucleotide single-stranded overhangs called **sticky ends**. Two DNA fragments cut by the same enzyme have complementary sticky ends and can therefore be joined by DNA ligase.

Step 1. **Substrate.** Any double-stranded DNA molecule containing the hexanucleotide recognition site. Example below: a plasmid (vector) and a foreign DNA fragment, both containing one $5'$-GAATTC-$3'$ site.

Step 2. **Recognition site (palindrome).** Reading $5' \rightarrow 3'$ on either strand gives the *same* sequence:

$$5'\,\mathrm{G\,A\,A\,T\,T\,C}\,3'$$
$$3'\,\mathrm{C\,T\,T\,A\,A\,G}\,5'$$

Step 3. **Site of cleavage.** EcoRI cuts the phosphodiester bond *between G and A* on each strand, indicated by the arrows below:

$$5'\,\mathrm{G}\downarrow\mathrm{A\,A\,T\,T\,C}\,3'$$
$$3'\,\mathrm{C\,T\,T\,A\,A}\uparrow\mathrm{G}\,5'$$

Because the cuts are staggered (not directly opposite each other), each fragment carries a four-base single-stranded $5'$-overhang.

Step 4. **Products.** Two DNA fragments with complementary $5'$-AATT-$3'$ sticky ends:

$$5'\,\mathrm{G} \qquad \mathrm{AATT\ C}\,3' \qquad\qquad 3'\,\mathrm{CTTAA} \qquad \mathrm{G}\,5'$$

Mixing vector-cut DNA with foreign-DNA-cut fragments allows their AATT overhangs to base-pair; **DNA ligase** then seals the nicks to form a single **recombinant DNA** molecule.

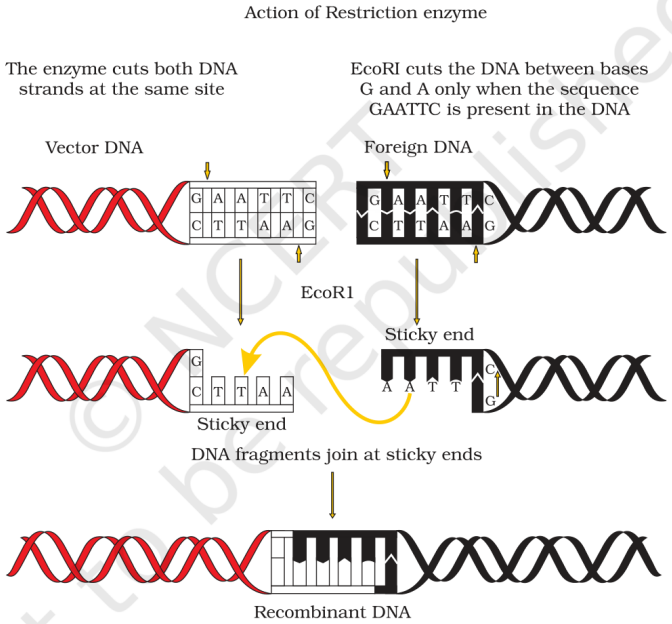
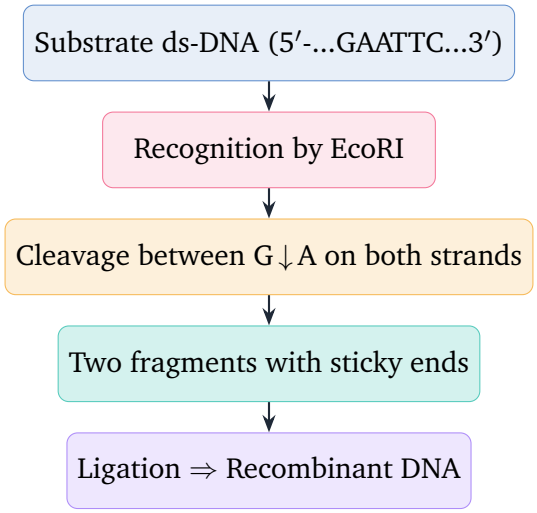


Figure 9.1 Steps in formation of recombinant DNA by action of restriction endonuclease enzyme - EcoRI

Fig. 9.1, NCERT Class 12 Biology, Chapter 9. Action of restriction enzyme EcoRI *producing sticky ends and the resulting recombinant DNA.*



Final Answer: EcoRI recognises 5′-GAATTC-3′, cuts between G and A on both strands, and produces DNA fragments with 4-base 5′-AATT sticky ends that ligate to give recombinant DNA.

✗ Common Mistake

Restriction enzymes do *not* cut at random. They demand an exact palindromic recognition site; one base mismatch and the enzyme will not cleave. This sequence-specificity is what makes them *molecular scissors* rather than blunt scissors.

EXPERT'S SOLUTION *: Priya Reddy, M.Sc Biotechnology, AIIMS Delhi*

Strategic angle (chart-format framing). Examiners love a clean two-row table: enzyme name + source on the left, substrate / cut-site / product on the right. Present the four required pieces of information explicitly.

Step 1. **Enzyme + source.** *EcoRI*, isolated from *Escherichia coli* strain RY13. Naming convention: first letter of genus (E), first two of species (co), strain (R), order of discovery (I).

Step 2. **Substrate.** Double-stranded DNA bearing the palindromic recognition hexamer GAATTC; could be a viral genome, plasmid or any genomic DNA.

Step 3. **Cut site.** Between the 5′-G and the next A on each strand. Because the cuts on the two strands are offset by four bases, the enzyme makes a *staggered cut* rather than a flush cut.

Step 4. **Product.** Two DNA fragments, each ending in a 5′-AATT single-stranded overhang. These complementary overhangs are *sticky ends* — they base-pair spontaneously with any other fragment cut by EcoRI, allowing recombination across species barriers.

Step 5. **Why sticky ends matter.** They are the reason recombinant DNA technology works: a human gene cut with EcoRI and a bacterial plasmid cut with EcoRI will join up because both carry the same AATT overhang.

Why this matters. The chart should always have four columns: *Enzyme* | *Source* | *Recognition + cut site* | *Product*. Examiners reward the source organism and the staggered-cut detail.

> **Final Answer:** Chart: EcoRI (from *E. coli*) recognises 5′-G↓AATTC-3′ on ds-DNA and produces two fragments with complementary 5′-AATT sticky ends.

Q 9.3 **From what you have learnt, can you tell whether enzymes are bigger or DNA is bigger in molecular size? How did you know?**

SOLUTION

Concept used. *Molecular size* can be compared two ways: by **molecular weight** (number of atoms / mass in daltons) or by **physical length** of the molecule. For biological macromolecules a genome's DNA is typically thousands to billions of base-pairs long, while a single enzyme is a folded polypeptide of a few hundred amino acids. Gel-electrophoresis behaviour (Section 9.3.1 of the chapter) is the direct evidence: in an agarose gel the DNA migrates more slowly and gets caught higher up, while a protein of comparable nominal mass slips through.

☞ **Reference numbers**

The *E. coli* genome is $\sim 4.6 \times 10^6$ base pairs $\Rightarrow$ molecular weight $\sim 3 \times 10^9$ Da. A typical restriction-enzyme polypeptide is $\sim$ 300 amino acids $\Rightarrow$ molecular weight $\sim 3 \times 10^4$ Da ($\sim$ 30 kDa). Therefore the genomic DNA is roughly 10^5 times heavier than the enzyme that cuts it.

Step 1. **Compare on the same gel.** In agarose-gel electrophoresis, DNA fragments and proteins behave very differently. DNA, being uniformly negatively charged and much larger, migrates only short distances and is held back in the gel matrix; proteins of a few tens of kDa run far toward the electrode. The direct visual evidence: DNA stays near the well, the protein runs away.

Step 2. **Count the monomers.** A bacterial chromosome is $\sim 10^6$–10^7 nucleotide pairs long. Even the largest enzymes are $\leq 10^3$ amino acids long. By monomer count alone, DNA wins by 3–4 orders of magnitude.

Step 3. **Translate to mass.** Average nucleotide pair ≈ 650 Da; average amino acid ≈ 110 Da. So 1 million bp of DNA $\approx 6.5 \times 10^8$ Da, whereas a 300-aa enzyme $\approx 3.3 \times 10^4$ Da. The DNA is $\sim 2 \times 10^4$ times heavier than the enzyme.

Step 4. **Visual evidence in the chapter.** Section 9.3 figures show one tiny restriction enzyme nibbling at a much longer DNA strand. The enzyme docks onto a 6-bp recognition site that occupies a vanishing fraction of the substrate molecule.

Final Answer: DNA is far bigger than enzymes — by mass (10^4–10^5 times) and by physical length (kilobases vs nanometre-scale folded proteins). The proof: on agarose-gel electrophoresis the DNA stays near the well while a $\sim$ 30 kDa enzyme migrates far down.

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

Quick-reading angle. The question is testing whether you remember the gel-electrophoresis intuition and the order-of-magnitude comparison. Frame the answer as: *evidence + numbers + visual*.

Step 1. **Evidence from gel electrophoresis.** On a 1% agarose gel run with the same voltage and the same dye, DNA bands appear near the wells (large molecules can barely thread through the pore network) while protein bands run off the bottom unless the gel is much denser (which is why proteins are usually resolved on polyacrylamide, not agarose).

Step 2. **Numerical comparison.** Molecular weight of one nucleotide pair ≈ 650 Da; molecular weight of one amino-acid residue ≈ 110 Da. The shortest functional DNA (a gene of ~ 1000 bp) already weighs 6.5×10^5 Da while an enzyme of

~ 300 residues weighs only 3.3×10^4 Da. DNA wins by $\sim 20\times$ for a single gene and by millions of times for a chromosome.

Step 3. **Physical-length comparison.** 1 bp of B-form DNA $= 0.34$ nm, so a 1-kb gene stretches ~ 340 nm. A folded globular enzyme of 300 aa is ~ 5–8 nm across. Even unfolded as a polypeptide chain, 300×0.38 nm ≈ 114 nm, still well under a single kilobase.

Step 4. **Visualisation.** Picture the restriction enzyme as a small *molecular pacman* (~ 30 kDa) docking onto a substrate DNA strand that is thousands of base-pairs long — a tiny machine reading along a very long tape.

Why this matters. The size disparity is the reason a single enzyme can cleave a whole genome at every occurrence of its 6-bp site, generating thousands of fragments from one continuous piece of DNA.

> **Final Answer:** DNA is much bigger than enzymes; the evidence is the slow agarose-gel migration of DNA versus the fast migration of any typical 30-kDa protein.

Q 9.4 What would be the molar concentration of human DNA in a human cell? Consult your teacher.

SOLUTION

Concept used. Molar concentration (molarity, M) is defined as the number of moles of a substance per litre of solution:

$$M = \frac{n}{V} = \frac{N}{N_A \cdot V},$$

where N is the number of molecules of the substance, $N_A \approx 6.022 \times 10^{23}\,\text{mol}^{-1}$ is Avogadro's number, and V is the volume of solution in litres. A diploid human somatic cell contains *two copies* of the haploid genome (one set of 23 chromosomes from each parent), so $N = 2$ molecules of nuclear DNA per cell. The remaining unknown is the cell volume, which for a typical mammalian cell is $\sim 1000\,\mu\text{m}^3 = 1000 \times 10^{-15}$ L $= 10^{-12}$ L.

> ☞ **A Fermi-style estimation**
> We are estimating an extremely small concentration. Order-of-magnitude errors in cell volume are fine; the key insight is that with only 2 copies of the genome in a picolitre, the molarity has to be in the femto-molar range.

Step 1. **Count the DNA molecules per cell.** A diploid human cell contains 46 chromosomes $= 2$ complete genomes (one maternal, one paternal). Treating each genome as one DNA molecule (it is actually 23 linear molecules, but the

question asks for "human DNA" as a whole):

$$N = 2 \text{ copies.}$$

Step 2. **Convert to moles.** Number of moles of DNA per cell:

$$n = \frac{N}{N_A} = \frac{2}{6.022 \times 10^{23}} = 3.32 \times 10^{-24}\,\text{mol.}$$

Step 3. **Estimate cell volume.** A typical mammalian cell has a diameter ~ 10–$20\,\mu$m. Take $V \approx 1000\,\mu\text{m}^3$:

$$V = 1000 \times (10^{-6}\,\text{m})^3 = 10^{-15}\,\text{m}^3 = 10^{-12}\,\text{L.}$$

(Recall $1\,\text{m}^3 = 1000\,\text{L.}$)

Step 4. **Apply the molarity definition.**

$$M = \frac{n}{V} = \frac{3.32 \times 10^{-24}\,\text{mol}}{10^{-12}\,\text{L}} = 3.32 \times 10^{-12}\,\text{mol/L.}$$

In SI prefixes, $M \approx 3.3\,\text{pmol/L}$, i.e. ~ 3 picomolar.

Final Answer: Molar concentration of human DNA in a single diploid cell $\approx 3.32 \times 10^{-12}\,\text{M}$ (~ 3 picomolar).

☞ NEET / CUET angle

The exam rewards the method, not the exact number. Always show N/N_A for moles and V for litres; the final answer will land in the picomolar range no matter which reasonable cell-volume estimate you take.

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

Strategic angle (Fermi-estimation framing). This is a *back-of-envelope* question. The answer hangs on three pieces of information: number of DNA molecules per cell, Avogadro's number, and a sensible cell volume. Show each clearly.

Step 1. **Number of DNA molecules.** A diploid human somatic cell carries two complete genomes:

$$N = 2 \text{ molecules of genomic DNA.}$$

(One could refine this to 46 chromosomes; the molarity scales linearly and only changes the prefactor.)

Step 2. **Convert to moles using Avogadro's number.**

$$n = \frac{N}{N_A} = \frac{2}{6.022 \times 10^{23}\,\text{mol}^{-1}}.$$

Numerator $= 2$; denominator $= 6.022 \times 10^{23}$; ratio $= 0.332 \times 10^{-23}\,\text{mol} = 3.32 \times 10^{-24}\,\text{mol}$.

Step 3. **Estimate cell volume.** Typical somatic-cell diameter $d \approx 10$–$15\,\mu$m. Treating the cell as a sphere of $d = 12\,\mu$m:

$$V = \frac{4}{3}\pi\left(\frac{d}{2}\right)^3 = \frac{4}{3}\pi(6\,\mu\text{m})^3 \approx 9 \times 10^2\,\mu\text{m}^3 \approx 10^{-12}\,\text{L}.$$

Step 4. **Compute molarity.**

$$M = \frac{n}{V} = \frac{3.32 \times 10^{-24}\,\text{mol}}{10^{-12}\,\text{L}} = 3.32 \times 10^{-12}\,\text{M}.$$

Step 5. **Sanity check.** The answer is far below the molarity of typical buffer salts (10^{-3} M) or even rare metabolites (10^{-9} M), which makes sense — there are only two copies of the genome in a tiny picolitre volume. The relevant *number* of molecules, not concentration, is what matters in molecular biology.

Why this matters. This exercise shows why analytical techniques (PCR, qPCR) had to be invented: with only $\sim 10^{-12}$ M DNA per cell, you cannot detect a target gene by classical chemistry. Amplification is mandatory.

Final Answer: $M_{\text{human DNA per cell}} \approx 3.3 \times 10^{-12}\,\text{M}$ (~ 3 picomolar).

Q 9.5 Do eukaryotic cells have restriction endonucleases? Justify your answer.

SOLUTION

Concept used. A **restriction endonuclease** is a sequence-specific enzyme that cleaves *foreign* double-stranded DNA at a palindromic recognition site, while the host's *own* DNA is protected by methylation at the same sites. This pair of activities (*restriction* + *modification*) constitutes a prokaryotic defence system against invading viruses (bacteriophages): phage DNA is unmethylated, the host enzymes shred it; the host's own chromosomal DNA carries methyl groups on key bases (typically the adenine of GATC or the cytosine of CCGG) and therefore escapes cleavage. Eukaryotic cells use a fundamentally different anti-viral defence (RNA interference, type-I interferon signalling, restriction factors of the APOBEC/TRIM family) and do *not* encode the classical sequence-specific restriction enzymes used in genetic engineering.

Step 1. **Direct answer. No.** Eukaryotic cells do *not* possess restriction endonucleases of the type used in recombinant DNA technology (Types I, II, III).

Step 2. **Justification — why prokaryotes have them.** Restriction-modification systems evolved as a microbial *immune system* against bacteriophages. Bacteria carry a methylase and a restriction enzyme that share the same recognition site: the methylase tags host DNA so it is ignored; the restriction enzyme cleaves any unmethylated invading phage DNA into fragments.

Step 3. **Justification — why eukaryotes do not need them.** Eukaryotic cells face viruses but defend through different mechanisms: **RNA interference** (siRNA / miRNA pathway targeting viral mRNA), interferon-induced antiviral state, cell-autonomous restriction factors (APOBEC3, SAMHD1, TRIM5), and the adaptive immune system in metazoans. Carrying promiscuous DNA-cutting enzymes in the cytoplasm would risk fragmenting their own enormous nuclear genome.

Step 4. **Caveat — eukaryotic nucleases that cut DNA.** Eukaryotes do encode other nucleases: *endonuclease G* (apoptotic DNA fragmentation), *DNase I/II* (extracellular digestion), *Cas9* (in archaea; introduced into eukaryotes for genome editing). These are *not* sequence-specific restriction enzymes — they cut after a defined biological trigger (apoptosis, RNA guidance), not at a palindrome.

> **Final Answer:** No. Eukaryotic cells lack the bacterial restriction-modification systems used in cloning. The enzyme evolved as a phage-defence tool in prokaryotes; eukaryotes use RNA interference and the immune system instead.

♥ Evolutionary lens

The fact that only prokaryotes carry restriction enzymes is the single reason genetic engineering became possible. Hamilton Smith, Werner Arber and Daniel Nathans shared the 1978 Nobel Prize in Medicine for purifying them from bacteria — a tool that nature gave us because of the bacterium–phage arms race.

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

Structural-defence angle. Treat this as a comparative immunology question: *which lineage uses which weapon against viruses?*

Step 1. **Bacteria**: restriction–modification systems. A methylase protects self DNA; an endonuclease cuts unmethylated invading DNA. The two activities share the same recognition site (e.g. GATC).

Step 2. **Archaea and bacteria also have CRISPR–Cas**: a sequence-programmable adaptive immune system. Cas9 is the eukaryote-friendly cousin that we now exploit for genome editing.

Step 3. **Eukaryotes**: do not encode classical restriction endonucleases. Their genome is huge (3×10^9 bp in humans) — releasing a promiscuous palindrome-cutter would shred it. Instead, they encode:

- **RNAi machinery** (Dicer, Argonaute) targeting viral RNA.
- **Interferon-stimulated genes** (PKR, OAS, IFIT) inducing an antiviral state.
- **Restriction factors** (APOBEC3G, SAMHD1, TRIM5α) blocking specific viral life-cycle steps.
- **Adaptive immunity** in vertebrates (T cells, B cells, antibodies).

Step 4. **Why the difference?** Genome size and architecture. A 4-Mb bacterial chromosome can be protected by methylating a few thousand recognition sites; a 3-Gb mammalian genome cannot tolerate the metabolic burden of methylating millions of sites just to keep a self-destruct enzyme in check.

Why this matters. The answer also explains why bacterial hosts (*E. coli*) must be re-engineered to be *restriction-minus* (e.g. DH5α) before they can accept foreign DNA: otherwise the host's own restriction enzymes would shred the incoming plasmid.

> **Final Answer:** Eukaryotic cells do not have restriction endonucleases because they use RNA-interference, interferon signalling and adaptive immunity instead of restriction–modification systems.

Q 9.6 Besides better aeration and mixing properties, what other advantages do stirred tank bioreactors have over shake flasks?

SOLUTION

Concept used. A **shake flask** is a small (50 mL to 2 L) Erlenmeyer-flask culture agitated on an orbital shaker; it supports research-scale growth but offers no real-time monitoring or control. A **stirred-tank bioreactor** is an industrial-grade vessel (100–1000 L, sometimes much larger) equipped with an internal stirrer, an aeration sparger, and an integrated control system that monitors and adjusts pH, temperature, dissolved oxygen, foam, and substrate feed in real time. Beyond the obvious aeration/mixing advantage stated in the question, the control architecture is what makes bioreactors the only viable platform for industrial-scale recombinant-protein production.

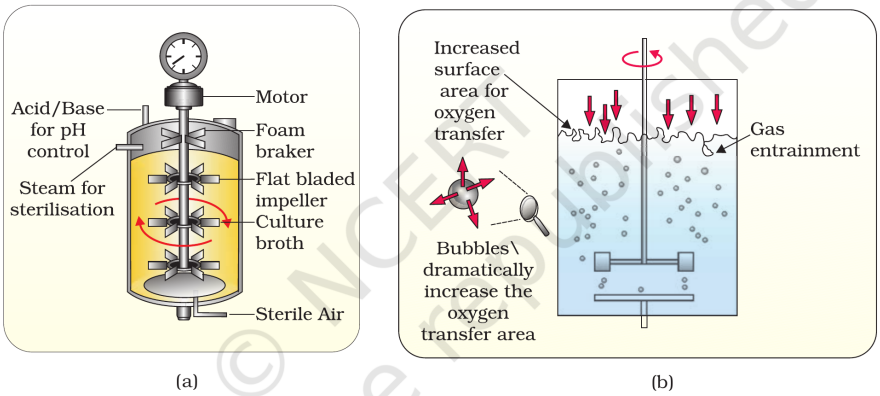


Figure 9.7 (a) Simple stirred-tank bioreactor; (b) Sparged stirred-tank bioreactor through which sterile air bubbles are sparged

Fig. 9.7, NCERT Class 12 Biology, Chapter 9. (a) Simple stirred-tank bioreactor showing motor, foam breaker, flat-bladed impellers, pH control, and sterile-air inlet. (b) Sparged stirred-tank bioreactor in which sterile-air bubbles dramatically increase the oxygen-transfer area.

Step 1. **Online process monitoring and control.** The bioreactor carries probes for temperature, pH, dissolved oxygen, and (often) glucose and cell-density. Each probe feeds a controller that adjusts heater, base/acid addition, sparger flow rate, and feed pumps in real time. A shake flask offers none of this — you sample manually and adjust offline.

Step 2. **Foam control.** Vigorous aeration in protein-rich media creates foam that traps cells and clogs sparger filters. A bioreactor has a mechanical *foam breaker* and/or an antifoam-addition system; a shake flask just overflows.

Step 3. **Sterile environment maintained at scale.** The bioreactor body is built for in-situ steam sterilisation (the "Steam for sterilisation" inlet in Fig. 9.7 a) and carries a sterile-air filter on the sparger. Shake flasks rely on a cotton plug and quickly contaminate above 5 L.

Step 4. **Sampling ports for at-line analytics.** Aseptic sampling ports let the operator pull aliquots without opening the vessel. This is essential for tracking the **log/exponential phase** so that downstream processing is triggered at peak yield.

Step 5. **Scalable working volume (100–1000 L+).** Shake flasks plateau at ~ 5 L; a single bioreactor processes 100–10 000 L per run, giving the biomass needed for commercial recombinant-protein quantities. Production bioreactors for monoclonal antibodies routinely reach $20\,000$ L.

Step 6. **Controlled substrate feeding (fed-batch / continuous).** Bioreactors support *fed-batch* mode (slow addition of glucose to prevent overflow metabolism) and *continuous* mode (medium added and harvested simultaneously to keep cells in exponential phase indefinitely). Shake flasks are strictly batch — you set up

once and harvest at the end.

Step 7. **Reproducibility for GMP compliance.** The digital-control loop produces a documented batch record (cell density vs time, pH vs time, etc.) that meets Good Manufacturing Practice requirements for therapeutic products. Regulators will not accept shake-flask product for clinical use.

> **Final Answer:** Stirred-tank bioreactors offer online pH/temperature/DO monitoring and control, foam control, sterile in-situ steam sterilisation, aseptic sampling ports, scalability to thousands of litres, fed-batch and continuous feeding, and GMP-grade reproducibility.

☞ NEET / CUET cue

When a 3-mark question asks for "advantages of bioreactors over shake flasks", the expected answer is a 5–7 bullet list. Mention *at least* pH control, temperature control, foam control, sterility, sampling ports and scale-up volume.

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

Process-engineering angle. Frame the advantages around the *four classical bioprocess parameters*: pH, temperature, oxygen and substrate. A shake flask gives you no real-time handle on any of them; a bioreactor gives you all four.

Step 1. **pH control.** Acid/base reservoirs feed the vessel via peristaltic pumps as the inline pH probe drifts. Microbial fermentations release organic acids — without active control the pH crashes from 7 to 5 in hours and cells die.

Step 2. **Temperature control.** Jacketed walls circulate hot/cold water; the heater/cooler is locked to a thermistor. Recombinant-protein expression in *E. coli* often uses a deliberate temperature shift ($37\,^{\circ}\text{C} \rightarrow 25\,^{\circ}\text{C}$) to improve soluble-protein folding — impossible in a shake flask.

Step 3. **Dissolved oxygen.** A polarographic DO probe drives sparger flow and stirrer speed to maintain $\geq 30\%$ saturation. Shake flasks cannot deliver oxygen beyond surface diffusion, capping cell density at $\sim \text{OD}_{600} = 3$; bioreactors easily reach $\text{OD}_{600} > 100$.

Step 4. **Substrate feeding.** Fed-batch glucose addition prevents acetate overflow in *E. coli*; constant glucose in a shake flask leads to acetate poisoning at $\text{OD}_{600} > 5$. Bioreactors can also operate in **continuous mode**, where used medium is drained out of one side while fresh medium is added from the other, keeping cells in the exponential phase and producing far more biomass.

Step 5. **Aseptic operation at scale.** In-situ steam sterilisation, sterile-air filters, and

aseptic sampling ports keep contamination at $< 1\%$ of batches; shake-flask contamination above 10 L is endemic.

Step 6. **Compliance and traceability.** Every controlled parameter is logged digitally, generating a batch record regulators (CDSCO, USFDA) require for any therapeutic-grade biologic.

Why this matters. The shake-flask-to-bioreactor transition is the same conceptual leap as moving from a kitchen pot to a chemical plant: only the bioreactor lets you control *every* variable a microbe cares about, and produce protein at clinical scale.

> **Final Answer:** Stirred-tank bioreactors add online process control (pH, temperature, DO, substrate feed), foam handling, scalability to thousands of litres, fed-batch / continuous operation, in-situ sterilisation, aseptic sampling and GMP-compliant batch records.

Q 9.7 **Collect 5 examples of palindromic DNA sequences by consulting your teacher. Better try to create a palindromic sequence by following base-pair rules.**

SOLUTION

Concept used. A **palindromic DNA sequence** is a double-stranded sequence that reads the *same* $5' \to 3'$ on *both* strands. Because of Watson–Crick base-pairing rules (A pairs with T, G pairs with C), this requires the sequence on one strand to be the **reverse complement** of itself. Palindromic sites are the recognition sites for nearly all Type-II restriction enzymes used in cloning.

> ☞ **Reverse-complement rule**
> To check whether $5'$-$X_1X_2 \ldots X_n$-$3'$ is palindromic: write the complement strand below it, reverse-read it $5' \to 3'$, and compare. If the two strings match, it is a palindrome.

Step 1. **Five classical palindromic restriction sites.**

Enzyme	Source organism	Recognition site ($5' \to 3'$)
EcoRI	*Escherichia coli* RY13	G A A T T C
BamHI	*Bacillus amyloliquefaciens* H	G G A T C C
HindIII	*Haemophilus influenzae* Rd	A A G C T T
PstI	*Providencia stuartii*	C T G C A G
SalI	*Streptomyces albus* G	G T C G A C

Each site is six base pairs long and reads identically in the $5' \to 3'$ direction on both strands.

Step 2. **Demonstrate the palindrome property for EcoRI.**

$$\begin{aligned} &5'\,\mathrm{G\,A\,A\,T\,T\,C}\,3' \quad \text{(forward strand)} \\ &3'\,\mathrm{C\,T\,T\,A\,A\,G}\,5' \quad \text{(complement, written } 3' \to 5'\text{)} \end{aligned}$$

Now read the lower strand in the $5' \to 3'$ direction (i.e. right to left): G-A-A-T-T-C. Identical to the top strand. Therefore $5'$-GAATTC-$3'$ is a true palindrome.

Step 3. **Construct a new palindrome.** Pick the first half freely, then write its reverse complement as the second half. Example construction:

- Choose first three bases on the top strand: $5'$-**ATC**...-$3'$.
- Reverse complement of ATC is GAT (complement of A,T,C is T,A,G; reverse it to get G,A,T).
- Append GAT to the top strand: $5'$-**ATCGAT**-$3'$.

Verify: complement is $3'$-TAGCTA-$5'$. Read in $5' \to 3'$: ATCGAT. Match. So $5'$-**ATCGAT**-$3'$ is a 6-bp palindromic sequence (this is, in fact, the recognition site of *ClaI*).

Final Answer: Five palindromic recognition sites: EcoRI (GAATTC), BamHI (GGATCC), HindIII (AAGCTT), PstI (CTGCAG), SalI (GTCGAC). A custom palindrome built by base-pair rules: ATCGAT (= ClaI site).

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

Construction-recipe angle. The trick to creating a palindrome is to think of it as "write the first half, then mirror its reverse complement to the second half".

Step 1. **Standard examples to memorise.** EcoRI – GAATTC, BamHI – GGATCC, HindIII – AAGCTT, PstI – CTGCAG, SalI – GTCGAC. Add NotI (GCGGCCGC, an 8-cutter) for variety in board exams.

Step 2. **Recipe to build your own palindrome (even-length).**

- Decide length $2n$ (typical $n = 3$ for a 6-bp site).
- Write any sequence of n bases as the first half: say $\mathrm{B_1B_2B_3}$ = T G C.
- Reverse: C G T. Complement each base: G C A. That is the second half.
- Concatenate: TGCGCA. Verify by writing the complement strand and reading $5' \to 3'$ — it must match.

Step 3. **Worked check on TGCGCA.** Top: $5'$-TGCGCA-$3'$. Complement, written $3' \to 5'$: $3'$-ACGCGT-$5'$. Reading bottom strand in $5' \to 3'$: TGCGCA. Match ✓. So $5'$-TGCGCA-$3'$ is a palindrome (*MluI* site).

Step 4. **What about odd-length sequences?** A perfect palindrome of odd length is impossible in double-stranded DNA because the middle base would have to pair with itself (A=A or G=G), which violates Watson–Crick rules. Hence every classical restriction-enzyme site is of even length (typically 4, 6 or 8 bp).

Why this matters. The palindrome rule is what enables restriction enzymes to bind as homodimers (one monomer per strand) and cut both strands symmetrically — that symmetric cut is what creates the sticky ends that make cloning possible.

> **Final Answer:** Five palindromes: GAATTC, GGATCC, AAGCTT, CTGCAG, GTCGAC. Custom-built palindrome: write half, append its reverse complement ⇒ e.g. TGCGCA (MluI).

Q 9.8 Can you recall meiosis and indicate at what stage a recombinant DNA is made?

SOLUTION

Concept used. Meiosis is the reductional cell division that produces gametes; it consists of two successive nuclear divisions, Meiosis I and Meiosis II, with a single round of DNA replication beforehand. The defining feature of Meiosis I is the formation of *bivalents* (tetrads), the pairing of homologous chromosomes in Prophase I, and the physical exchange of chromosome segments between non-sister chromatids through crossing over. Crossing over is the natural, intracellular generation of recombinant DNA — and it occurs at the pachytene sub-stage of Prophase I.

> ☞ **Five sub-stages of Prophase I**
>
> Leptotene → **Zygotene** (synapsis begins, synaptonemal complex forms) → **Pachytene** (crossing over occurs at recombination nodules) → Diplotene (chiasmata become visible) → Diakinesis.

Step 1. **Set-up — pre-meiotic S phase.** Before meiosis begins, each chromosome is duplicated so that every chromosome consists of two identical sister chromatids joined at the centromere.

Step 2. **Prophase I — Leptotene.** Chromosomes condense and become visible. No recombination yet.

Step 3. **Prophase I — Zygotene.** Homologous chromosomes pair side-by-side along their length, a process called synapsis. The pair is held together by a protein scaffold (the synaptonemal complex), forming a *bivalent* of four chromatids (a tetrad).

Step 4. **Prophase I — *Pachytene* (the key stage).** Recombination nodules — large

protein complexes — assemble on the synaptonemal complex. They catalyse a programmed double-strand break on one chromatid, invasion of the homologous chromatid, and reciprocal exchange of segments between non-sister chromatids of homologous chromosomes. This physical exchange of DNA is **crossing over**, and the chromatids that result carry *recombinant DNA* — DNA molecules with sequences from both maternal and paternal origin joined by covalent bonds.

Step 5. **Prophase I — Diplotene.** The synaptonemal complex dissolves; the homologues partially separate but remain joined at the sites of crossover, now visible as **chiasmata**. These X-shaped points are the cytological evidence of the recombinant DNA molecules made at pachytene.

Step 6. **Independent assortment in Metaphase I + Anaphase I.** Bivalents line up at the equator with random maternal/paternal orientation; segregation in Anaphase I separates the recombinant chromosomes into daughter cells, propagating the new combinations into the gametes.

Final Answer: Recombinant DNA is generated naturally during *Pachytene* of Prophase I of Meiosis I, when non-sister chromatids of homologous chromosomes undergo crossing over, exchanging equivalent segments.

♥ The same enzymes, two worlds

The intracellular machinery that makes natural recombinant DNA at pachytene includes Spo11 (programmed double-strand break), Dmc1/Rad51 (strand invasion) and DNA ligase. The in-vitro recombinant-DNA technology you study in this chapter uses different enzymes (restriction endonucleases + DNA ligase) but produces the same chemical outcome: a covalent joint between two distinct DNA sequences.

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

Sub-stage-tracking angle. The examiner is looking for one phrase: "Pachytene of Prophase I". Frame the answer as a brief recap of meiosis followed by a sharp pinpoint.

Step 1. **Meiosis in one line.** A reductional division that halves the chromosome number (diploid → haploid) and produces four genetically unique daughter cells from one precursor.

Step 2. **Two divisions, one DNA replication.** S-phase → Meiosis I (Prophase I, Metaphase I, Anaphase I, Telophase I) → Meiosis II (PM, MM, AM, TM).

Step 3. **Prophase I has five sub-stages.** Leptotene → Zygotene → **Pachytene** → Diplotene → Diakinesis. Zygotene aligns homologues via the synaptonemal

complex; **Pachytene** is where crossing over actually happens.

Step 4. **What "crossing over" creates.** Two physically recombinant chromatids: each carries a stretch of maternal DNA covalently joined to a stretch of paternal DNA. This is *nature's own* recombinant DNA, predating the recombinant DNA technology you study in this chapter by about 2 billion years.

Step 5. **Cytological evidence at the next stage.** In Diplotene the recombination joints become visible as *chiasmata* — direct microscopic proof that recombination at pachytene actually occurred.

Why this matters. The pachytene–crossover answer connects the cell-biology chapter (Chapter 10 of Class 11) with this biotechnology chapter: both produce DNA molecules with sequences from two different sources joined together.

> **Final Answer:** Recombinant DNA is naturally produced at the *Pachytene* sub-stage of Prophase I in Meiosis I via crossing over between non-sister chromatids of homologous chromosomes.

Q 9.9 Can you think and answer how a reporter enzyme can be used to monitor transformation of host cells by foreign DNA in addition to a selectable marker?

SOLUTION

Concept used. **Transformation** is the uptake of foreign DNA by a host cell. To prove that a host cell has indeed taken up the recombinant plasmid (and not merely survived the treatment), molecular biologists use two markers carried on the vector:

- **Selectable marker** — typically an antibiotic-resistance gene (e.g. amp^R). Cells lacking the plasmid die on the antibiotic plate; cells carrying the plasmid live. This tells you the cell took up *some* plasmid.
- **Reporter enzyme / gene** — a gene whose product is easily and visibly assayed (colour, fluorescence, light, enzyme activity). The reporter is engineered so that the cloning site sits *inside* the reporter gene; a successful insert disrupts the reporter, producing a detectable colour change.

The classical example is **insertional inactivation of β-galactosidase** (blue-white screening) using the *lacZ*α gene.

Step 1. **The set-up — blue/white screening with lacZ.** The cloning vector (e.g. pUC18) carries:

- An antibiotic-resistance gene (amp^R) as the selectable marker.
- The *lacZ*α fragment as the reporter, with a multiple-cloning site (MCS)

embedded inside it.

The host *E. coli* carries the complementary *lacZ*ω fragment; together with α they produce active β-galactosidase.

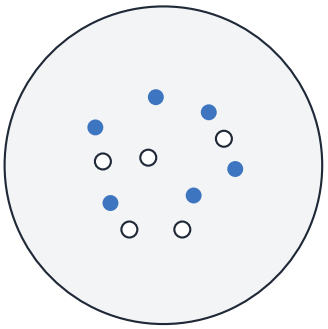
Step 2. **Plate on ampicillin + X-gal + IPTG.**

- Untransformed cells $\Rightarrow$ no *amp*^{R} $\Rightarrow$ die.
- Transformed cells carrying *empty vector* $\Rightarrow$ intact *lacZ*α $\Rightarrow$ β-galactosidase active $\Rightarrow$ cleaves X-gal to a blue dye $\Rightarrow$ **blue colony**.
- Transformed cells carrying *recombinant vector* (insert disrupts *lacZ*α) $\Rightarrow$ no active β-galactosidase $\Rightarrow$ X-gal stays colourless $\Rightarrow$ **white colony**.

Step 3. **Why both markers are needed.** The selectable marker (antibiotic resistance) alone tells you *which cells took up plasmid*, but not whether the plasmid carries your insert. The reporter (lacZ) alone cannot pre-enrich for plasmid-carrying cells — you would be screening millions of background colonies. Combining the two: select on antibiotic, then *screen* the survivors for the recombinant (white) colonies.

Step 4. **Modern reporter enzymes.** Beyond *lacZ*, the same logic uses *GFP* (green fluorescent protein, visible under UV), *luciferase* (emits visible light in the presence of luciferin), *chloramphenicol acetyltransferase*, and *β-glucuronidase* (GUS) in plants.

Ampicillin + X-gal + IPTG plate



● **Blue** = empty vector (lacZα intact)
○ **White** = recombinant (lacZα disrupted)
No growth = untransformed (no *amp*^{R})

Final Answer: A reporter gene (e.g. *lacZ*α) is engineered so that successful insertion of foreign DNA disrupts it; the host loses the reporter activity (no blue colour with X-gal) while the selectable antibiotic-resistance gene still works, giving white colonies on a blue background that are the true recombinants.

✗ Common Mistake

A common confusion: thinking the reporter and the selectable marker are interchangeable. They are not. The selectable marker *kills* non-transformants; the reporter merely *flags* recombinants. Both are needed: selection first, screen second.

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

Two-marker logic angle. Frame the answer as "selection filters out the 99%; the reporter identifies the 1% you want".

Step 1. **Why insertional inactivation works.** Engineer the multiple-cloning site (MCS) of the vector *inside* a reporter gene. With no insert, the reporter gene is intact and produces colour. With an insert, the reading frame of the reporter is broken and no colour is made.

Step 2. **Blue-white screening flow (with lacZ).** Plate on ampicillin + X-gal + IPTG.

- No plasmid $\Rightarrow$ dies on ampicillin.
- Empty vector $\Rightarrow$ active β-galactosidase $\Rightarrow$ cleaves colourless X-gal to blue product $\Rightarrow$ blue colony.
- Recombinant vector $\Rightarrow$ disrupted lacZ $\Rightarrow$ no β-gal $\Rightarrow$ white colony.

Step 3. **Why this beats antibiotic-only selection.** Even a plate of all *amp*^{R} colonies contains $\geq 90\%$ self-ligated empty vector. The reporter distinguishes recombinants from non-recombinants without DNA isolation and gel checks for each colony.

Step 4. **Other reporters in current use.**

- **GFP** (*Aequorea victoria*): green fluorescence under UV light. Live, non-destructive readout.
- **Luciferase** (firefly): emits photons when given luciferin and ATP; ultra-sensitive.
- **GUS** (*β-glucuronidase*): cleaves X-Gluc to a blue product; used in plant biology.

Step 5. **Practical pay-off.** A single agar plate gives you thousands of colonies; without a reporter you would need to mini-prep and restriction-digest each one. With the reporter, you pick only the white (or fluorescent, or luminescent) colonies and skip the rest.

Why this matters. Reporter genes are at the heart of every modern molecular-biology workflow — fluorescent reporters (GFP, mCherry) track gene expression in living cells; luciferase reporters quantify transcription factor activity in cancer assays.

> **Final Answer:** Reporter enzymes (lacZα, GFP, luciferase, GUS) sit inside the cloning site of the vector. A successful insert disrupts the reporter, so recombinant cells lose the colour/fluorescence while still being antibiotic-resistant — letting you identify recombinants visually on the same plate.

Q 9.10 **Describe briefly the following:**
(a) Origin of replication
(b) Bioreactors
(c) Downstream processing

SOLUTION

Concept used. Each sub-part names a single component of the recombinant-DNA pipeline: the molecular feature that lets the plasmid copy itself (origin), the engineering vessel that lets us grow host cells at scale (bioreactor), and the post-fermentation purification steps that convert culture broth into a marketable product (downstream processing).

(a) Origin of replication (*ori*). A specific sequence of nucleotides in a vector at which DNA replication *initiates*. When the host's DNA-replication machinery (DNA polymerase III holoenzyme in bacteria) recognises the *ori*, it loads on, unwinds the double helix and begins synthesising a new strand bidirectionally. Any piece of foreign DNA ligated downstream of the *ori* rides along and is amplified to the same copy number as the vector. The *ori* also determines that copy number: high-copy plasmids (pUC series, *ori* derived from ColE1) maintain 500–700 copies per cell, while low-copy plasmids (pBR322 derivatives, 15–20 copies) trade yield for stability of large inserts.

(b) Bioreactors. Vessels typically of 100–10 000 L working volume, in which raw materials (medium, substrates, inoculum) are biologically converted into specific products (recombinant proteins, enzymes, secondary metabolites, biomass) by microbial, plant, animal or human cells. A bioreactor provides *optimal* conditions: controlled temperature, pH, dissolved oxygen, substrate concentration, agitation. The most common design is the **stirred-tank bioreactor** (Fig. 9.7 a): cylindrical or curved-base vessel with a vertical-shaft impeller, sparger for sterile-air supply, jacketed walls for temperature control, and probes for pH/DO. A **sparged stirred-tank bioreactor** (Fig. 9.7 b) bubbles sterile air through a perforated ring to multiply oxygen-transfer area.

(c) Downstream processing. After fermentation is complete, the product must be separated from cells/medium and purified before sale. **Downstream processing** is the collective name for this sequence:

1. **Cell separation** — centrifugation, microfiltration or settling to recover cells (if intracellular product) or supernatant (if secreted).
2. **Cell disruption** — sonication, French press, bead-milling or lysozyme treatment if the product is inside the cell.
3. **Purification** — successive chromatography steps (ion-exchange, affinity, gel-filtration), precipitation, ultrafiltration. Affinity chromatography (His-tag on Ni-NTA, for example) can give $\geq 90\%$ purity in a single step.
4. **Formulation** — adding stabilisers, preservatives, excipients to the active ingredient and adjusting pH/tonicity.

5. **Quality control + clinical trials** — strict QC testing for purity, potency, sterility, endotoxin and residual host DNA. For drugs, formal Phase I–III trials.

Downstream processing typically accounts for $\geq 60\%$ of the total manufacturing cost of a recombinant therapeutic.

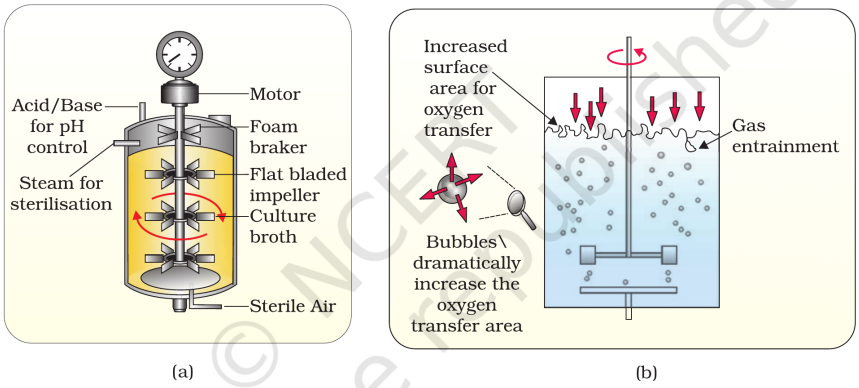


Figure 9.7 (a) Simple stirred-tank bioreactor; (b) Sparged stirred-tank bioreactor through which sterile air bubbles are sparged

Fig. 9.7, NCERT Class 12 Biology, Chapter 9. Two stirred-tank bioreactor designs used in industrial fermentation.

Final Answer: (a) Origin of replication = vector sequence where DNA replication starts; controls copy number. (b) Bioreactors = engineered vessels (100–10 000 L) for controlled large-scale cell culture. (c) Downstream processing = separation, purification, formulation and QC of the product after fermentation.

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

Three-card definition framing. Treat each sub-part as a flash card: *what it is, where it sits in the workflow, why it matters.*

Step 1. **(a) Origin of replication (ori).**

- *What:* a short stretch of DNA at which replication is initiated.
- *Where:* on every plasmid and every chromosome; in the vector, the ori sits adjacent to the selectable marker.
- *Why:* the host's polymerase only loads at the ori; without an ori, the foreign DNA cannot replicate inside the cell. The ori also sets the copy number (high-copy ColE1 ori $\sim$500; low-copy pSC101 ori $\sim$5).

Step 2. **(b) Bioreactors.**

- *What:* closed vessels in which cells are grown under controlled conditions to produce a desired metabolite or protein.
- *Where:* immediately after the cloning step; they convert millilitre lab cultures

to multi-kilolitre industrial production.

- *Why:* only a bioreactor delivers consistent temperature, pH, dissolved oxygen, foam control and aseptic operation at the volumes (100–10 000 L) needed for commercial protein production.

Step 3. **(c) Downstream processing.**

- *What:* the post-fermentation pipeline of separation, purification, formulation and quality control.
- *Where:* immediately after the cells reach the target density and the product has accumulated.
- *Why:* the molecule is no good unless it is pure, sterile and stable. Affinity chromatography, ultrafiltration and lyophilisation transform cloudy culture broth into vials of clinical-grade drug.

Step 4. **Workflow stitch-together.** A recombinant gene carries an ori to replicate, is grown in a bioreactor for biomass, and the product is purified by downstream processing.

Why this matters. Examiners often ask these three as a single question because together they cover the upstream (cloning), midstream (fermentation) and downstream (purification) thirds of biotechnology.

> **Final Answer:** Origin of replication starts plasmid copying; bioreactors grow host cells at scale; downstream processing purifies and formulates the final product.

Q 9.11 Explain briefly

(a) PCR

(b) Restriction enzymes and DNA

(c) Chitinase

SOLUTION

Concept used. Each part names a tool of recombinant DNA technology: *PCR* amplifies a chosen DNA region in vitro; *restriction enzymes* are sequence-specific molecular scissors for cutting DNA at defined sites; *chitinase* is the enzyme used to break the chitin-rich cell wall of fungi during DNA extraction.

(a) Polymerase Chain Reaction (PCR). PCR is an *in-vitro* method to make millions to billions of copies of a chosen DNA region using two short primer DNAs and a thermostable DNA polymerase. A single PCR cycle has three temperature steps:

- **Denaturation ($\sim 94\,^{\circ}$C, 30 s)**: the double-stranded DNA template melts into two

single strands.

- **Annealing (~ 50–$65\,^\circ$C, 30 s)**: two oligonucleotide primers (typically 18–25 nt long, chemically synthesised, complementary to the flanks of the target region) base-pair with their target sites on each single strand.
- **Extension ($\sim 72\,^\circ$C, 30–60 s)**: thermostable **Taq DNA polymerase** (from *Thermus aquaticus*) extends each primer in the $5' \to 3'$ direction using the provided deoxynucleotides (dNTPs).

Because each new product itself becomes a template in the next cycle, copy number doubles per cycle. After n cycles the amplification factor is 2^n; 30 cycles give $2^{30} \approx 10^9$ copies, matching the figure shown in Fig. 9.6.

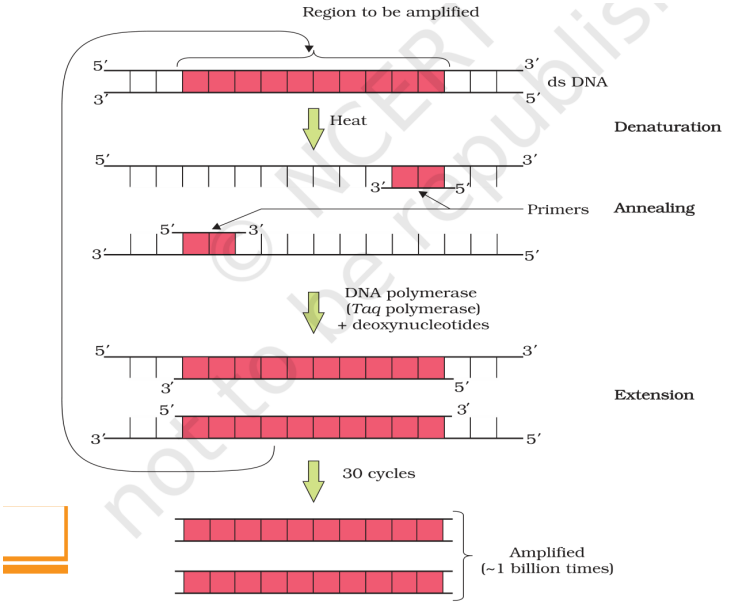


Figure 9.6 Polymerase chain reaction (PCR) : Each cycle has three steps: (i) Denaturation; (ii) Primer annealing; and (iii) Extension of primers

Fig. 9.6, NCERT Class 12 Biology, Chapter 9. PCR cycle: denaturation, annealing of primers, and extension by Taq *DNA polymerase. Thirty cycles amplify the chosen DNA region $\sim 10^9$-fold.*

(b) Restriction enzymes and DNA. *Restriction endonucleases* are bacterial enzymes that recognise specific short *palindromic* sequences (4–8 bp) in double-stranded DNA and cleave both strands. The first one was isolated by Smith & Wilcox (1970) from *Haemophilus influenzae* and named *HindII*. By 2024 over 4000 restriction enzymes from > 230 strains of bacteria are commercially available, each with a unique recognition site. They fall into three categories — Type I, II (used in cloning) and III — based on how cleavage and methylation activities are organised. The Type-II workhorses (EcoRI, BamHI, HindIII, PstI, NotI) leave defined **sticky ends** that ligase can rejoin, making them the cutting tools at the heart of recombinant DNA technology.

(c) Chitinase. An enzyme that hydrolyses the β-1,4-glycosidic bonds of **chitin**, the structural polysaccharide of fungal cell walls (and of arthropod exoskeletons). In molecular biology, chitinase is the enzyme of choice for *digesting fungal cell walls* during DNA isolation: fungal cells, unlike bacterial or plant cells, resist lysozyme and cellulase

because their walls are chitin-based. Treating the fungal pellet with chitinase releases the protoplast, after which standard SDS-based lysis liberates the DNA. The same role for cell-wall digestion is played by lysozyme (for bacteria) and cellulase (for plants).

> **Final Answer:** (a) PCR amplifies a defined DNA region in vitro through denaturation–annealing–extension cycles using two primers and *Taq* polymerase; 30 cycles $\rightarrow\sim 10^9$ copies. (b) Restriction enzymes are bacterial endonucleases that cut DNA at specific palindromic sites and are the cutting tools of gene cloning. (c) Chitinase digests the chitin in fungal cell walls during DNA isolation from fungi.

☞ NEET / CUET focus

PCR is one of the most asked board / NEET questions in this chapter. Memorise the three temperatures ($94, 55, 72\,^\circ$C), the role of *Taq* polymerase (thermostability), and the 2^n amplification factor.

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

Three-card framing. For each tool: *what it is, what it does, where it sits in the workflow.*

Step 1. **(a) PCR.**

- *What:* an in-vitro DNA-amplification method.
- *Components:* template DNA, two primers, four dNTPs, Mg^{2+}, *Taq* polymerase, buffer.
- *Mechanism:* thermal cycling between ~ 94, ~ 55 and $\sim 72\,^\circ$C drives denaturation, annealing and extension respectively.
- *Output:* after n cycles, target sequence is amplified $\sim 2^n$-fold; 30 cycles give $\sim 10^9$ copies.
- *Use cases:* diagnostics (RT-PCR for SARS-CoV-2), forensics (DNA fingerprinting from picogram samples), cloning (preparing the insert before ligation).

Step 2. **(b) Restriction enzymes and DNA.**

- *What:* bacterial endonucleases that cut DNA at specific palindromic recognition sites.
- *Types:* Type I (cut at random away from site), Type II (cut within or adjacent to the site — workhorses of cloning), Type III.
- *Action on DNA:* cleaves both strands; staggered cuts produce sticky ends, blunt cuts produce blunt ends.
- *Examples:* EcoRI (GAATTC), BamHI (GGATCC), HindIII (AAGCTT), PstI (CTGCAG), NotI (GCGGCCGC).

Step 3. **(c) Chitinase.**

- *What:* an enzyme that hydrolyses chitin into its monomeric units of N-acetylglucosamine.
- *Substrate:* chitin, a β-1,4 polymer of N-acetylglucosamine; major component of fungal cell walls and arthropod exoskeletons.
- *Where in DNA isolation:* added to fungal pellet to break down the cell wall, releasing protoplasts which can then be lysed by SDS detergent to liberate genomic DNA.
- *Parallel enzymes: lysozyme* digests bacterial peptidoglycan; *cellulase* digests plant cell-wall cellulose. The choice depends on source organism.

Step 4. **Stitching together.** Restriction enzymes cut the DNA; PCR amplifies it; chitinase (or its analogues) provides the starting material by releasing the DNA from the cell.

Why this matters. These three tools, in their order, constitute the upstream half of recombinant DNA technology — *liberate, cut, amplify*.

> **Final Answer:** (a) PCR = 2^n amplification of a target DNA via thermal cycling with *Taq* polymerase. (b) Restriction enzymes = bacterial endonucleases that cut DNA at palindromic sites, producing sticky/blunt ends. (c) Chitinase = enzyme that digests chitin in fungal cell walls during DNA extraction.

Read the Full Chapter 9 Revision Notes →

Q 9.12 **Discuss with your teacher and find out how to distinguish between**
(a) Plasmid DNA and Chromosomal DNA
(b) RNA and DNA
(c) Exonuclease and Endonuclease

SOLUTION

Concept used. Each pair contrasts two molecules / enzymes that are biochemically similar but functionally and structurally distinct. A well-formed answer is a side-by-side table of *property : entity-1 : entity-2*.

(a) Plasmid DNA vs Chromosomal DNA.

Property	Plasmid DNA	Chromosomal DNA
Location	Cytoplasm (extrachromosomal)	Nucleoid (bacteria) / nucleus (eukaryotes)
Shape	Small, circular, double-stranded	Large; circular in bacteria, linear in eukaryotes
Size	1–250 kbp (commonly 3–10 kbp)	Mbp to Gbp ($10^3\times$ larger)
Genes	Few accessory genes (drug resistance, virulence, conjugation)	Carries essential genes for growth, metabolism, reproduction
Replication	Replicates independently from its own ori	Replicates once per cell cycle from the chromosomal ori
Essential?	Not essential for cell survival under normal conditions	Essential for life
Inheritance	Can be lost during division	Strictly inherited by every daughter cell

(b) RNA vs DNA.

Property	DNA	RNA
Sugar	Deoxyribose (2′-OH replaced by 2′-H)	Ribose (2′-OH present)
Bases	A, T, G, C	A, U, G, C (T replaced by U)
Strands	Double-stranded helix (B-form)	Mostly single-stranded; can fold into secondary structures
Stability	Stable (no 2′-OH; bases protected inside helix)	Less stable; the 2′-OH catalyses base hydrolysis
Genetic role	Stores genetic information	Conveys (mRNA), translates (tRNA, rRNA) and regulates (miRNA, siRNA) the information
Length	Continuous chromosome (Mb to Gb)	Discrete molecules (mRNA tens of kb, tRNA $\sim$ 80 nt, rRNA hundreds–thousands nt)
Reaction to alkali	Stable	Hydrolysed (the 2′-OH attacks the adjacent phosphodiester)

(c) Exonuclease vs Endonuclease.

Property	Exonuclease	Endonuclease
Site of cleavage	At the ends (5′ or 3′) of a DNA/RNA strand	At internal phosphodiester bonds
Mode of action	Removes nucleotides one at a time from a terminus	Cuts the strand in the middle, generating two new ends
Specificity	Independent of sequence (in many cases); just chews from the end	Often sequence-specific (palindromic site for Type-II restriction enzymes)
Examples	DNA Pol I ($5' \to 3'$ exo), Exonuclease III	EcoRI, BamHI, HindIII, DNase I
Role in the cell	DNA repair, proofreading during replication, processing of Okazaki fragments	Restriction–modification, DNA repair, apoptosis (caspase-activated DNase)
Used in cloning?	Limited (Bal31 to trim ends)	Central (every cloning step uses a restriction endonuclease)

Final Answer: (a) Plasmid DNA = small, circular, extrachromosomal, self-replicating, dispensable; chromosomal DNA = large, essential, houses all life-support genes. (b) DNA = deoxyribose + A,T,G,C + ds-helix + stable + stores info; RNA = ribose + A,U,G,C + mostly ss + alkali-labile + executes info. (c) Exonucleases cut from ends, one nucleotide at a time; endonucleases cut internally at specific sites and generate two new ends.

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

Comparison-table angle. Examiners reward tabulated answers because they make the contrast unmissable. Stick to 5–7 rows per pair.

Step 1. **(a) Plasmid vs Chromosomal DNA — three killer points.** Size (plasmids are 1–250 kbp vs Mbp-Gbp), independence (plasmid replicates from its own *ori*), and dispensability (cell can survive without the plasmid but not without the chromosome).

Step 2. **(b) RNA vs DNA — three killer points.** Sugar (ribose 2′-OH vs deoxyribose 2′-H), base (U vs T), strand topology (single-stranded vs double-helical). Combine these and you also explain why RNA is less stable — the 2′-OH attacks the adjacent phosphodiester under alkaline conditions.

Step 3. **(c) Exonuclease vs Endonuclease — two killer points.** Position of cleavage (end vs middle) and mode of attack (sequential nibbling vs single internal cut). Add the sequence-specificity contrast (endos often palindromic; exos generally not).

Step 4. **Quick-recall mnemonic.** *Endo = inside cut* $\Rightarrow$ gives 2 fragments; *Exo = exit cut* $\Rightarrow$ chews from the end. *Plasmid = passenger DNA*; *chromosome = essential cargo*. *R(NA) for read, D(NA) for data*.

Why this matters. These three pairwise distinctions surface in every cloning experiment: you pick a plasmid (not the chromosome) as a vector, you make a cDNA from mRNA (RNA $\rightarrow$ DNA), and you cut with restriction endonucleases (not exonucleases) to avoid chewing back your insert.

Final Answer: Plasmid DNA $\neq$ chromosomal DNA by size, copy independence and dispensability. RNA $\neq$ DNA by sugar (2′-OH), base (U), strand topology, and chemical stability. Exonuclease $\neq$ endonuclease by site of cleavage (terminus vs internal phosphodiester).

Key Takeaways

- **Recombinant DNA technology** cuts a foreign gene with a **restriction endonuclease** (EcoRI, BamHI . . .), joins it to a vector through complementary **sticky ends** sealed by **DNA ligase**, transforms it into a host cell, and amplifies it for protein production.
- Restriction enzymes cut DNA at **palindromic** sites (e.g. EcoRI: GAATTC) and produce sticky-end fragments that re-anneal across species barriers — the cornerstone of all cloning.
- **PCR** amplifies a chosen DNA region $\sim 2^n$-fold through repeated cycles of denaturation, annealing and extension, using two primers and thermostable *Taq* polymerase.
- **Vectors** carry an origin of replication, a selectable marker and (usually) a reporter gene; in *E. coli* the workhorse vector pBR322 carries amp^{R} and tet^{R} markers.
- Eukaryotic cells do *not* carry classical restriction endonucleases; they defend against viruses through RNA interference, interferon signalling and adaptive immunity.
- Natural recombinant DNA is generated in vivo at the **pachytene** sub-stage of Prophase I of meiosis, through **crossing over** between non-sister chromatids of homologues.
- Industrial production needs a **stirred-tank bioreactor**: controlled temperature, pH, dissolved oxygen, sterile-air sparging, foam control, and sampling ports for 100–10 000 L cultures.
- **Downstream processing** (separation, purification, formulation, quality control) is where the recombinant product becomes a clinical-grade drug.

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