Class 12 Biology Formula Sheet

Chapter 9 Biotechnology: Principles and Processes — NCERT 2026-27

Chapter 9: Biotechnology — Principles & Processes

Restriction enzymes | rDNA workflow | PCR | Bioreactors | Downstream processing

Also see for this chapter: NCERT Solutions | Revision Notes | Exemplar Solutions

Chapter-Wide Key Quantitative Reference

Parameter	Typical Value / Range	Significance
Restriction-enzyme recognition site	4–8 bp (commonly 6 bp palindrome)	Type II endonucleases (e.g. *Eco*RI cuts GAATTC)
Number of restriction enzymes known	> 900 from > 230 bacterial strains	Each cleaves a unique sequence
First restriction endonuclease	*Hind* II (1968)	Six-base recognition; isolated five years after the 1963 discovery
PCR amplification factor	$N = N_0 \times 2^n$ (n = cycles)	30 cycles $\Rightarrow \sim 10^9$ copies ("billion-fold")
PCR cycle temperatures	~ 94–$95\,^\circ$C / 50–$65\,^\circ$C / $72\,^\circ$C	Denaturation / Annealing / Extension (*Taq*)
Taq polymerase source / stable at	*Thermus aquaticus*; $> 95\,^\circ$C	Survives denaturation step — no re-addition needed each cycle
Plasmid copy number per cell	1–2 (low) to 15–100 (high)	Set by *ori*; high-copy vectors $\Rightarrow$ more product
Heat-shock transformation profile	ice $\rightarrow 42\,^\circ$C (~ 45 s) $\rightarrow$ ice	With Ca^{2+}-treated competent cells
Bioreactor working volume	100–1000 L (industrial)	vs. shake-flask: 0.1–1 L
Stirred-tank impeller / aeration	flat-bladed impeller; sparged air	Mixing + O_2 transfer + foam & pH control
pBR322 vector size / hosts	~ 4361 bp; *E. coli*	Carries amp^R, tet^R, *ori*, *rop*
Heat-shock $CaCl_2$ concentration	~ 50 mM (divalent cation)	Neutralises DNA charge $\Rightarrow$ uptake through pores

1 9.1 Principles of Biotechnology

Sets up biotechnology as the use of **live organisms / enzymes / cells** for products and services. The chapter's two core enabling techniques are genetic engineering and bioprocess engineering, and the three universal steps of genetic modification.

EFB definition of biotechnology

European Federation of Biotechnology (EFB): "The integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services."

Modern (narrow) sense ⇒ **processes using genetically modified organisms** (GMOs) at scale — antibiotics, vaccines, recombinant insulin, GM crops, gene therapy.

Two core techniques of modern biotechnology

(i) Genetic engineering: techniques to alter the chemistry of **DNA / RNA** ⇒ introduce these into a host ⇒ change the host's **phenotype**.

(ii) Bioprocess engineering: maintain a **sterile** (contamination-free) ambience in chemical engineering processes so that **only the desired microbe / eukaryotic cell** grows in large quantities.

Genetic engineering specifies *what* to make; bioprocess engineering specifies *how to make it at scale*. Both are mandatory for any commercial recombinant product (insulin, vaccines, enzymes).

Three universal steps of genetic modification

(i) Identification of DNA carrying the **desirable gene**.

(ii) Introduction of the identified DNA into the host.

(iii) Maintenance of the introduced DNA in the host **and** its transfer to progeny.

Step (iii) is why the alien DNA **must carry an *ori*** or be integrated into a host chromosome — otherwise it dies out in the next cell division.

Why an alien DNA needs the origin of replication

A chromosome carries a specific sequence called the **origin of replication (ori)** that initiates DNA replication. Any alien DNA **linked to an ori** can replicate and multiply itself in the host ⇒ this is **cloning** (making multiple identical copies of a template DNA).

Historical milestones — principles

1972: Stanley Cohen & Herbert Boyer construct the first recombinant DNA molecule — antibiotic-resistance gene from a *Salmonella typhimurium* plasmid ligated into another plasmid using restriction enzymes + DNA ligase.
Foundation of the discipline of biotechnology.

2 9.2 Tools of Recombinant DNA Technology

Covers the five toolkit pieces every rDNA experiment needs: **restriction enzymes** (cut), **polymerases** (copy), **ligases** (join), **vectors** (carry), and a **competent host** (express). Each has its own naming convention, optimal conditions and biological role.

Restriction-enzyme nomenclature — 3-letter rule

$\underbrace{E}_{\text{genus initial}}\ \underbrace{co}_{\text{species, first 2 letters}}\ \underbrace{\text{R}}_{\text{strain}}\ \underbrace{\text{I}}_{\text{order isolated}}$

Example: ***Eco*RI** = from ***E****scherichia* ***co****li* **R**Y 13, first (I) enzyme isolated from that strain.

Genus letter **italic capital**, species letters **italic lowercase**; strain Roman; Roman numeral indicates the order of isolation. Total > 900 such enzymes are known, each with its own recognition sequence.

Classes of nucleases

Class	Action	Example / use
Exonuclease	removes nucleotides from **ends** of DNA	exo-trimming; lambda exonuclease
Endonuclease	cuts at **internal specific sites**	*Hind* II, *Eco*RI, *Bam*HI
Restriction endonuclease	subclass of endonuclease, sequence-specific (Type II)	"molecular scissors" — cuts at recognition sites

Endonucleases enable **cloning at precise positions**; exonucleases only trim ends. The recombinant-DNA toolkit relies on Type II restriction endonucleases.

Palindromic recognition sequence (*Eco*RI)

A **DNA palindrome** reads the same on both strands when read $5' \rightarrow 3'$:

$5'$ — G A A T T C — $3'$

$3'$ — C T T A A G — $5'$

*Eco*RI cuts between G | A on each strand $\Rightarrow$ leaves 4-nt $5'$-overhangs ("**sticky ends**" `AATT`).

Sticky ends from **any** DNA cut by the same enzyme are complementary — they H-bond to each other, allowing DNA ligase to seal foreign + vector DNA into one recombinant molecule.

Sticky ends vs blunt ends

Sticky ends = short single-stranded overhangs produced when an enzyme cuts off-centre in a palindrome (e.g. *Eco*RI $\rightarrow$ `AATT`). They base-pair with complementary overhangs and dramatically raise the ligation efficiency.

Blunt ends = produced when an enzyme cuts straight through the centre (e.g. *Sma*I, *Hae*III). Ligatable but with much lower efficiency.

DNA ligase — the molecular glue

$\text{DNA}_1\ (3'\text{-OH}) + \text{DNA}_2\ (5'\text{-PO}_4) \longrightarrow$ phosphodiester bond

Catalyst: T4 DNA ligase; **cofactor:** ATP.

Action: forms a covalent phosphodiester bond between the $3'$-OH and the $5'$-

phosphate of two DNA fragments (sticky-end or blunt-end).

Required to **seal nicks** after sticky ends have H-bonded. Without ligase the joint is unstable. T4 DNA ligase (from phage T4) is the workhorse in rDNA labs.

Gel electrophoresis — size separation

Matrix: agarose (natural polymer from sea weeds), typical 0.8–2 **%**.

Driving force: DNA is **negatively charged** $\Rightarrow$ migrates to the **anode (+)**.

Resolution rule: $v \propto \dfrac{1}{\log(\text{fragment size in bp})} \Rightarrow$ **smaller fragments move farther**.

Detection: ethidium bromide stains DNA $\Rightarrow$ bright **orange** bands under UV.

Elution: cut the band out of the gel; extract DNA for downstream ligation.

Sample is loaded in wells near the cathode end. The gel must be photographed under UV **quickly** — prolonged UV nicks the DNA.

Cloning vector — four mandatory features

Feature	Role
(i) ori (origin of replication)	sequence where replication starts; **controls copy number**. High-copy *ori* $\Rightarrow$ more recombinant product
(ii) Selectable marker	gene (e.g. amp^R, tet^R, kan^R, cam^R) that **kills non-transformants** and lets transformants grow
(iii) Cloning sites	one (preferably single) recognition site for each common restriction enzyme; multiple sites would shatter the vector
(iv) Small size	easy to manipulate, high transformation efficiency (pBR322 ≈ 4361 bp)

pBR322 is the textbook *E. coli* cloning vector — carries *ori*, *rop*, amp^R, tet^R and single sites for *Hind* III, *Eco*RI, *Bam*HI, *Sal* I, *Pvu* II, *Pst* I, *Cla* I.

Insertional inactivation — recombinant selection

Principle: insert the foreign DNA into the **middle of a selectable marker gene** $\Rightarrow$ that gene becomes non-functional in recombinants.

Two-antibiotic example (pBR322, BamHI in tet^R):

	Ampicillin plate	Tetracycline plate
Non-transformant	no growth	no growth
Non-recombinant (vector only)	grows	grows
Recombinant (insert)	grows	**no growth**

Chromogenic alternative: insert into the **lacZ** (β-galactosidase) gene $\Rightarrow$ on X-gal/IPTG

plates non-recombinants give **blue** colonies, recombinants give **white/colourless** colonies — single-plate screen.

Vectors for plant and animal cells

Vector	Source pathogen	Delivered into / use
Ti plasmid (disarmed)	*Agrobacterium tumefaciens*	dicot plants — delivers T-DNA
Retroviral vectors (disarmed)	retroviruses	animal cells — integrate into host chromosome
Plasmids (e.g. pBR322)	*E. coli*	bacterial host
Bacteriophages (λ, M13)	phages	high-copy bacterial cloning

Ti plasmid naturally causes crown-gall tumours; biotechnologists strip its tumour genes ("disarm") and use the remaining DNA-delivery machinery. Same logic for retroviral vectors in gene therapy.

Making a competent host + transformation regime

Step 1: treat host cells (*E. coli*) with a **divalent cation**, typically **Ca^{2+}** ($\sim$ 50 mM $CaCl_2$, ice) $\Rightarrow$ "competent" cells.

Step 2: add recombinant DNA; incubate **on ice**.

Step 3: **heat shock** at $42\,^{\circ}$**C** for $\sim$ 45 s $\Rightarrow$ DNA enters through transient pores in the cell wall.

Step 4: return to ice; plate on selection medium.

Alternative delivery methods:

- **Micro-injection** — direct injection into the nucleus (animal cells).
- **Biolistics / gene gun** — high-velocity gold or tungsten micro-particles coated with DNA (plant cells).
- **Disarmed-pathogen vectors** — Ti plasmid (plants), retroviruses (animals).

Ca^{2+} neutralises the negative phosphate backbone of DNA, allowing the hydrophilic DNA molecule to slip through bacterial pores during the brief heat shock.

Restriction enzyme $\neq$ ligase

Restriction endonucleases CUT; **DNA ligase JOINS.** Both are needed in every rDNA experiment, but they are opposite reactions — never substitute one for the other. The **same** enzyme cuts both source and vector DNA so that their sticky ends are complementary.

NEET extension — restriction site density

Probability of a random 6-bp recognition site occurring in random DNA $\approx \frac{1}{4^6} = \frac{1}{4096}$ bp. In a 3×10^9 bp human genome, an enzyme like *Eco*RI cuts roughly $\sim 7 \times 10^5$ times — which is why genomic DNA is fragmented into a smear, not a single band, on a gel.

3 9.3 Processes of Recombinant DNA Technology

The full rDNA workflow has **seven sequential steps**, from breaking open the cell to extracting the marketed product. Each step is summarised below with its biological gloss; PCR and bioreactors get their own boxes.

The seven steps of rDNA technology

(1) Isolation of DNA (lyse cell, digest other macromolecules, precipitate DNA with chilled ethanol).
(2) Cutting the DNA with the chosen restriction endonuclease (source DNA and vector both).
(3) Amplification of the gene of interest using **PCR**.
(4) Ligation of gene of interest into the vector with DNA ligase $\Rightarrow$ recombinant DNA.
(5) Insertion of recombinant DNA into the competent host (transformation).
(6) Culture of transformed cells at large scale in a **bioreactor**.
(7) Downstream processing — separation, purification, formulation, QC, marketing.

Steps 1–5 are bench-scale molecular biology; steps 6–7 are industrial bioprocess engineering. Both halves are essential to a commercial product.

Step 1 — DNA isolation enzymes

Enzyme	Cell-wall target	Used for
Lysozyme	peptidoglycan	bacteria
Cellulase	cellulose	plant cells
Chitinase	chitin	fungi
Ribonuclease (RNase)	RNA	remove RNA contamination
Protease	proteins (histones, etc.)	remove protein contamination

Final precipitant: **chilled ethanol** $\Rightarrow$ pure DNA appears as **fine fibrous threads (spool)** that can be lifted out on a glass rod. Visible to the naked eye — a classic textbook experiment.

Why an alien DNA is not destroyed by host nucleases

After ligation into a vector, the recombinant DNA carries a host-compatible **ori** and methylation pattern. Once transformed, **the host's own DNA polymerase** replicates the alien DNA along with its chromosome — so the new strand is methylated like host DNA and escapes the cell's restriction defence.

PCR — the three-step thermal cycle

Step	Temperature	Event
Denaturation	94–95 °C, ∼ 30 s	H-bonds break ⇒ dsDNA → two ssDNA templates
Annealing	∼ 50–65 °C, ∼ 30 s	Forward + reverse **primers** (chemically synthesised oligonucleotides, ∼ 18–25 nt) H-bond to their complementary regions
Extension	72 °C, ∼ 1 min / kb	***Taq* polymerase** extends primers in 5′ → 3′ direction using dNTPs

One cycle doubles the target DNA ⇒ amplification is **exponential**.

Each cycle takes ∼ 2–5 min; a typical 30-cycle programme runs ∼ 1.5 h. Without a thermostable polymerase the enzyme would have to be added fresh every cycle, which is why the discovery of *Taq* was transformative.

PCR amplification factor

$$N = N_0 \times 2^n$$

where N_0 = initial template copies; n = number of cycles; N = copies after n cycles.

Example: $n = 30$ cycles ⇒ $2^{30} = 1.07 \times 10^9$ ⇒ approximately **one billion** copies from a single template molecule.

Real-world yield is slightly below ideal (2^n) because of reagent depletion and primer mis-binding in later cycles — the textbook "∼ 1 billion times" is a near-exact match to 2^{30}.

Primer melting temperature (T_m) — Wallace rule

For short primers (≤ 20 nt), the melting temperature in °C:

$$T_m = 2\,(\mathrm{A} + \mathrm{T}) + 4\,(\mathrm{G} + \mathrm{C})$$

where A, T, G, C are the counts of each base in the primer.

Annealing rule of thumb: $T_{\text{anneal}} \approx T_m - 5$ °C.

G–C pairs have **three** H-bonds vs A–T's **two** ⇒ GC-rich primers melt at higher T_m. Setting the annealing step too low gives non-specific products; too high gives no product.

Taq polymerase — the enabling enzyme

Source: *Thermus aquaticus* — a thermophilic bacterium isolated from hot springs.

Optimum activity: ∼ 72 °C; **remains active** after exposure to ∼ 95 °C denaturation step.

Why it matters: avoids the need to **add fresh polymerase** after every denaturation cycle ⇒ enables automation in a thermal cycler.

Lacks 3′ → 5′ proof-reading exonuclease activity; high-fidelity work uses *Pfu* polymerase instead. The amplified fragment can then be ligated to a vector or directly sequenced.

Bioreactor — principle and basic equation

A **bioreactor** is a vessel (~ 100–1000 L) in which raw materials are biologically converted into a desired product using microbes, plant or animal cells.

Mass-balance (working volume):

$$V_{\text{working}} \approx (0.7\text{–}0.8) \times V_{\text{total}}$$

Cell-growth (exponential phase):

$$N_t = N_0\, e^{\mu t} \quad \Longleftrightarrow \quad t_d = \frac{\ln 2}{\mu}$$

where μ = specific growth rate (h^{-1}); t_d = doubling (generation) time (h); N_t = cell density at time t.

20–30 **%** headspace is left for foam, gas & mixing. Continuous cultivation drains spent medium and adds fresh medium so cells stay in their **log/exponential phase** — the most productive state.

Bioreactor anatomy — six control systems

System	Function
Agitator (impeller)	flat-bladed; even mixing of substrate, cells, O_2
Oxygen delivery / sparger	sterile air bubbled in $\Rightarrow$ raises O_2 transfer area
Foam control	foam-breaker arm prevents foam-out
Temperature control	jacketed walls / coils maintain optimum (e.g. $30\,^\circ$C or $37\,^\circ$C)
pH control	acid/base feed lines hold pH at set point
Sampling port	withdraw small volumes for analysis without contamination

Two NCERT types: **(a) simple stirred-tank** (impeller-mixed) and **(b) sparged stirred-tank** (sterile air bubbled in for higher O_2 transfer). Both use steam for in-place sterilisation.

Volumetric scale-up rule of thumb

For geometrically similar stirred-tank bioreactors, hold the **volumetric power input constant**:

$$\frac{P_1}{V_1} = \frac{P_2}{V_2}$$

where P = impeller power (W); V = working volume (L).

Implication: doubling the volume requires doubling the power $\Rightarrow$ stirrer torque, motor rating and gas-flow rate all scale with V, not with surface area.

This is why a lab shake-flask (0.25 L) cannot simply be "run larger" — a 1000-L fermenter needs purpose-built impellers, spargers and cooling jackets to keep $k_L a$ (O_2 transfer coefficient) above the cell's demand.

Downstream processing — the five sub-steps

(i) Separation (centrifugation, filtration) → **(ii) Purification** (chromatography, precipitation) → **(iii) Formulation** (add preservatives, stabilisers) → **(iv) Clinical trials** (for drugs) → **(v) Quality-control testing**.

Downstream processing varies enormously product-to-product; for a therapeutic protein it can account for $\geq$ **70 %** of total production cost. "Downstream" = everything **after** the biosynthetic stage in the bioreactor.

Recombinant protein — definition

When any **protein-encoding gene is expressed in a heterologous host** (e.g. human insulin gene in *E. coli*), the resulting protein is called a **recombinant protein**. Examples: recombinant human insulin, hepatitis B vaccine, recombinant blood-clotting factor VIII, recombinant erythropoietin.

"Cloning" has two unrelated meanings

Molecular cloning = making multiple identical copies of a DNA fragment in a host cell (via vector + *ori*).
Reproductive cloning = producing a genetically identical organism (e.g. Dolly the sheep).
NCERT Ch 9 only deals with **molecular cloning**. Mixing the two costs marks.

Heat shock $\neq$ heat killing

The **42 °C heat shock** is a **45-second pulse** that creates transient pores in the bacterial cell wall so DNA can enter. It is **not** long or hot enough to kill the bacteria, which is why cells must be returned to ice immediately afterwards and then plated.

"I CALI BD" — rDNA workflow order

Isolate → **C**ut (restriction) → **A**mplify (PCR) → **L**igate → **I**nsert (transform) → **B**ioreactor → **D**ownstream.
The same seven steps work for insulin, vaccines, GM crops, and recombinant clotting factors — only the gene and host change.

"3-5-7" rule of rDNA

3 steps of genetic modification (Identify, Introduce, Maintain).
5 tools of rDNA (Restriction enzymes, Polymerases, Ligases, Vectors, Host).
7 steps of the full workflow (Isolate → Cut → Amplify → Ligate → Insert → Bioreactor → Downstream).

NEET extension — transformation efficiency

Transformation efficiency = number of transformants per μg of plasmid DNA.

$$\text{TE} = \frac{\text{cfu on selection plate}}{\mu\text{g DNA added}} \times \frac{V_{\text{total}}}{V_{\text{plated}}}$$

A standard $CaCl_2$-treated *E. coli* run gives 10^5–10^7 cfu/μg; electroporation can push this to 10^9 or above.

Download the Full Revision Notes PDF

4 Conventions & Unit Conversions Used in This Chapter

Quick cheat-card of the units, prefixes and conversions that appear inside biotechnology problems.

DNA-length & concentration unit conversions

Quantity	Conversion	Use case
bp $\leftrightarrow$ kb	1 kb $= 10^3$ bp	gene / plasmid size
kb $\leftrightarrow$ Mb	1 Mb $= 10^6$ bp	chromosome size
DNA mass per bp (ds-DNA)	1 bp ≈ 650 Da	convert mass $\leftrightarrow$ copy number
μg $\leftrightarrow$ pmol of 1 kb ds-DNA	$1\ \mu$g ≈ 1.52 pmol	ligation set-up
OD_{260} of dsDNA (1 cm path)	$1.0 = 50\ \mu$g/mL	spectrophotometric quantitation
OD_{260} of ssDNA / RNA	$1.0 = 33$ / $40\ \mu$g/mL	primers / RNA
A_{260} / A_{280} purity	~ 1.8 pure DNA; ~ 2.0 pure RNA	contamination check

These conversions let students translate spectrophotometer readings into the molar ratios needed for ligation, PCR and transformation reactions.

Common temperature / pH / time conventions

Process	Set-point	Notes
Restriction digest (*Eco*RI)	$37\,^\circ$C, 1 h	vendor-specified buffer
DNA ligation (T4 ligase)	4–$16\,^\circ$C, overnight	sticky ends; ATP required
Heat shock (transformation)	$42\,^\circ$C, 45 s	ice $\rightarrow 42\,^\circ$C $\rightarrow$ ice
E. coli growth	$37\,^\circ$C, pH 7.0	doubling time ~ 20 min in rich medium
Bacterial colony plating	$37\,^\circ$C, 12–16 h	visible colonies overnight
PCR denaturation / anneal / extend	95 / 50–65 / $72\,^\circ$C	one-cycle thermal profile

Memorise the heat-shock and PCR temperatures — both are popular one-line questions in NEET and Class 12 boards.

Related Collegedunia Resources

Same chapter — other resources:

- NCERT Solutions
- Revision Notes
- NCERT Book PDF
- Exemplar Book PDF
- Exemplar Solutions
- Handwritten Notes

Continue learning:

- Ch 8: Microbes in Human Welfare
- Ch 10: Biotechnology and its Applications
- Class 12 Biology — All Chapters

Quick Reference — Term → Definition Index

Term / Tool	Category	One-line definition / key datum
Biotechnology (EFB)	Definition	Use of organisms / cells / molecular analogues for products & services
Genetic engineering	Technique	Altering DNA / RNA chemistry to change host phenotype
Bioprocess engineering	Technique	Sterile large-scale cultivation of desired microbe / cell
Recombinant DNA	Product	Hybrid DNA from two sources joined *in vitro*
Restriction endonuclease	Enzyme	Cuts DNA at a specific sequence (palindrome); > 900 known
*Eco*RI recognition / cut	Sequence	`GAATTC`; cut between G and A $\Rightarrow$ `AATT` sticky end
Hind II	First enzyme	Six-base recognition; isolated 1968
Sticky ends	Structural	4-nt $5'$ overhangs; H-bond with complementary overhangs
DNA ligase	Enzyme	Joins $3'$-OH and $5'$-PO_4 via phosphodiester bond
Plasmid	Vector	Small autonomous circular DNA; carries *ori*
pBR322	Vector	*E. coli*; ~ 4361 bp; amp^R, tet^R, *ori*, *rop*
ori	Vector feature	Origin of replication; sets copy number
Selectable marker	Vector feature	Antibiotic-resistance gene (amp^R, tet^R, kan^R)
Insertional inactivation	Selection technique	Insert disrupts marker; recombinants lose that resistance
β-galactosidase (*lacZ*)	Chromogenic marker	Blue/white screening on X-gal plate
Ti plasmid	Vector	*Agrobacterium tumefaciens*; delivers T-DNA into dicot plants
Disarmed retrovirus	Vector	Animal cells; gene therapy
Competent cell	Host state	Ca^{2+}-treated $\Rightarrow$ DNA-uptake capable
Heat shock	Transformation step	Ice $\rightarrow 42\,^{\circ}$C (~ 45 s) $\rightarrow$ ice
Biolistics / gene gun	Delivery method	DNA-coated gold/tungsten particles into plant cells

Term / Tool	Category	One-line definition / key datum
Micro-injection	Delivery method	Direct injection into animal-cell nucleus
Gel electrophoresis	Separation	Agarose; DNA $\rightarrow$ anode; smaller fragments move farther
Ethidium bromide	Stain	Intercalator; orange bands under UV
Elution	Recovery	Cut band out, extract DNA
Lysozyme / cellulase / chitinase	Lysis enzymes	Break bacterial / plant / fungal cell wall
RNase / protease	Cleanup	Remove RNA / protein from DNA prep
Chilled ethanol	Precipitant	Fibrous DNA spool
PCR	Amplification	$N = N_0 \times 2^n$; 30 cycles $\Rightarrow 10^9$ copies
Taq polymerase	Enzyme	*Thermus aquaticus*; thermostable; extends at $72\,^\circ$C
Primer	PCR component	$\sim$ 18–25 nt synthetic oligonucleotide; pair brackets target
T_m (Wallace rule)	Primer design	$T_m = 2(A+T) + 4(G+C)\,^\circ$C
Bioreactor	Equipment	100–1000 L vessel; biological $\rightarrow$ product conversion
Stirred-tank reactor	Bioreactor type	Impeller + sparged air + foam / pH / T control
Doubling time	Kinetics	$t_d = \ln 2/\mu$
Downstream processing	Stage	Separate, purify, formulate, trial, QC
Recombinant protein	Product	Gene from one species expressed in heterologous host