

Unit 3: Genomic and Proteomic Tools, Recombinant DNA Technology and Gene Therapy

Free notes at pharmacolearn.fyi

3.1 DNA electrophoresis

Gel electrophoresis is the basic separation tool of the molecular laboratory. DNA carries a uniform negative charge on its phosphate backbone, so in an electric field it migrates toward the positive electrode through a gel that acts as a sieve: short fragments thread through the pores faster than long ones, and fragments separate strictly by size. For DNA the gel is **agarose**, its concentration setting the size range resolved; the run is read by staining with a fluorescent dye that binds DNA (ethidium bromide classically, safer modern alternatives now) and comparing band positions against a ladder of fragments of known size. The pharmacological uses are everyday ones: checking that a PCR worked and made a product of the expected size, verifying that a restriction enzyme cut a plasmid where it should, separating the alleles of a length polymorphism in genotyping, and the older forensic and paternity technique of DNA fingerprinting.

3.2 The polymerase chain reaction

PCR copies a chosen stretch of DNA in a tube, a billion fold, from starting amounts too small to detect. The reaction holds template DNA, two synthetic **primers** that bracket the target sequence, the four deoxynucleotide building blocks, buffer with magnesium, and a heat stable DNA polymerase (Taq, from the hot spring bacterium *Thermus aquaticus*) that survives the temperatures used to melt DNA apart. A thermal cycler repeats three steps:

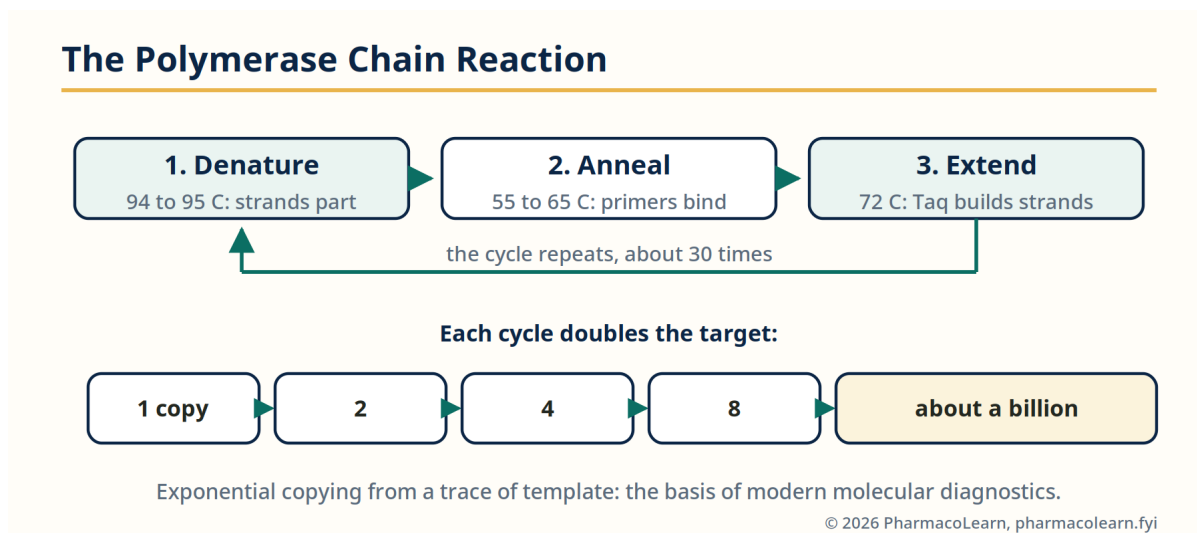


Figure: The PCR cycle. Heat separates the strands, primers mark the target, polymerase extends them; each cycle doubles the product, so about 30 cycles make about a billion copies.

Denaturation at about 94 to 95 degrees C separates the strands; cooling to roughly 55 to 65 degrees C lets the primers **anneal** to their complementary sites; at 72 degrees C the polymerase **extends** each primer along the template. Because the products of one cycle become templates of the next, growth is exponential. The applications define modern diagnostics: detecting pathogen DNA in blood and tissue, identifying mutations, quantifying templates in the real time form below, and preparing DNA for sequencing

and cloning.

3.3 Reverse transcription PCR and real time PCR

Two extensions of PCR carry separate names that examinations love to confuse. **Reverse transcription PCR (RT-PCR)** adds a first step in which the enzyme reverse transcriptase copies an RNA template into complementary DNA, after which ordinary PCR amplifies it. It is the only way to PCR an RNA target, so it detects RNA viruses and measures which genes a cell is expressing. **Real time PCR (quantitative PCR, qPCR)** watches the reaction as it happens: a fluorescent signal grows with the product, either from a dye such as SYBR Green that lights up on binding any double stranded DNA, or from a sequence specific probe (the TaqMan design, in which polymerase activity separates a reporter dye from its quencher only when the true target is amplified). The cycle at which fluorescence crosses a threshold, the **Ct value**, is inversely related to the starting amount: a low Ct means a lot of template. Combined, reverse transcription real time PCR is the standard for viral load measurement in HIV and hepatitis C, for the respiratory virus testing that became familiar during the COVID-19 pandemic (targeting SARS-CoV-2 RNA), and for quantitative gene expression studies.

3.4 Gene sequencing

Sequencing reads the base order of DNA. The classical **Sanger method** runs a copying reaction spiked with small amounts of **dideoxynucleotides**, modified bases that terminate the chain wherever they are incorporated; the result is a ladder of fragments each ending at a known base, separated by size and read off in order, in modern instruments by four fluorescent labels and capillary electrophoresis. It remains the accuracy standard for a single gene or a variant confirmation. **Next generation sequencing** changed the scale: millions of fragments are sequenced in parallel on a flow cell, the short reads are assembled by computer against the reference genome, and a whole exome or genome becomes a routine clinical order. The pharmacological yield is direct: sequencing finds the mutation behind a genetic disease, panels the mutations of a tumour to select targeted therapy (EGFR in lung cancer is the prototype), and supplies the pharmacogenomic genotypes of Unit 4.

3.5 DNA microarray technique

A **DNA microarray** measures the activity of thousands of genes in one experiment. A chip carries a grid of microscopic spots, each holding many copies of a probe DNA for one gene. Messenger RNA from the sample is converted to labelled complementary DNA and washed over the chip; each labelled molecule hybridises to the spot whose probe matches it, and a scanner reads the fluorescence at every spot as a measure of how strongly that gene was expressed. Comparing two samples, tumour and normal tissue, or cells with and without a drug, gives an expression signature across the genome at once. Microarrays classified leukaemias and breast tumours into molecular subtypes with different prognoses, identified the genes a drug switches on or off, and genotyped single nucleotide polymorphisms across the genome for the association studies of Unit 4. RNA sequencing now does the expression job with greater range, but the microarray remains the clearer teaching example of hybridisation based measurement.

3.6 SDS-PAGE: separating proteins by size

Proteins vary in charge as well as size, so the protein gel first forces them into a common shape. In **SDS-PAGE**, the detergent sodium dodecyl sulfate coats each protein with a uniform negative charge per unit length, and a reducing agent breaks disulfide bonds, so every protein becomes an unfolded rod whose migration through the **polyacrylamide** gel depends on size alone: small proteins run far, large proteins lag. Staining (Coomassie blue, or silver for sensitivity) reveals the bands, sized against a marker ladder. The technique answers everyday questions of molecular pharmacology: is the recombinant protein the right size

and how pure is it, which subunits make up a receptor preparation, has a protease cut its target, and it is the obligatory first half of the western blot. Two dimensional electrophoresis, separating first by charge and then by size, was the original engine of proteomics, resolving thousands of protein spots from one sample.

3.7 ELISA

The **enzyme linked immunosorbent assay (ELISA)** measures an antigen or an antibody by turning its binding into a colour. One partner is immobilised on the wells of a plastic plate; the sample is added and the target, if present, is captured; an enzyme labelled detection antibody (horseradish peroxidase or alkaline phosphatase) completes the complex; washing removes everything unbound; and a substrate is added that the enzyme converts into a coloured product, read as absorbance in a plate reader and quantified against standards. The format varies with the job:

Format	Arrangement	Typical use
Direct	Labelled antibody detects antigen fixed on the plate	Quick screens, less specific
Indirect	Unlabelled primary antibody, labelled secondary antibody	Detecting antibodies in a patient sample, signal amplified
Sandwich	Capture antibody on the plate, sample antigen, labelled detection antibody	Most specific and sensitive; cytokines, hormones, viral antigens
Competitive	Sample analyte competes with a labelled reference	Small molecules and haptens, thyroid hormones, some drug assays

ELISA is the workhorse serology of medicine: screening blood donations for HIV antibodies and hepatitis B surface antigen, the urine pregnancy test's laboratory ancestor for hCG, antibody titres after vaccination, cytokine measurement in research, and the screening tier of therapeutic drug and antidrug antibody monitoring for the biologics of Unit 4.

3.8 Western blotting

Western blotting identifies a specific protein within a mixture and reports its size at the same time. The sample is first separated by SDS-PAGE (section 3.6); the protein bands are then transferred electrically onto a membrane of nitrocellulose or PVDF that preserves their pattern; the membrane is blocked with protein solution so antibodies cannot stick non specifically; a primary antibody against the target protein is applied, then an enzyme or fluorescent labelled secondary antibody against the first; and a substrate reaction reveals a band exactly where the target sits. The blot answers questions no other single technique combines: is the protein present, is it the expected size (so is it cut, modified or the wrong isoform), and roughly how much is there compared with a control. It was the classic confirmatory test for HIV antibodies, is routine for verifying receptor and signaling protein expression (phosphorylated ERK and STAT blots are the standard readout of the pathways of Unit 2), and remains the credibility check behind antibody based claims in research. It is slow and semi quantitative, which is why ELISA and blotting are taught as a complementary pair: ELISA quantifies in bulk, the blot verifies identity.

3.9 Recombinant DNA technology: restriction enzymes and vectors

Recombinant DNA technology joins DNA from different sources in a carrier and lets a host cell copy it. The cutting tools are **restriction endonucleases**, bacterial enzymes that recognise short, usually palindromic, DNA sequences and cut them, many leaving short single stranded **sticky ends** that anneal to matching ends cut by the same enzyme; DNA ligase seals the joins permanently. The carrier is a **vector**, a DNA molecule able to enter a host cell and replicate there, carrying selectable markers (usually antibiotic resistance genes) so the rare cells that took it up can be found:

Vector	Host	Approximate insert size	Notes
Plasmid	Bacteria (E. coli)	Up to about 10 kb	The everyday cloning and protein production vector
Bacteriophage lambda	Bacteria	About 15 to 20 kb	Efficient delivery by infection
Cosmid	Bacteria	About 35 to 45 kb	Plasmid with phage packaging signals
Bacterial artificial chromosome (BAC)	Bacteria	About 100 to 300 kb	Stable carriage of whole genes with their control regions
Yeast artificial chromosome (YAC)	Yeast	Up to about 1 Mb	Largest capacity, more fragile and harder to handle

A cloning experiment is then simple in plan: cut vector and foreign DNA with matching enzymes, ligate, introduce into host cells, select on antibiotic medium, and screen the colonies for the insert. The pharmacology begins when the insert is a human gene and the host becomes a factory.

3.10 Applications of recombinant DNA technology

Recombinant production replaced extraction from animal tissues and cadavers with fermentation, and several foundation medicines exist only because of it:

MAKING A RECOMBINANT MEDICINE

```

human gene identified (Unit 4.1 cloning)
|
gene inserted into a plasmid vector
|
vector introduced into host cells (E. coli, yeast, mammalian cells)
|
fermentation: the host copies the gene and expresses the protein
|
protein purified, checked (SDS-PAGE, western blot), formulated
|
medicine: insulin, growth hormone, vaccine antigen, antibody

```

- **Recombinant human insulin** (1982) was the first medicine of the technology, made in E. coli, ending dependence on pig and cattle pancreases and their allergy and supply problems.
- **Recombinant growth hormone** replaced hormone extracted from cadaver pituitary glands, which had transmitted the prion disease Creutzfeldt-Jakob disease to some recipients.
- **Hepatitis B vaccine** is the viral surface antigen made in yeast, a subunit vaccine with no virus at any stage.
- Erythropoietin, granulocyte colony stimulating factor, interferons, tissue plasminogen activator and the monoclonal antibodies of Unit 4 all come from recombinant cell lines, usually mammalian cells such as Chinese hamster ovary cells where human style glycosylation matters.

3.11 Gene therapy: gene transfer techniques

Gene therapy treats disease by delivering a working gene, or a gene modifying tool, to the patient's cells. Strategy decides the technique. In **ex vivo** therapy, cells are removed, corrected in the laboratory and returned, which suits blood and immune cells that are easy to harvest and reinfuse. In **in vivo** therapy, the vector is given directly to the patient, by injection into a tissue such as the retina or muscle, or intravenously to reach the liver. The delivery vehicles divide into viral and non viral:

Vehicle	Properties	Strengths and limits
Retrovirus	Integrates into the host genome, needs dividing cells	Permanent expression; integration carries a cancer risk
Lentivirus	Integrates, infects dividing and non dividing cells	The ex vivo standard for blood stem cells and CAR-T manufacture
Adenovirus	Stays outside the genome, strongly immunogenic	High expression but transient; immune reactions limit repeat use
Adeno associated virus (AAV)	Stays mainly outside the genome, weakly immunogenic	Small cargo (about 4.7 kb); the in vivo standard for eye, muscle, liver and nerve targets
Non viral (plasmid, lipid nanoparticles, electroporation)	No viral proteins	Safer and repeatable, but delivery is far less efficient

3.12 Clinical applications and recent advances in gene therapy

Clinical gene therapy now has licensed products, and each one teaches a principle. **Voretigene neparvovec** (an AAV carrying the RPE65 gene, injected under the retina) restores a missing enzyme in an inherited blindness, showing in vivo therapy in an immune sheltered site. **Onasemnogene abeparvovec** (AAV9 carrying the SMN1 gene, one intravenous dose) treats spinal muscular atrophy in infants, showing systemic delivery across the blood brain barrier at a young age. Ex vivo therapy began with ADA deficient severe combined immunodeficiency, corrected in the patient's own blood stem cells, and matured into the **CAR-T cell** therapies, in which a patient's T cells are lentivirally equipped with a synthetic receptor against CD19 and returned to attack B cell cancers. The field's standing cautions come from its history: early retroviral trials in SCID-X1 cured the immune defect but caused leukaemia in some children when the vector integrated beside a growth gene, and a fatal immune reaction to an adenoviral vector in 1999 reset safety practice across the field. The **recent advance** the syllabus asks for is genome editing: in 2023 the first CRISPR based therapy, exagamglogene autotemcel, was approved for sickle cell disease and transfusion dependent beta thalassaemia, editing the patient's stem cells ex vivo to restart fetal haemoglobin production, the same delivery logic with a far more precise tool.

*More free M.Pharm notes and unit wise PDFs at pharmacolearn.fyi
Free video lectures on the PharmacoLearn YouTube channel*