

Introduction to Molecular Biology

Introduction to Molecular Biology Techniques

Key concepts, common methods, and practical considerations

1. Overview

Molecular biology explores the structure and function of nucleic acids (DNA, RNA) and proteins. Modern techniques enable researchers to:

Goal	Typical Technique(s)	What It Reveals
DNA/RNA isolation	Phenol?chloroform extraction, silica? column kits	Pure nucleic acids for downstream work
Amplification	PCR, RT?PCR, qPCR, LAMP	Generate many copies of a specific sequence
Cloning & manipulation	Restriction?enzyme cloning, Gibson assembly, CRISPR?Cas9	Insert, delete, or edit DNA in vectors or genomes
Sequencing	Sanger, Illumina (NGS), Oxford Nanopore	Determine nucleotide order
Expression analysis	Northern blot, RT?qPCR, RNA?seq, microarrays	Measure RNA levels
Protein analysis	Western blot, ELISA, mass spectrometry, flow cytometry	Detect and quantify proteins
Functional assays	Reporter genes, RNAi, CRISPR screens	Test gene function in cells or organisms

2. Sample Preparation

Step	Principle	Tips
Cell lysis	Disrupt membranes (detergents, mechanical shear)	Keep samples on ice; add protease/RNase inhibitors
Nucleic?acid purification	Bind nucleic acids to silica in presence of chaotropic salts; wash; elute	Use ethanol precipitation for large volumes; verify purity (A260/A280)
Quality check	Spectrophotometry, agarose gel electrophoresis, Bioanalyzer	A260/A280 >1.8 (DNA) or 2.0 (RNA); RIN >7 for RNA?seq

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3. Polymerase Chain Reaction Techniques

Component	Function
Template DNA	Source of target sequence
Primers	Short oligos (18-30nt) that flank the region
DNA polymerase	Thermostable enzyme (e.g., Taq, Phusion)
dNTPs	Building blocks
Buffer + Mg ²⁺	Optimizes enzyme activity
Cycling conditions	Denaturation ? Annealing ? Extension (30-35 cycles)

Variants

Variant	When to use	Key feature
RT-PCR	RNA ? cDNA	Reverse transcriptase added
qPCR (real-time PCR)	Quantify DNA/RNA	Fluorescent dyes (SYBR) or probes (TaqMan)
Multiplex PCR	Detect several targets simultaneously	Multiple primer sets, careful optimization
LAMP	Field diagnostics	Isothermal amplification, high speed

4. DNA Cloning & Genome Editing

4.1 Traditional Restriction Enzyme Cloning

1. **Digest** vector and insert with compatible restriction enzymes.
2. **Purify** fragments (gel extraction).
3. **Ligate** with T4 DNA ligase.
4. **Transform** into competent bacteria; select on antibiotic plates.

4.2 Seamless Assembly (Gibson, In-Fusion, Golden Gate)

- Overlap-based methods that join fragments without scars.

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- Useful for large constructs, multi-gene pathways.

Techniques

4.3 CRISPR-Cas Systems

| Component | Role