BP403T · B.PHARM THEORY

BP403T Pharmaceutical Biotechnology

Unit II — Genetic Engineering & Recombinant DNA Technology

Pills & Python Notes

Genetic Engineering · rDNA Technology · PCR · Recombinant Biopharmaceuticals

Syllabus coverage: Unit II

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Chapter 3 — Genetic Engineering Principles, rDNA Technology, PCR and Mutations

3.1 Learning Objectives

1. Define genetic engineering and recombinant DNA.
2. Describe the tools of rDNA technology (enzymes, vectors, hosts).
3. Outline the steps of making recombinant DNA and expressing a gene.
4. Explain PCR principle, steps and applications.
5. Define mutation and classify types of mutations and mutants.

3.2 Definitions

- **Genetic engineering:** Deliberately changing the genetic material (DNA) of an organism to give it a new, useful property — e.g., making bacteria produce human insulin.
- **Recombinant DNA (rDNA):** DNA made by joining pieces from different sources (e.g., a human gene pasted into a bacterial plasmid).
- **Vector:** A DNA carrier that transports a foreign gene into a host cell — most commonly a plasmid (small circular DNA of bacteria).
- **Host cell:** The living cell (bacterium, yeast, animal cell) that receives the recombinant DNA and manufactures the product.
- **PCR (Polymerase Chain Reaction):** A test-tube method that photocopies a chosen DNA piece millions of times.
- **Mutation:** A permanent change in the DNA sequence. **Mutant:** An organism carrying such a change.

3.3 Terminology

- **Restriction enzymes ("molecular scissors"):** Bacterial enzymes that cut DNA at specific sequences (e.g., EcoRI cuts at GAATTC), leaving "sticky ends" that can rejoin matching pieces.
- **DNA ligase ("molecular glue"):** Enzyme that seals the cut ends together.
- **Cloning:** Making identical copies — of a gene, a cell, or (in this context) a DNA fragment inside host cells.
- **Expression:** The host cell actually reading the inserted gene and making the protein (insertion alone is not enough — the gene must be "switched on").
- **Primer:** A short starter piece of DNA that tells the PCR copying enzyme where to begin.
- **Taq polymerase:** The heat-proof DNA-copying enzyme from a hot-spring bacterium — the engine of PCR.

3.4 Explanation

The rDNA story in one line: Cut the wanted gene out, paste it into a plasmid, put the plasmid into bacteria, grow the bacteria, collect the product.

Tools of the trade:

1. **Restriction enzymes** cut both the donor DNA (containing our gene) and the plasmid vector at matching sites → complementary sticky ends.
2. **DNA ligase** glues the gene into the opened plasmid → recombinant plasmid.
3. **Transformation** — the plasmid is inserted into host cells (e.g., *E. coli* made permeable by calcium chloride and heat shock, or by electroporation).

4. **Selection** — plasmids carry a marker (e.g., antibiotic resistance gene); only cells that received the plasmid grow on antibiotic plates.
5. **Expression and harvest** — the culture is grown in fermenters; cells make the protein; the protein is purified.

Simple explanation: rDNA is cut-and-paste for genes. Scissors (restriction enzyme) cut out a paragraph (gene) from one book (human DNA) and glue (ligase) pastes it into a notebook (plasmid) that a student (bacterium) carries everywhere and reads aloud (expression).

PCR — the DNA photocopier (3 repeating steps):

1. **Denaturation (~94 °C):** Heat separates the double helix into two single strands (like unzipping).
2. **Annealing (~55 °C):** Cooling lets short primers stick to their matching spots on each strand.
3. **Extension (~72 °C):** Taq polymerase builds new strands starting from the primers.
   - Each cycle doubles the DNA: 1 → 2 → 4 → 8… after 30 cycles, over a billion copies. All done in a thermal cycler machine in a couple of hours.

PCR applications: detecting infections (TB, COVID-19, HIV viral load), forensic DNA testing, cancer mutation screening, checking food contamination, and amplifying genes for cloning.

Mutations and mutants:

- **Types of mutation by change:** point mutation (one letter changed; subtypes: silent — no effect, missense — different amino acid, nonsense — premature stop), insertion/deletion (letters added or removed — can cause frameshift), chromosomal (large-scale).
- **By cause:** spontaneous (natural copying errors) vs induced (radiation, chemicals called mutagens).
- **Types of mutants (useful ones!):** auxotrophic mutants (cannot make something they need — used to select engineered cells), antibiotic-resistant mutants (used as selectable markers), overproducing mutants (make extra of a product — prized in industry).

Analogy: DNA is a recipe book. A point mutation is a typo in one word (sometimes harmless, sometimes ruins the dish). PCR is a photocopier that copies only the page you point at, a billion times.

Key takeaway: Genetic engineering = cut (restriction enzymes), paste (ligase), deliver (vector into host), select (markers), produce (expression). PCR amplifies tiny DNA traces into detectable amounts through heat cycles. Mutations are DNA changes — some harmful, some industrially useful.

3.5 Classifications

- rDNA steps: isolation → cutting → ligation → transformation → selection → expression → purification.
- PCR steps: denaturation | annealing | extension.
- Mutations: point (silent/missense/nonsense) | insertion/deletion (frameshift) | chromosomal; spontaneous | induced.
- Mutants: auxotrophs | resistant mutants | overproducers | conditional (temperature-sensitive) mutants.

3.6 Mechanisms

Sticky-end ligation: EcoRI cuts GAATTC between G and A on both strands, leaving single-stranded overhangs (AATT). A gene cut with the same enzyme has matching overhangs; base-pairing joins them temporarily, then ligase forms the permanent phosphodiester bonds.

3.7 Diagrams (describe for drawing)

- Draw the rDNA pipeline: donor DNA + plasmid → cut with scissors → paste with glue → recombinant plasmid → into bacterium → colony on antibiotic plate → fermenter → purified protein.
- Draw one PCR cycle as a circular 3-step diagram with temperatures: 94 °C (unzip) → 55 °C (primers attach) → 72 °C (copy).

3.8 Flowcharts

rDNA workflow: Isolate gene → Cut gene + plasmid (restriction enzyme) → Ligate (DNA ligase) → Transform host → Select on antibiotic → Grow & express → Harvest & purify.

3.9 Infographic Concept Box

✂️ **CUT–PASTE–COPY:** Scissors = restriction enzymes, glue = ligase, notebook = plasmid, student = host cell. PCR = DNA photocopier: 94 °C unzip → 55 °C primers → 72 °C copy, ×30 cycles = billion copies. Mutation = DNA typo; mutants can be useful factory workers.

3.10 Tables

Tool	Nickname	Job
Restriction enzyme	Molecular scissors	Cuts DNA at specific sites
DNA ligase	Molecular glue	Seals cut ends
Plasmid	Delivery notebook	Carries gene into host
Taq polymerase	Heat-proof copier	Copies DNA in PCR

Mutation type	Change	Example effect
Silent	Letter changes, same amino acid	No effect
Missense	Different amino acid	May alter protein
Nonsense	Premature stop signal	Short, usually dead protein
Frameshift	Insertion/deletion shifts reading frame	Scrambled protein

3.11 Examples

- Insulin gene cut from human DNA and pasted into a plasmid, then into *E. coli* — the basis of modern insulin supply (details in Chapter 4).
- PCR detection of tuberculosis bacteria from a sputum sample in a district lab.

3.12 Analogies

- Plasmid as a USB pen drive carrying a file (gene) from one computer (human cell) to another (bacterium).

3.13 Pharmacy Relevance

- Every recombinant drug (insulin, interferons, vaccines, growth factors) is made by rDNA; pharmacists must understand the technology to explain biosimilars, handle cold-chain biologics, and interpret PCR-based diagnostic reports.

3.14 India Context

- India manufactures recombinant hepatitis-B vaccine and insulin at large scale, supplying immunization programmes and affordable diabetes care domestically and to other countries.

3.15 Key Points

1. Genetic engineering = deliberate DNA change; rDNA = joined DNA from different sources.
2. Tools: restriction enzymes (cut), ligase (paste), plasmid vector (carry), host cell (produce).
3. Steps: cut → ligate → transform → select (antibiotic marker) → express → purify.
4. PCR: denaturation (94 °C) → annealing (55 °C) → extension (72 °C); ~30 cycles = billion-fold copies.
5. Mutations: point (silent/missense/nonsense), frameshift, chromosomal; spontaneous or induced; useful mutants include auxotrophs and overproducers.

3.16 Common Confusions

- **Vector vs host:** Vector is the DNA carrier (plasmid); host is the living cell that receives it.
- **Transformation vs transfection:** Transformation = DNA into bacteria; transfection = DNA into animal cells.

3.17 Common Mistakes

- Saying PCR "makes proteins" — PCR copies DNA only; protein comes later via expression.
- Forgetting the selection step — without antibiotic markers you cannot find the few cells that received the plasmid.

3.18 Mnemonics

- rDNA steps: **"C-L-T-S-E"** — Cut, Ligate, Transform, Select, Express ("Cells Love To Share Everything").
- PCR temperatures: **"94-55-72"** — "Unzip (94), Attach (55), Build (72)".

3.19 Exam Summary

Genetic engineering deliberately alters DNA; recombinant DNA joins gene + vector using restriction enzymes and ligase, delivered into host cells and selected via markers for protein production. PCR amplifies specific DNA through repeated denaturation–annealing–extension cycles and is used in infection diagnosis, forensics and cloning. Mutations are permanent DNA changes classified as point, frameshift or chromosomal; useful mutants serve as industrial and selection tools.

3.20 One-Minute Revision

⚡ Cut (scissors) → paste (glue) → deliver (plasmid) → select (antibiotic) → produce (expression). PCR: 94 unzip, 55 attach, 72 build ×30 = billion copies. Mutation = DNA typo; mutants can be useful.

3.21 Exam Questions

Short answer:

1. Define rDNA and list its tools.
2. What are sticky ends?
3. Name the three PCR steps with temperatures.
4. Define mutant and give two useful types.

Long answer:

5. Describe the steps of recombinant DNA technology with a flowchart.
6. Explain the principle and applications of PCR.

MCQs:

7. Molecular scissors are: (a) ligase (b) restriction enzymes (c) primers (d) plasmids — Answer: (b)
8. Taq polymerase is used in PCR because it is: (a) cheap (b) heat-stable (c) fast only (d) human-made — Answer: (b)
9. A nonsense mutation causes: (a) no change (b) premature stop (c) frameshift (d) duplication — Answer: (b)

Viva:

10. Why does PCR need primers? (Polymerase can only extend an existing strand; primers mark the start.)

Application-based:

11. A lab wants to detect TB bacteria present in very low numbers in sputum. Which technique and why? (PCR — amplifies tiny DNA amounts to detectable levels.)

3.22 References

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- Primrose, S. B., Twyman, R. M. and Old, R. W. Principles of Gene Manipulation and Genomics. Wiley-Blackwell.

Chapter 4 — Production of Insulin, Interferons and Hepatitis-B Vaccine

4.1 Learning Objectives

1. Explain why recombinant insulin replaced animal insulin.
2. Describe the production of human insulin by rDNA technology.
3. Classify interferons and describe their production and uses.
4. Describe the production of recombinant hepatitis-B vaccine.
5. Compare conventional vs recombinant production of biopharmaceuticals.

4.2 Definitions

- **Human insulin:** The exact human hormone (51 amino acids in two chains, A and B, joined by disulfide bonds) that lowers blood glucose.
- **Interferons (IFNs):** Natural signalling proteins that "interfere" with viral replication and activate immune cells.
- **Hepatitis-B vaccine:** A vaccine containing the hepatitis-B surface antigen (HBsAg) that trains immunity without any live virus.
- **Subunit vaccine:** A vaccine containing only a piece of the pathogen (e.g., one protein), not the whole germ — very safe.

4.3 Terminology

- **Proinsulin:** The single-chain precursor that is cut to give mature two-chain insulin.
- **Inclusion bodies:** Clumps of misfolded protein inside *E. coli* that must be unfolded and refolded correctly during insulin manufacture.
- **IFN-α, IFN-β, IFN-γ:** The three main interferon families (alpha: antiviral; beta: antiviral/anti-inflammatory; gamma: immune activation).
- **HBsAg:** Hepatitis-B surface antigen — the protein piece used in the vaccine.
- **Yeast expression system:** Using yeast (*Saccharomyces cerevisiae* or *Pichia*) as host — a eukaryote that folds proteins better than bacteria.

4.4 Explanation

Why recombinant insulin was a revolution: Earlier, insulin came from cow/pig pancreas — slightly different from human insulin (could cause allergies), supply depended on slaughterhouses, and purification was hard. Recombinant human insulin is identical to human insulin, allergy risk is lower, and supply is unlimited.

Production of recombinant human insulin (two classic routes):

- **Route A — *E. coli*:** The A-chain and B-chain genes are cloned separately into bacteria. Each chain is made (often as inclusion bodies), then the chains are purified, refolded, and joined by forming the disulfide bonds chemically.
- **Route B — yeast:** The proinsulin gene is expressed in yeast, which secretes folded proinsulin; an enzyme then trims it to mature insulin.
- Both end with extensive purification and the full characterization battery from Chapter 1.

Simple explanation: Old insulin = borrowing slightly-wrong keys from animals. New insulin = manufacturing exact human keys in microbial factories.

Interferons: Discovered as substances that "interfere" with viruses. Types: IFN-α (from white blood cells, antiviral), IFN-β (from fibroblasts), IFN-γ (from T-cells, immune booster). Production: the human interferon gene is cloned into *E. coli* or yeast, expressed, and purified. Uses: chronic hepatitis B and C (historically), multiple sclerosis (IFN-β), some cancers, and immune disorders. Side effects like flu-like symptoms are common — an honest counselling point.

Hepatitis-B vaccine (the first recombinant vaccine success story):

1. The gene for HBsAg (surface protein of the virus) is cloned into yeast.
2. Yeast cells produce HBsAg, which self-assembles into virus-like particles (look like the virus surface but contain no viral DNA — cannot cause infection).
3. Particles are purified, adsorbed onto an aluminium adjuvant (which boosts the immune response), filled and QC-tested.
4. Given as injections (birth, 6, 10, 14 weeks in India's immunization schedule, plus catch-up doses).

Analogy: The hepatitis-B vaccine is like showing the police a photograph of a criminal's face (surface protein) — the body learns to recognize the face without ever meeting the criminal (no live virus).

Key takeaway: Insulin, interferons and hepatitis-B vaccine are flagship rDNA products: insulin replaced animal extracts, interferons weaponize the body's antiviral signals, and the Hep-B vaccine proved a single recombinant protein can safely immunize millions.

4.5 Classifications

- Insulin types: rapid-acting, short-acting (regular), intermediate (NPH), long-acting analogues (glargine, detemir) — by onset/duration.
- Interferons: Type I (IFN-α, IFN-β) | Type II (IFN-γ).
- Vaccines by technology: live-attenuated | inactivated | subunit/recombinant | toxoid | mRNA (newer).

4.6 Mechanisms

Insulin action (brief): Insulin binds its receptor on muscle/fat/liver cells → glucose transporters (GLUT4) move to the cell surface → blood glucose enters cells → blood sugar falls.

4.7 Diagrams (describe for drawing)

> - Draw proinsulin (single chain with C-peptide loop) → scissors cutting C-peptide → mature insulin (A + B chains with 2 disulfide bridges).
> - Draw HBsAg particles assembling in yeast → purification → vial labelled "Hep-B vaccine (recombinant)".

4.8 Flowcharts

> **Recombinant insulin (E. coli route):** Human insulin genes → Plasmids → *E. coli* → Fermentation → Harvest inclusion bodies → Solubilize & refold → Join A + B chains → Purify (chromatography) → Characterize → Formulate & fill.

4.9 Infographic Concept Box

> 💉 **rDNA BLOCKBUSTERS:** Insulin — exact human hormone, no more animal extracts. Interferons — body's own antiviral alarm, bottled. Hep-B vaccine — one yeast-made protein, zero live virus, millions protected. All made by: gene → plasmid → host → fermenter → purify → test.

4.10 Tables

Product	Host	Key step
Human insulin	*E. coli* / yeast	A+B chain joining / proinsulin trimming
Interferon-α	*E. coli* / yeast	Refolding from inclusion bodies
Hepatitis-B vaccine	Yeast	HBsAg self-assembly into particles

Insulin type	Onset	Use
Regular (short-acting)	~30 min	Mealtime control
NPH (intermediate)	1–2 h	Basal cover
Glargine (long-acting analogue)	Slow, ~24 h	Once-daily basal

4.11 Examples

- A type-1 diabetic student in Delhi takes basal glargine at night and rapid-acting insulin before meals — both recombinant.
- India's Universal Immunization Programme gives hepatitis-B vaccine to newborns.

4.12 Analogies

- Inclusion bodies: crumpled paper balls of protein that must be smoothed out (refolded) before use.

4.13 Pharmacy Relevance

- Pharmacists counsel on insulin storage (2–8 °C, in-use pen at room temperature per label), injection site rotation, hypoglycaemia signs, and vaccine schedules and cold-chain maintenance.

4.14 India Context

- India is among the world's largest producers of recombinant hepatitis-B vaccine and insulin, supplying its own immunization programme and exporting affordable biologics.

4.15 Key Points

1. Recombinant insulin = exact human sequence; replaced animal insulin (allergy + supply problems).
2. *E. coli* route joins A and B chains; yeast route trims proinsulin.
3. Interferons (α, β, γ) are antiviral/immune proteins made by rDNA; used in hepatitis, MS, cancers.
4. Hep-B vaccine = yeast-made HBsAg particles + aluminium adjuvant; no live virus.
5. All rDNA products need purification + full characterization before release.

4.16 Common Confusions

- **Subunit vs live vaccine:** Subunit (Hep-B) contains only a protein piece — cannot cause disease; live-attenuated contains weakened whole germ.
- **Insulin analogue vs animal insulin:** Analogues are slightly modified human insulin for better timing; animal insulin is a different species' hormone.

4.17 Common Mistakes

- Saying "insulin is extracted from bacteria" — bacteria MAKE it from an inserted human gene; it is not naturally theirs.
- Confusing interferons with interleukins — both are cytokines but different families and jobs.

4.18 Mnemonics

- rDNA blockbusters: **"I-I-H"** — Insulin, Interferons, Hepatitis-B vaccine.
- Insulin chain joining: **"A+B = Active"**.

4.19 Exam Summary

Recombinant human insulin (identical to human insulin) replaced animal insulin via *E. coli* (A+B chain joining) or yeast (proinsulin trimming) routes. Interferons α/β/γ are rDNA-made antiviral and immune-modulating proteins used in viral hepatitis, multiple sclerosis and cancers. Recombinant hepatitis-B vaccine consists of yeast-produced HBsAg particles with aluminium adjuvant — safe, with no live virus — and is part of national immunization.

4.20 One-Minute Revision

⚡ Insulin: human gene → bacteria/yeast → exact human hormone. Interferons: α/β antiviral, γ immune. Hep-B vaccine: yeast-made surface protein, no live virus. All: purify + characterize + cold chain.

4.21 Exam Questions

Short answer:

1. Why was animal insulin replaced?
2. What is HBsAg?
3. Name the three interferon types and one use each.
4. What is a subunit vaccine?

Long answer:

5. Describe the production of recombinant human insulin.
6. Describe the production and advantages of recombinant hepatitis-B vaccine.

MCQs:

7. HBsAg is produced in: (a) *E. coli* (b) yeast (c) plants (d) mice — Answer: (b)
8. Proinsulin is converted to insulin by: (a) heating (b) enzymatic trimming of C-peptide (c) PCR (d) filtration — Answer: (b)
9. IFN-β is mainly used in: (a) diabetes (b) multiple sclerosis (c) hypertension (d) asthma — Answer: (b)

Viva:

10. Why does the Hep-B vaccine need an adjuvant? (Purified protein alone gives a weak immune response; aluminium adjuvant boosts it.)

Application-based:

11. A patient on animal-origin insulin reports frequent rashes. What would you suggest to the prescriber and why? (Switch to recombinant human insulin — identical to human sequence, lower immunogenicity.)

4.22 References

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