LAB 1: Laboratory Equipment and Materials in Molecular Biology

Introduction: A molecular biology laboratory is designed specifically for the analysis of genetic material (DNA and RNA) and biomolecules. Unlike routine microbiology laboratories, molecular labs deal with very small quantities of nucleic acids, often in microliter or nanogram ranges.

Because enzymes such as DNA polymerase, reverse transcriptase, and restriction enzymes are extremely sensitive, even minor errors in handling or contamination can lead to false results. Therefore, understanding laboratory equipment is not optional—it is essential for experimental success and result reliability.

1. Micropipettes

Function: Measure and transfer microliter volumes with precision.

Types: P10 (0.5–10 µL), P200 (20–200 µL), P1000 (100–1000 µL).

Usage Notes:

1. Always select the correct pipette for the volume range.
2. Use sterile tips and change them between samples.
3. Pre-wet tips for accuracy.

Principle: Uses air displacement to aspirate and dispense precise liquid volumes.

2. Centrifuges

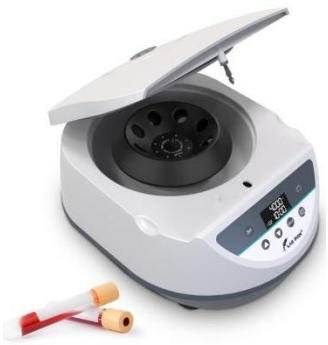
Function: Separate mixtures by density using high-speed rotation.

Principle: Applies centrifugal force to sediment particles based on size and density.

Types:

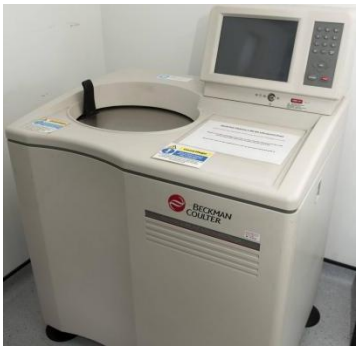
1. **Mini-centrifuge**

- **Speed range**: Typically up to 12,500 rpm (~9,800 × g).
- **Uses**:
    - Quick spin-down of droplets in PCR tubes.
    - Rapid separation of small particulates.
    - Useful for protocols requiring short, fast spins (e.g., microfilter separations).
- **Advantages**: Compact, easy to use, ideal for routine quick tasks.



2. **Bench-top centrifuge**

- **Speed range**: 3,000–20,000 rpm depending on rotor type.
- **Uses**:
    - Pelleting bacterial cells from culture.
    - Isolating nucleic acids (DNA/RNA) after extraction.
    - Protein precipitation and purification.
- **Advantages**: Versatile, accommodates larger sample volumes, widely used in molecular biology labs.



3. **Ultracentrifuge**

- **Speed range**: 100,000–150,000 rpm (forces exceeding 1,000,000 × g).
- **Uses**:
    - Separation of macromolecules such as proteins, nucleic acids, ribosomes, viruses, and vesicles.
    - Density gradient centrifugation for precise fractionation.
- **Advantages**: Extremely powerful, allows resolution of very small and delicate biological components.

Usage Notes:

1. Balance tubes opposite each other.
2. Use appropriate rotors and tube types.

3. Spectrophotometer / Nanodrop

Function: Measure DNA, RNA, and protein concentration and purity.

Key Values:

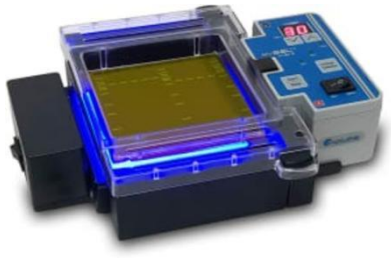
- DNA purity: A260/A280 ~1.8.
- RNA purity: A260/A280 ~2.0.

Usage Notes:

1. Clean pedestal with ethanol between samples.
2. Avoid bubbles in sample drops.

Principle: Measures absorbance of specific wavelengths by nucleic acids or proteins to quantify concentration.

4. Gel Electrophoresis Apparatus

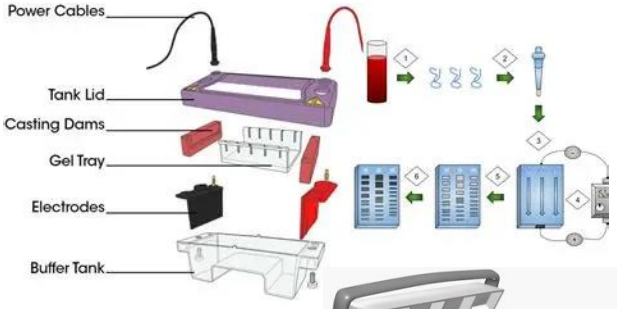


Function: Separate DNA, RNA, or proteins by size using an electric field.

Components: Gel tank, casting tray, combs, power supply.

Usage Notes:

1. Add loading dye to samples.
2. Use DNA ladder for size comparison.
3. Handle staining dyes carefully.



Principle: Molecules migrate through a gel matrix at rates inversely proportional to their size under an electric field.

5. Thermal Cycler (PCR Machine)

Function: Amplify DNA through cycles of denaturation, annealing, and extension.

Features: Programmable temperature cycles, heated lid.

Usage Notes:

1. Keep reagents on ice before loading.
2. Use sterile PCR tubes.

Principle: Uses temperature cycling to denature DNA 95C, anneal primers 45-60C, and extend new DNA strands 72C via polymerase.

6. Incubators and Shakers

Function: Maintain bacterial cultures at optimal temperature.

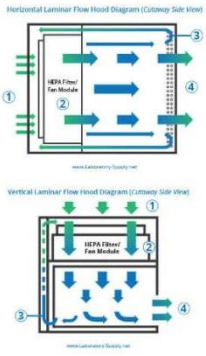
Types: Static incubator, shaking incubator.

Usage Notes:

1. Set correct temperature (usually 37°C for E. coli).
2. Label plates and flasks clearly.

Principle: Provides controlled temperature and agitation to promote microbial growth.

7. Laminar Flow Hood / Biosafety Cabinet



Function: Provide sterile environment for DNA/RNA work and cell culture.

Features: HEPA filter, controlled airflow.

Usage Notes:

1. Wipe surfaces with ethanol before and after use.
2. Work at least 10 cm inside the hood.

Principle: Uses HEPA-filtered airflow to prevent contamination.

8. Autoclave

Function: Sterilize media, glassware, and instruments using high-pressure steam.

Usage Notes:

1. Do not seal containers tightly.
2. Use autoclave tape to confirm sterilization.

Principle: Uses pressurized steam at high temperature to kill microorganisms.

9. Refrigerators and Freezers

1. 4°C: short-term storage.
2. −20°C: enzymes, primers, DNA samples.
3. −80°C: long-term storage of RNA, cell stocks.

Usage Notes:

1. Avoid repeated freeze-thaw cycles.
2. Use aliquots for sensitive reagents.

Principle: Low temperatures slow down enzymatic and microbial activity to preserve samples.

10. Electroporator / Heat Block

Electroporator: Introduce DNA into cells using electric pulses.

Heat Block: Maintain samples at specific temperatures for transformation.

Usage Notes:

1. Use sterile cuvettes.
2. Calibrate voltage settings.

Principle: Electroporator uses electrical pulses to create temporary pores in cell membranes; heat block provides consistent heat for reactions.

11. Consumables and Reagents

1. **Plasticware**: Microtubes, PCR tubes, pipette tips, Petri dishes.
2. **Glassware**: Beakers, flasks, cylinders.
3. **Reagents**: Buffers, agarose, acrylamide, DNA ladders.
4. **Enzymes**: Restriction enzymes, ligases, polymerases.
5. **Culture Media**: broth/agar, selective media with antibiotics.

LAB 2: DNA Isolation and Extraction

1. Introduction: DNA isolation is one of the most fundamental techniques in molecular biology. It provides the starting material for almost all downstream applications such as PCR, sequencing, cloning, and diagnostics. The goal is to obtain pure, intact DNA free from proteins, RNA, and other cellular contaminants. Different biological sources (bacteria, tissue, blood, plants) require adapted protocols, but the underlying principle remains the same.

Two related but distinct processes are used in the laboratory:

- **DNA Isolation**: The process of separating DNA from other cellular components (proteins, lipids, RNA, metabolites) to obtain a clean preparation.
- **DNA Extraction**: The physical removal of DNA from biological material (cells, tissues, blood, viruses) into solution, often using specific protocols tailored to the sample type.

2. Principle of Work: The process of DNA isolation relies on:

- **Cell lysis:** Breaking open the cell membrane and nuclear envelope to release DNA.
- **Removal of proteins and lipids:** Using detergents (SDS), proteases (Proteinase K), or organic solvents (phenol/chloroform).
- **DNA precipitation:** DNA is insoluble in alcohol (ethanol or isopropanol) in the presence of salts (NaCl, sodium acetate).
- **Purification and resuspension:** Washing the DNA pellet with 70% ethanol and dissolving it in a buffer (TE or nuclease-free water).

3. Methods of DNA Isolation

- **Organic extraction (phenol–chloroform):** High yield, but uses toxic chemicals.
- **Salting-out method:** Safer, relies on high salt concentration to precipitate proteins, leaving DNA in solution.
- **Silica column kits:** DNA binds to silica in the presence of chaotropic salts, then is washed and eluted. Fast and widely used in teaching and clinical labs.
- **Magnetic bead-based methods:** DNA binds to coated beads, allowing easy washing and elution. Increasingly common in automated systems.

4. DNA Extraction examples

A. DNA Extraction from Bacteria

Principle: Bacteria have rigid cell walls (especially Gram-positive), so enzymatic or chemical lysis is required before DNA can be released.

Materials:

- Bacterial culture (e.g., *E. coli*)
- Lysis buffer (Tris-HCl, EDTA, SDS)
- Lysozyme (for Gram-positive bacteria)
- Proteinase K
- RNase A (optional)
- Phenol:chloroform:isoamyl alcohol **(organic extraction)**
- Ethanol or isopropanol
- Tris EDTA (TE buffer)

Protocol:

1. Pellet 1–2 mL bacterial culture at 10,000 × g for 5 min.
2. Resuspend pellet in 500 µL lysis buffer.
3. Add lysozyme (Gram-positive) or proceed directly with SDS + Proteinase K.
4. Incubate at 55 °C for 30–60 min until lysate clears.
5. Add 500 µL phenol:chloroform:isoamyl alcohol, vortex 15 s, centrifuge 12,000 × g for 10 min.
6. Transfer aqueous phase (~400 µL) to new tube.
7. Add 50 µL sodium acetate + 1.25 mL cold ethanol. Mix gently, incubate −20 °C for 30 min.
8. Centrifuge 12,000 × g for 10 min, discard supernatant.
9. Wash pellet with 500 µL 70% ethanol, centrifuge, air-dry 5–10 min.
10. Resuspend DNA in 50–100 µL TE buffer

B. DNA Extraction from Viruses

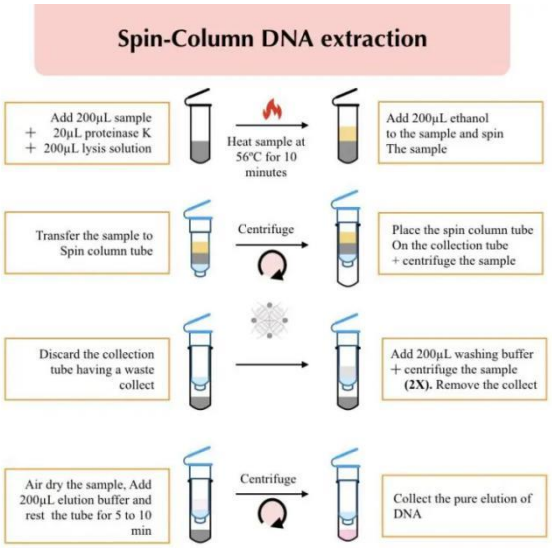
Principle: DNA viruses (e.g., adenovirus, herpesvirus) have their genomes protected by protein capsids or lipid envelopes. Lysis and protease digestion are required to release DNA.

Materials:

- Viral suspension
- Lysis buffer with SDS
- Proteinase K

- silica spin column kit
- Ethanol/isopropanol
- TE buffer

Protocol:



1. Mix 200 µL viral suspension + 200 µL lysis buffer + 20 µL Proteinase K. Incubate at 56 °C for 20 min.
2. Add 200 µL ethanol, mix well.
3. Transfer mixture to spin column, centrifuge 8,000 × g for 1 min. Discard flow-through.
4. Wash column with 500 µL wash buffer 1, centrifuge.
5. Wash with 500 µL wash buffer 2, centrifuge.
6. Dry spin at 12,000 × g for 2 min.
7. Place column in clean tube, add 50–100 µL elution buffer. Incubate 1 min, centrifuge.
8. Collect purified viral DNA.

C. DNA Extraction from Blood

Principle: DNA is obtained from **white blood cells (WBCs)**, since red blood cells lack nuclei. Hemoglobin and proteins must be removed for purity.

Materials:

- Whole blood (EDTA tube)
- RBC lysis buffer (NH_4Cl, $KHCO_3$, EDTA)
- WBC lysis buffer (Tris-HCl, EDTA, SDS)
- Proteinase K
- Saturated NaCl solution (salting-out)
- Isopropanol
- TE buffer

Protocol (Salting-Out Method):

1. Mix 2 mL blood + 6 mL RBC lysis buffer. Incubate 10 min at room temp. Centrifuge 500 × g for 10 min. Discard supernatant. Repeat until pellet is white (WBCs).
2. Resuspend WBC pellet in 500 µL WBC lysis buffer.
3. Add 20 µL Proteinase K, incubate at 55 °C for 1 hour.

4. Add 200 µL saturated NaCl, vortex, centrifuge 12,000 × g for 10 min. Proteins pellet.
5. Transfer supernatant to new tube. Add 1 mL cold isopropanol, mix gently. DNA precipitates as threads.
6. Centrifuge 12,000 × g for 10 min, discard supernatant.
7. Wash pellet with 500 µL 70% ethanol, centrifuge, air-dry.
8. Resuspend DNA in 100 µL TE buffer.

4. Genral Applications

- **Research:** PCR, cloning, sequencing, gene expression studies.
- **Clinical diagnostics:** Pathogen detection (bacteria, viruses, and fungi), genetic testing, cancer profiling.
- **Forensics:** DNA fingerprinting and identity verification.
- **Biotechnology:** Recombinant DNA technology, transgenic organism development.
- **Education:** Training students in molecular biology techniques.

5. Purity Verification: Two main approaches are used to check DNA quality:

A. NanoDrop Spectrophotometry

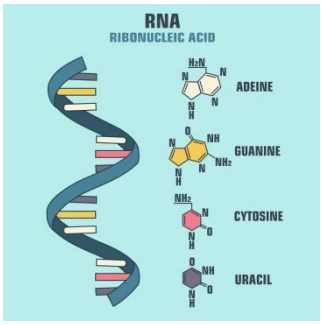
- DNA absorbs UV light at **260 nm**.
- **Concentration:** Calculated from absorbance (A260 × dilution factor × 50 µg/mL for dsDNA).
- **Purity ratios:** A260/A280 ≈ 1.8 → pure DNA (protein contamination lowers ratio).

B. Agarose Gel Electrophoresis

- DNA is negatively charged and migrates toward the positive electrode.
- **Intact genomic DNA:** Appears as a high-molecular-weight band.
- **Smearing:** Indicates degraded DNA.
- **Extra bands:** Suggest RNA contamination.
- Stains (ethidium bromide or SYBR Safe) allow visualization under UV light.

LAB 3 : RNA Isolation

1. **Introduction:** RNA isolation is a fundamental technique in molecular biology used to obtain pure RNA from cells, tissues, or blood. Unlike DNA, RNA is more fragile and prone to degradation by ubiquitous RNases. Therefore, RNA isolation requires **strict precautions** to prevent contamination and degradation. Pure RNA is essential for downstream applications such as RT-PCR, transcriptome analysis, RNA sequencing, and gene expression studies.



2. Principle of Work: The process of RNA isolation relies on:

- **Cell lysis:** Breaking open cells to release RNA.
- **Inactivation of RNases:** Using strong chaotropic agents (guanidinium salts) and β-mercaptoethanol to denature proteins and protect RNA.
- **Separation of RNA from DNA and proteins:** Often achieved by organic extraction (phenol/chloroform) or silica column binding.
- **RNA precipitation:** Using alcohol (isopropanol or ethanol) in the presence of salts.
- **Purification and resuspension:** Washing pellet with 70% ethanol and dissolving RNA in nuclease-free water.

3. Methods of RNA Isolation

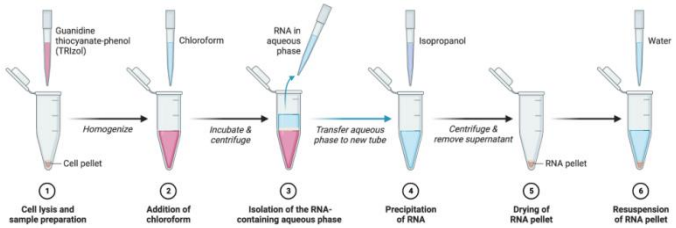
- **Organic extraction (TRIzol/phenol–chloroform):** High yield, widely used, but requires toxic reagents.
- **Silica column kits:** Safer, faster, RNA binds to silica in chaotropic salts, contaminants are washed away.
- **Magnetic bead-based methods:** RNA binds to beads, allowing automation and high throughput.

4. Example Protocols of RNA Isolation

A. RNA Isolation from Tissue/Cells (TRIzol Method)

Materials:

- Tissue sample (~20–50 mg) or cultured cells (~1 × 10^6).
- TRIzol reagent (1 mL per 50 mg tissue or 1 × 10^6 cells).
- Chloroform (200 µL).
- Isopropanol (500 µL).
- 70% ethanol (1 mL).
- Nuclease-free water (30–50 µL).



Steps:

1. Homogenize tissue or pellet cells in 1 mL TRIzol.
2. Incubate 5 min at room temperature.
3. Add 200 µL chloroform, shake vigorously 15 s.
4. Incubate 2–3 min, centrifuge 12,000 × g for 15 min.
5. Transfer aqueous phase (~500 µL) to new tube.
6. Add 500 µL isopropanol, incubate 10 min, centrifuge 12,000 × g for 10 min.
7. Wash pellet with 1 mL 70% ethanol, centrifuge 7,500 × g for 5 min.
8. Air-dry pellet, resuspend in 30–50 µL nuclease-free water.
9. Store at −80 °C.

B. RNA Isolation from Blood (Column Method)

Materials:

- 200 µL whole blood (EDTA tube).
- Lysis buffer with guanidinium salts (from kit).
- Proteinase K.
- Ethanol (200 µL).
- Spin column kit wash buffers (500 µL each).
- Elution buffer (30–50 µL nuclease-free water).

Steps:

1. Mix 200 µL blood with 600 µL lysis buffer + Proteinase K.
2. Incubate 10 min at room temperature.
3. Add 200 µL ethanol, transfer to spin column.
4. Centrifuge → RNA binds to silica.
5. Wash with buffer 1 and buffer 2.
6. Dry spin to remove ethanol.
7. Elute RNA with 30–50 µL nuclease-free water.
8. Store at −80 °C.

5. Purity Verification

- **NanoDrop spectrophotometry:**
    - RNA absorbs at 260 nm.
    - A260/A280 ≈ 2.0 → pure RNA.

- A260/A230 > 2.0 → minimal contamination.

- **Agarose gel electrophoresis:**
  - Intact RNA shows two sharp rRNA bands (28S and 18S in eukaryotes).
  - Smearing indicates degraded RNA.

6. Applications

- Reverse transcription PCR (RT-PCR).
- RNA sequencing (NGS).
- Gene expression profiling.
- Transcriptome studies.
- Detection of RNA viruses.

Functional studies of non-coding RNAs.

LAB 4: Quantification of DNA and RNA Concentration with NanoDrop

1. Introduction: Quantification of nucleic acids is a critical step in molecular biology. Accurate measurement of DNA and RNA concentration and purity ensures that downstream applications (PCR, qPCR, sequencing, cloning, transcriptomics) are performed with the correct template amount and without contamination. The NanoDrop spectrophotometer is a micro-volume UV-Vis instrument that allows rapid measurement of DNA and RNA concentration and purity using only 1–2 µL of sample. Unlike traditional cuvette-based spectrophotometers, NanoDrop uses a pedestal system, making it ideal for teaching labs and research settings.

2. Principle of Work: The NanoDrop spectrophotometer works on the principle of UV absorbance spectroscopy:

- **Nucleic acids absorb UV light at 260 nm** because of the aromatic nitrogenous bases (adenine, thymine, cytosine, guanine, uracil).
- The instrument measures absorbance across a spectrum (220–350 nm) and identifies peaks at 260 nm (nucleic acids), 280 nm (proteins, due to tryptophan and tyrosine residues), and 230 nm (organic compounds, salts, phenol, carbohydrates).
  - **Conversion factors** are standardized:
    - dsDNA: 1 A260 = 50 µg/mL
    - ssDNA: 1 A260 = 33 µg/mL
    - RNA: 1 A260 = 40 µg/mL

- **Purity ratios** are calculated:
- **A260/A280 ratio** → indicates protein contamination.
  - DNA ≈ 1.8, RNA ≈ 2.0.
- **A260/A230 ratio** → indicates contamination by salts, phenol, or carbohydrates.
- Both DNA and RNA should be > 2.0.

Thus, NanoDrop provides concentration (ng/µL) and purity ratios in seconds using only 1–2 µL of sample.

3. Materials & Equipment

- NanoDrop spectrophotometer.
- Nuclease-free water (blank).
- DNA and RNA samples (from previous isolation/extraction labs).
- Pipettes and sterile tips.

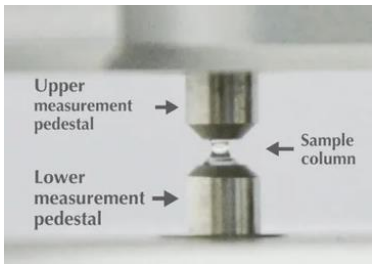
- Gloves and lint-free tissues for cleaning.

4. Procedure

Step 1: Instrument Setup

1. Turn on NanoDrop and select **Nucleic Acid** mode.
2. Clean both pedestal surfaces with nuclease-free water and lint-free tissue.
3. Perform a **blank measurement** using 1–2 µL nuclease-free water. This sets the baseline.

Step 2: Sample Measurement



1. Pipette 1–2 µL of DNA or RNA sample onto the lower pedestal.
2. Lower the arm to form a liquid column.
3. The instrument automatically scans absorbance from 220–350 nm.
4. Record displayed values: concentration (ng/µL), A260/A280, A260/A230.

Step 3: Cleaning Between Samples

- Wipe pedestal with nuclease-free water and tissue.
- Avoid bubbles or residual sample carryover.

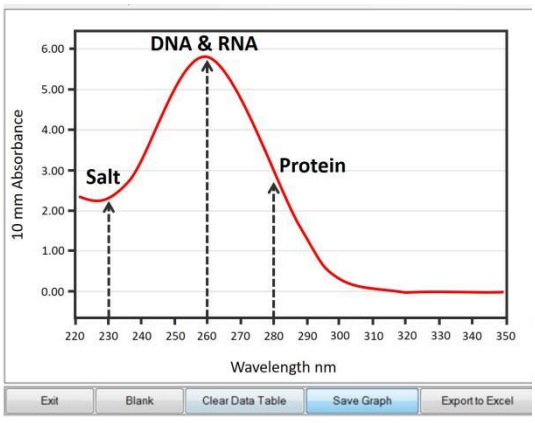
5. Interpretation of Results

A. DNA

- Example: A260 = 0.2 → 0.2 × 50 µg/mL = 10 µg/mL.
- **Purity check:**
  - A260/A280 ≈ 1.8 → pure DNA.
  - Lower ratio (<1.6) → protein contamination.
  - High ratio (>2.0) → RNA contamination.

B. RNA

- Example: A260 = 0.25 → 0.25 × 40 µg/mL = 10 µg/mL.
- **Purity check:**
  - A260/A280 ≈ 2.0 → pure RNA.
  - Lower ratio (<1.8) → protein contamination.
  - Low A260/A230 (<1.8) → salt or phenol contamination.



C. Spectral Curve

- Smooth peak at 260 nm → nucleic acids present.

- Shoulder at 280 nm → protein contamination.
- Broad absorbance at 230 nm → organic/salt contamination.

6. Applications

- **PCR/qPCR:** Ensuring correct DNA template concentration.
- **Sequencing:** Accurate input for Sanger or NGS libraries.
- **Cloning:** Reliable DNA concentration for ligation and transformation.
- **Transcriptomics:** RNA quantification for RT-PCR and RNA-seq.
- **Diagnostics:** Viral RNA/DNA quantification for clinical assays.
- **Education:** Teaching students how to assess nucleic acid quality.

7. Examples

Example 1: DNA Sample

- **Absorbance values:**
  - A260 = 0.25
  - A280 = 0.14
  - A230 = 0.10
- **Concentration calculation:**
  DNA concentration=A260*50 ug/mL
  0.25 * 50=12.5 µg/mL or = 12,5 ng/µL (since NanoDrop reports ng/µL).

Purity ratios:

- A260/A280 = 0.25 ÷ 0.14 = 1.79 → acceptable for DNA.
- A260/A230 = 0.25 ÷ 0.10 = 2.5 → good, minimal contamination.

Example 2: RNA Sample

- **Absorbance values:**
  - A260 = 0.30
  - A280 = 0.15
  - A230 = 0.12
- **Concentration calculation:**
  RNA concentration=A260 * 40 ug/mL
  0.30 * 40=12 µg/mL or = 12 ng/µL.
- **Purity ratios:**
  - A260/A280 = 0.30 ÷ 0.15 = 2.0 → ideal for RNA.
  - A260/A230 = 0.30 ÷ 0.12 = 2.5 → good, minimal contamination.

Example 3: Contaminated DNA Sample

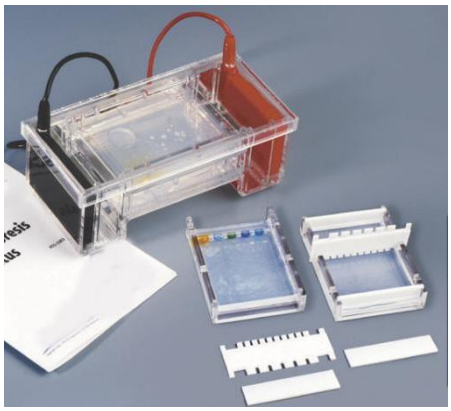
- **Absorbance values:**
  - A260 = 0.20
  - A280 = 0.20
  - A230 = 0.15
- **Concentration calculation:**
  0.20 * 50=10 µg/mL or = 10 ng/µL.
- **Purity ratios:**
  - A260/A280 = 0.20 ÷ 0.20 = 1.0 → too low, indicates protein contamination.
  - A260/A230 = 0.20 ÷ 0.15 = 1.33 → too low, indicates salt/phenol contamination.

LAB 5: Agarose Gel Electrophoresis

1. Introduction: Agarose gel electrophoresis is a fundamental technique in molecular biology used to separate, visualize, and analyze nucleic acids (DNA and RNA) based on size. It is widely applied in teaching labs, research, diagnostics, and forensics. The method relies on the migration of negatively charged nucleic acids through a porous agarose matrix under an electric field.

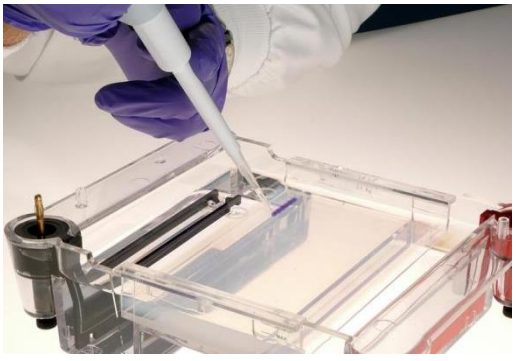
2. Principle of Work

- **Charge:** DNA and RNA are negatively charged due to phosphate groups; they migrate toward the positive electrode (anode).
- **Matrix:** Agarose gel acts as a molecular sieve; smaller fragments move faster, larger fragments move slower.
- **Visualization:** Using dyes (ethidium bromide, SYBR Safe, GelRed) bind nucleic acids and fluoresce under UV or blue light.
- **Size determination:** Migration distance is compared to a DNA ladder (molecular weight marker).



3. Materials and Equipment

- Agarose powder.
- Electrophoresis buffer 1X (TAE or TBE).
- DNA/RNA samples.
- DNA loading dye (contains glycerol and tracking dyes).
- DNA ladder (molecular weight marker).
- Ethidium bromide (EtBr) or safer alternatives (SYBR Safe, GelRed).
- Gel casting tray and comb.
- Electrophoresis chamber and power supply.
- UV transilluminator or gel documentation system.



4. Procedure

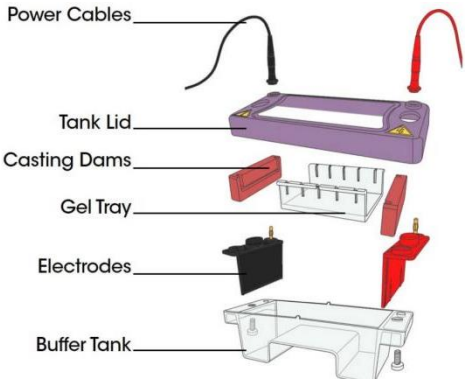
Step 1: Gel Preparation

1. Weigh agarose powder (e.g., 1 g for 100 mL of 1% gel).
2. Dissolve in 100 mL of 1X TAE or TBE buffer by heating (microwave or hot plate).
3. Cool to ~60 °C, add DNA stain (EtBr: 0.5 µg/mL or SYBR Safe as per manufacturer).
4. Pour into casting tray with comb inserted.

5. Allow gel to solidify (20–30 min). Remove comb to create wells.

Step 2: Sample Preparation

1. Mix DNA sample with 6× loading dye (e.g., 5 µL DNA + 1 µL dye).
2. Prepare DNA ladder in the same way.

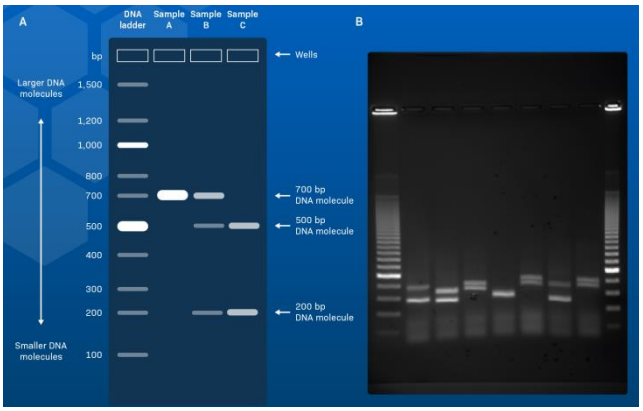


Step 3: Electrophoresis

1. Place gel in electrophoresis chamber, cover with buffer (TAE/TBE).
2. Load samples and DNA ladder into wells carefully.
3. Connect electrodes (DNA migrates toward positive electrode).
4. Run at 80–120 V for 30–45 min (depending on gel size and fragment length).

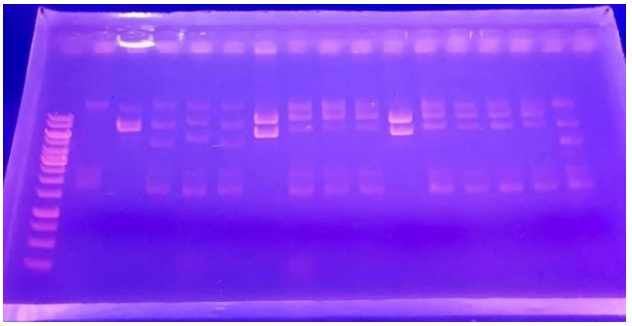
Step 4: Visualization

1. After run, place gel on UV transilluminator or blue light system.
2. Observe bands: DNA fragments fluoresce under UV.
3. Compare sample bands to DNA ladder to estimate fragment sizes.



5. Applications

- Checking DNA integrity after isolation.
- Confirming PCR product size.
- Restriction digestion analysis.
- RNA integrity assessment.
- Forensic DNA profiling.
- Teaching students nucleic acid separation principles.



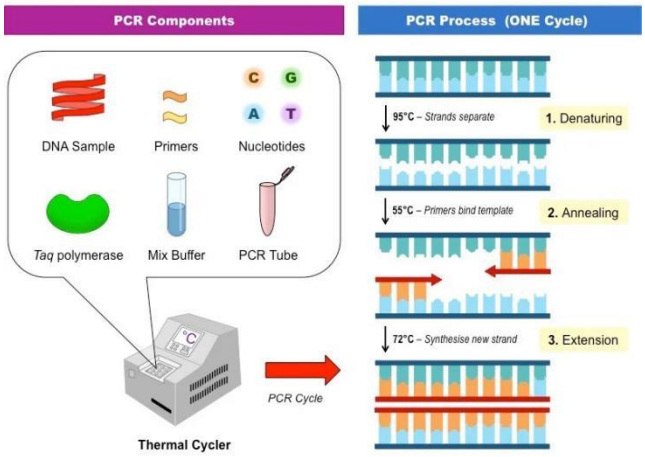
LAB 6: Polymerase Chain Reaction (PCR) Steps

1. Introduction: Polymerase Chain Reaction (PCR) is one of the most powerful techniques in molecular biology. It allows amplification of specific DNA fragments from minute amounts of template, producing millions of copies in just a few hours. PCR is essential for diagnostics, cloning, sequencing, forensic analysis, and research.

2. Principle of Work: PCR mimics natural DNA replication but in a controlled, cyclic manner:

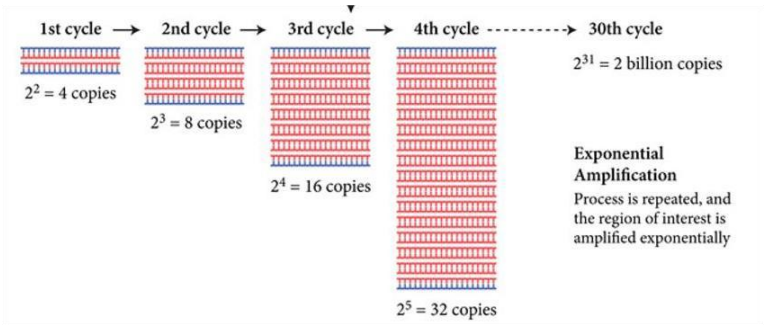
- **Template DNA** provides the sequence to be amplified.
- **Primers** (short synthetic oligonucleotides) define the start and end points of amplification.
- **DNA polymerase** (commonly Taq polymerase) synthesizes new DNA strands.
- **dNTPs** (nucleotides) are the building blocks.
- **Buffer and Mg^{2+} ions** provide optimal conditions.
- **Thermal cycling** alternates between denaturation, annealing, and extension to exponentially amplify DNA.

3. PCR Reaction Mixture (Typical Volumes for 25 µL Reaction)



- Template DNA: 1–2 µL (10–100 ng).
- Forward primer: 1 µL (10 µM).
- Reverse primer: 1 µL (10 µM).
- dNTPs: 0.5 µL (10 mM stock; final 200 µM each).
- 10× PCR buffer: 2.5 µL.
- $MgCl_2$: 1.5 µL (25 mM stock; final 1.5–2.5 mM).
- Taq DNA polymerase: 0.25 µL (5 U/µL stock; final 1.25 U).
- Nuclease-free water: up to 25 µL total volume.

4. PCR Steps (Thermal Cycling)



Step 1: Initial Denaturation

- Temperature: 94–95 °C
- Time: 2–5 minutes
- Purpose: Fully denature double-stranded DNA into single strands.

Step 2: Denaturation (per cycle)

- Temperature: 94–95 °C

- Time: 30 seconds
- Purpose: Separate DNA strands at the start of each cycle.

Step 3: Annealing (per cycle)

- Temperature: 50–65 °C (depends on primer Tm)
- Time: 30 seconds
- Purpose: Primers bind (anneal) to complementary sequences on single-stranded DNA.

Step 4: Extension (per cycle)

- Temperature: 72 °C (optimal for Taq polymerase)
- Time: 30–60 seconds (depending on fragment length; ~1 kb/min).
- Purpose: DNA polymerase extends primers, synthesizing new DNA strands.

Note: Typical cycle number: For Step2, Step3, and Step4 30–35 cycles → exponential amplification.

Step 5: Final Extension

- Temperature: 72 °C
- Time: 5–10 minutes
- Purpose: Ensure complete extension of all products.

Step 6: Hold

- Temperature: 4 °C
- Purpose: Preserve amplified DNA until collection.

5. Visualization of PCR Products: PCR products are typically analyzed using agarose gel electrophoresis:

- **Gel concentration:**
    - 1–2% agarose gel is standard for fragments between 100–1000 bp.
    - Lower % (0.8%) for larger fragments (>1 kb).
    - Higher % (2–3%) for small fragments (<200 bp).
- **DNA stain:**
    - Ethidium bromide (EtBr, 0.5 µg/mL) or safer alternatives (SYBR Safe, GelRed).
    - Stains intercalate into DNA and fluoresce under UV or blue light.
- **DNA ladder (marker):**
    - Loaded alongside samples to estimate fragment size.
    - Contains fragments of known lengths (e.g., 100 bp, 500 bp, 1 kb).

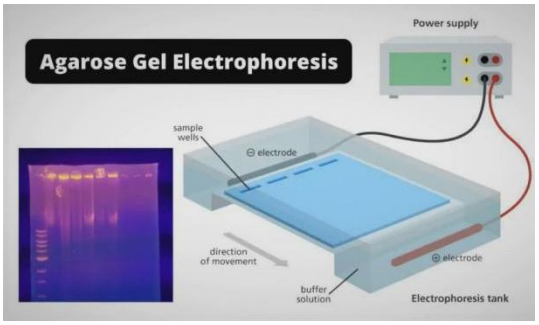
- **Loading dye:**
    - Mixed with PCR product before loading.
    - Contains glycerol (to sink sample into wells) and tracking dyes (bromophenol blue, xylene cyanol).
- **Electrophoresis conditions:**
- Run gel at 80–120 V for 30–45 minutes.
- DNA migrates toward the positive electrode

6. PCR Result Interpretation: When PCR products are analyzed by agarose gel electrophoresis, interpretation focuses on band presence, size, and quality:

- **Presence of bands:**
    - Confirms whether amplification occurred.
    - Absence of bands indicates failed amplification or technical issues.
- **Band size:**
    - Bands should correspond to the expected fragment length defined by primer design.
    - Comparison with a DNA ladder allows estimation of product size.
- **Band intensity:**
    - Strong, sharp bands indicate efficient amplification.
    - Faint bands suggest low template concentration or suboptimal reaction conditions.
- **Specificity:**
    - A single band at the expected size indicates specific amplification.
    - Multiple bands suggest non-specific primer binding or contamination.
- **Quality of bands:**
    - Clear, discrete bands reflect good reaction conditions.
    - Smearing indicates degraded template, excessive cycles, or poor reagent quality.
    - Very small bands near the bottom of the gel may represent primer-dimers.

7. Applications

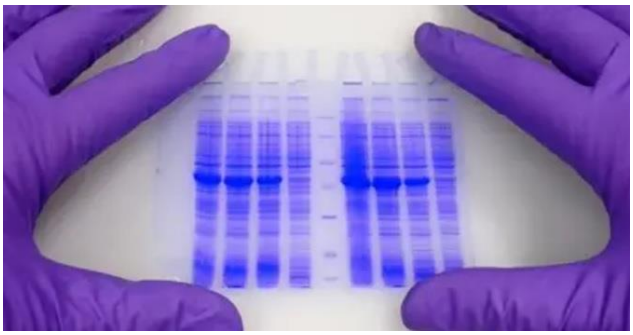
- **Diagnostics:** Detecting pathogens (viral, bacterial DNA).
- **Forensics:** DNA fingerprinting, identity testing.
- **Research:** Gene cloning, mutagenesis, sequencing.
- **Medicine:** Genetic testing, cancer mutation analysis.
- **Education:** Teaching DNA amplification principles.



LAB 7: Polyacrylamide Gel Electrophoresis (PAGE)

1. Introduction: Polyacrylamide Gel Electrophoresis (PAGE) is a widely used technique in molecular biology and biochemistry for the separation of proteins, DNA, and RNA. Compared to agarose gels, polyacrylamide gels provide higher resolution due to their smaller pore size, making them ideal for analyzing small nucleic acids and proteins. PAGE can be performed under native conditions (preserving structure) or denaturing conditions (e.g., SDS-PAGE for proteins, urea-PAGE for RNA).

2. Principle of Work: PAGE separates biomolecules based on their migration through a polyacrylamide matrix under an electric field.



- **Gel Matrix Formation:**
  - Acrylamide monomers polymerize with bis-acrylamide cross-linker to form a porous gel.
  - The pore size is controlled by acrylamide concentration (%T) and cross-linker ratio (%C).
  - Example: 10% acrylamide gel separates proteins in the 20–100 kDa range.
- **Electric Field:**
  - Molecules migrate toward the electrode of opposite charge.
  - **Proteins:** Migration depends on size, charge, and shape.
  - **DNA/RNA:** Negatively charged due to phosphate groups, migrate primarily by size.

3. Types of PAGE

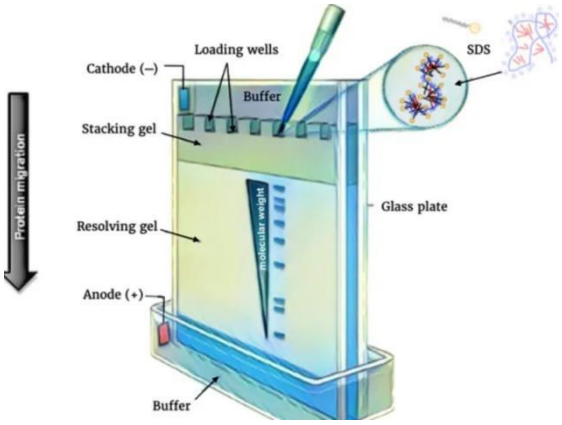
- **Native PAGE:** Molecules retain natural structure and charge; separation reflects both size and charge.
- **SDS-PAGE:** SDS detergent denatures proteins and imparts uniform negative charge, so migration depends only on molecular weight.
- **Urea-PAGE:** Urea denatures RNA secondary structures, allowing accurate size separation.
- **Stacking and Resolving Gel System (for proteins):**
  - **Stacking gel (low acrylamide, pH 6.8):** Concentrates proteins into sharp bands.
  - **Resolving gel (higher acrylamide, pH 8.8):** Provides actual size-based separation

4. Materials & Equipment

- Acrylamide/bis-acrylamide solution (30%).
- Tris buffer (pH 6.8 for stacking gel, pH 8.8 for resolving gel).
- SDS (for denaturing PAGE).

- Ammonium persulfate (APS) and TEMED (polymerization catalysts).
- Protein or nucleic acid samples.
- Sample loading buffer (Laemmli buffer for proteins).
- Molecular weight marker (protein ladder or DNA ladder).
- Electrophoresis apparatus and power supply.
- Staining reagents (Coomassie Blue, silver stain, ethidium bromide, SYBR Green).

5. Procedure (SDS-PAGE for Proteins)



Step 1: Gel Preparation

1. Prepare resolving gel (e.g., 10% acrylamide for proteins 20–100 kDa).
2. Pour resolving gel into gel cassette, overlay with water, allow to polymerize.
3. Prepare stacking gel (4% acrylamide), pour on top, insert comb to form wells.
4. Allow gel to polymerize (30 min).

Step 2: Sample Preparation

1. Mix protein sample with 2× Laemmli buffer (contains SDS, glycerol, bromophenol blue, β-mercaptoethanol).
2. Heat at 95 °C for 5 min to denature proteins.

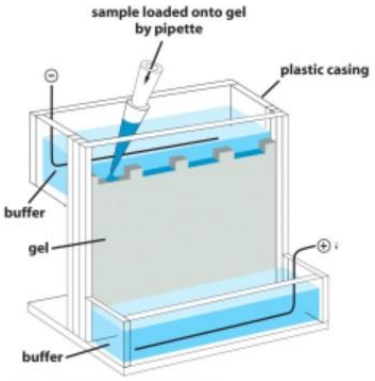
Step 3: Electrophoresis

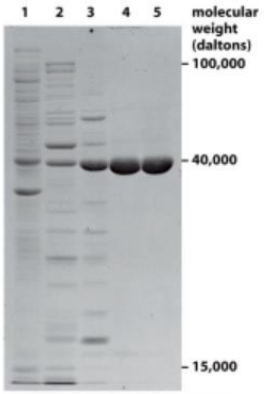
1. Place gel in electrophoresis chamber, add running buffer (Tris-glycine-SDS).
2. Load samples and protein ladder into wells.
3. Run gel at 80 V until samples enter resolving gel, then increase to 120 V.
4. Continue until dye front reaches bottom.

Step 4: Visualization

A. Protein Visualization

- **Coomassie Brilliant Blue:**
  - Most common stain.
  - Detects proteins in microgram quantities.
  - Simple and inexpensive.





- **Silver Stain:**
    - More sensitive than Coomassie (nanogram detection).
    - Used for low-abundance proteins.
- **Fluorescent Dyes (SYPRO Ruby, etc.):**
    - High sensitivity.
    - Compatible with imaging systems.

6. Result Interpretation

- **Band presence:** Confirms protein or nucleic acid sample.
- **Band size:** Compare migration distance to molecular weight marker.
- **Band intensity:** Reflects relative abundance.
- **Single sharp band:** Indicates purity.
- **Multiple bands:** Suggests mixture or degradation.
- **Smearing:** Indicates poor sample quality or overloading.

7. Applications

- Protein molecular weight determination.
- Checking protein purity.
- Detecting post-translational modifications.
- RNA integrity analysis (urea-PAGE).
- DNA fragment resolution (oligonucleotides, SSR markers).
- Forensic and diagnostic testing.

LAB 8: Hybridization Techniques

1. Introduction: Hybridization is a cornerstone technique in molecular biology used to detect, identify, and quantify specific DNA or RNA sequences. It exploits the natural property of complementary base pairing: adenine pairs with thymine (or uracil in RNA), and guanine pairs with cytosine. Hybridization methods are essential in genomics, transcriptomics, diagnostics, and cytogenetics. They form the basis of techniques such as Southern blotting, Northern blotting, in situ hybridization (ISH), fluorescence in situ hybridization (FISH), and microarray analysis.

2. Principle of Work

- **Denaturation:** Double-stranded nucleic acids are separated into single strands by heat or chemicals.
- **Hybridization:** A labeled probe (DNA, RNA, or oligonucleotide) binds specifically to its complementary target sequence.
- **Detection:** The probe is visualized using radioactivity, fluorescence, or chemiluminescence.

Hybridization thus provides specificity (sequence recognition) and sensitivity (signal detection).

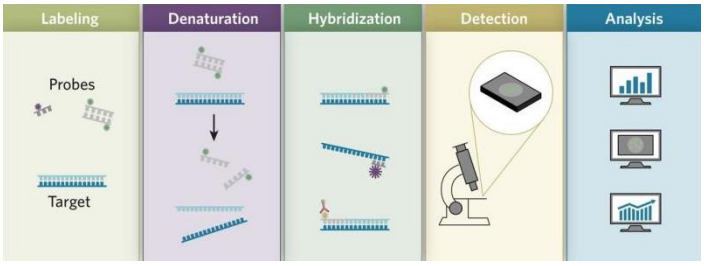
3. Types of Hybridization Techniques

A. **Southern Blotting**

- Detects specific DNA sequences.
- Workflow: DNA digestion → gel electrophoresis → transfer to membrane → probe hybridization → detection.
- Applications: Gene mapping, mutation detection, transgene confirmation.

B. **Northern Blotting**

- Detects specific **RNA sequences**.
- Workflow: RNA isolation → gel electrophoresis → transfer → probe hybridization → detection.
- Applications: Gene expression analysis, transcript size determination.



C. **In Situ Hybridization (ISH)**

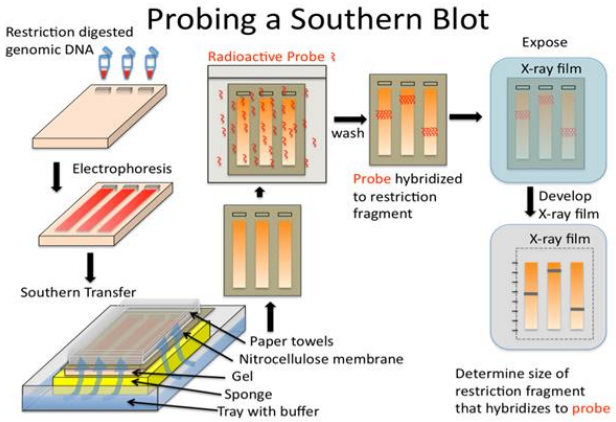
- Detects nucleic acids **within cells or tissues**.
- Probe hybridizes directly to target sequence in fixed samples.
- Applications: Localizing gene expression, detecting viral genomes in tissues.

D. **Fluorescence In Situ Hybridization (FISH)**

- Uses fluorescent probes for visualization under a microscope.
- Applications: Chromosome mapping, detecting genetic abnormalities, cancer diagnostics.

4. Materials & Equipment

- Target nucleic acids (DNA or RNA).
- Labeled probes (radioactive, fluorescent, or enzyme-linked).
- Denaturation agents (heat).
- Hybridization buffers (SDS).
- Membranes (nitrocellulose or nylon).
- Detection systems (autoradiography, fluorescence scanner, chemiluminescence).
- Microscopes (for ISH/FISH).



5. Procedure (General Hybridization Workflow)

Step 1: Sample Preparation

- Isolate DNA or RNA.
- Denature to single strands.
- Immobilize on membrane or prepare tissue sections.

Step 2: Probe Preparation

- Design probe complementary to target sequence.
- Label probe (radioactive, fluorescent, or enzyme-linked).

Step 3: Hybridization

- Incubate probe with sample under controlled stringency conditions.
- Allow probe to bind to complementary target sequence.

Step 4: Washing

- Remove unbound or non-specific probes.
- Adjust stringency to ensure specificity.

Step 5: Detection

- Visualize probe binding using autoradiography, fluorescence microscopy, or chemiluminescence.
- Analyze band patterns, signals, or fluorescence spots.

6. Visualization Methods

- **Radioactive probes:** Detected by autoradiography (high sensitivity, but safety concerns).

- **Fluorescent probes:** Visualized under fluorescence microscope or scanner (safe, multicolor detection possible).
- **Enzyme-linked probes:** Produce colorimetric or chemiluminescent signals (e.g., alkaline phosphatase, HRP).

7. Result Interpretation

- **Presence of signal:** Confirms target sequence is present.
- **Signal intensity:** Reflects abundance of target nucleic acid.
- **Signal location (ISH/FISH):** Indicates spatial distribution of gene expression or chromosomal region.
- **Multiple signals:** Suggests gene families, isoforms, or contamination.
- **No signal:** Target absent, degraded sample, or probe mismatch.

8. Applications

- Gene mapping and mutation detection.
- Gene expression profiling.
- Chromosome analysis (karyotyping with FISH).
- Pathogen detection (viral or bacterial genomes).
- Cancer diagnostics (oncogene amplification, translocations).
- Comparative genomics and evolutionary studies.

LAB 9: Comet Assay

1. Introduction: The Comet Assay, also known as Single Cell Gel Electrophoresis (SCGE), is a sensitive technique used to measure DNA damage and repair at the level of individual cells. It is widely applied in genotoxicity testing, environmental monitoring, cancer research, and molecular medicine. Cells with damaged DNA, when subjected to electrophoresis, produce a characteristic image resembling a comet:

- **Head:** Represents intact DNA.
- **Tail:** Represents fragmented or damaged DNA migrating away from the nucleus.

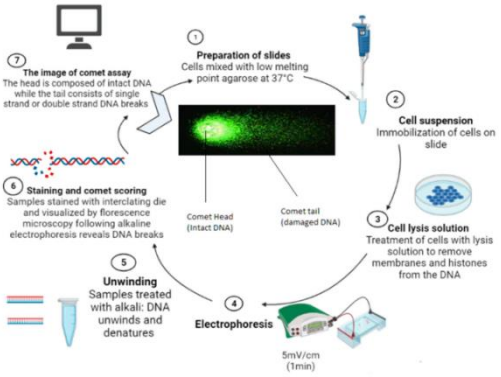
2. Principle of Work

- Cells are embedded in agarose on a microscope slide.
- Lysis removes membranes and proteins, leaving nucleoids containing supercoiled DNA.
- Electrophoresis under alkaline or neutral conditions causes fragmented DNA to migrate.
- DNA fragments form a comet tail, while intact DNA remains in the head.
- The extent of DNA migration reflects the degree of damage.
- Staining with fluorescent dyes (e.g., ethidium bromide, SYBR Green) allows visualization under a fluorescence microscope.

3. Types of Comet Assay

- **Alkaline Comet Assay:**
    - Detects single-strand breaks, double-strand breaks, alkali-labile sites.
    - Most commonly used.
- **Neutral Comet Assay:** Detects double-strand breaks specifically.

4. Materials & Equipment



- Low and normal melting point agarose.
- Microscope slides (pre-coated).
- Lysis buffer (with detergents and high salt).
- Electrophoresis buffer (alkaline or neutral).
- Electrophoresis chamber and power supply.
- Neutralization buffer (Tris).
- DNA stains (ethidium bromide, SYBR Green, propidium iodide).
- Fluorescence microscope.
- Image analysis software (e.g., CometScore, CASP).

5. Procedure (Alkaline Comet Assay)

Step 1: Cell Preparation

1. Collect cells (blood leukocytes, cultured cells, or tissue suspensions).
2. Adjust cell concentration (~1×10^5 cells/mL).

Step 2: Embedding in Agarose

1. Prepare slides with a thin layer of normal-melting agarose (base layer).
2. Mix cells with low-melting agarose and spread onto slides.
3. Cover with coverslip and allow agarose to solidify.

Step 3: Lysis

1. Remove coverslip.
2. Immerse slides in lysis buffer (high salt, detergent, pH ~10) at 4 °C for 1–2 h.
3. This removes membranes and proteins, leaving nucleoids.

Step 4: DNA Unwinding

1. Place slides in alkaline buffer (pH > 13) for 20–40 min.
2. DNA unwinds, exposing single-strand breaks.

Step 5: Electrophoresis

1. Run electrophoresis in alkaline buffer at 25 V (~300 mA) for 20–30 min.
2. Damaged DNA migrates toward the anode, forming comet tails.

Step 6: Neutralization

1. Wash slides with neutralization buffer (Tris, pH 7.5).
2. Dry slides gently.

Step 7: Staining

1. Stain DNA with ethidium bromide or SYBR Green.
2. Visualize under fluorescence microscope.

6. Result Interpretation

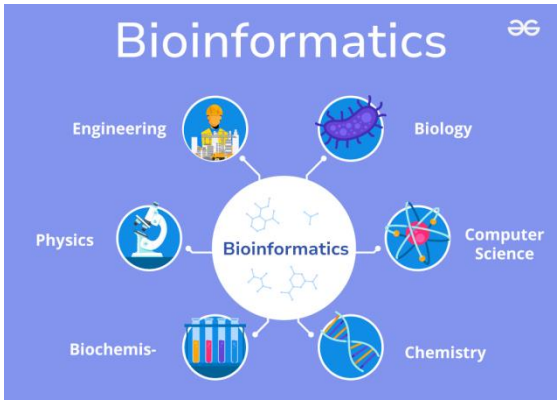
- **Comet Head:** Intact DNA remains in the nucleoid.
- **Comet Tail:** Fragmented DNA migrates, forming a tail.
- **Tail length and intensity:** Proportional to DNA damage.
- **Healthy cells:** Round head, minimal tail.
- **Damaged cells:** Prominent tail, reduced head intensity.

7. Applications

- **Genotoxicity testing:** Assess DNA damage caused by chemicals, radiation, or drugs.
- **Environmental monitoring:** Detect DNA damage in organisms exposed to pollutants.
- **Cancer research:** Study DNA repair capacity in tumor vs. normal cells.
- **Clinical diagnostics:** Evaluate DNA damage in patients with genetic disorders.
- **Pharmacology:** Screen protective or damaging effects of compounds.

LAB 10: Introduction to Bioinformatics

Introduction: Bioinformatics is the science of using computational tools and methods to store, analyze, and interpret biological data. With the rapid growth of genomics and sequencing technologies, bioinformatics has become essential for modern biology, medicine, and biotechnology.



Principles

- **Data storage and retrieval:** Biological sequences (DNA, RNA, proteins) are stored in specialized databases.
- **Sequence analysis:** Algorithms compare sequences to identify similarities, differences, and evolutionary relationships.
- **Functional prediction:** Bioinformatics tools predict gene functions, protein structures, and regulatory elements.
- **Integration:** Combines biology, computer science, mathematics, and statistics.

Core Components

- **Databases:**
  - GenBank (DNA sequences)
  - EMBL/DDBJ (international sequence databases)
  - UniProt (protein sequences)
  - PDB (Protein Data Bank for 3D structures)
- **Tools and Software:**
  - BLAST (Basic Local Alignment Search Tool) for sequence similarity searches.
  - ClustalW/Clustal Omega for multiple sequence alignment.
  - MEGA for phylogenetic tree construction.
  - Swiss-Model for protein structure prediction.

Applications

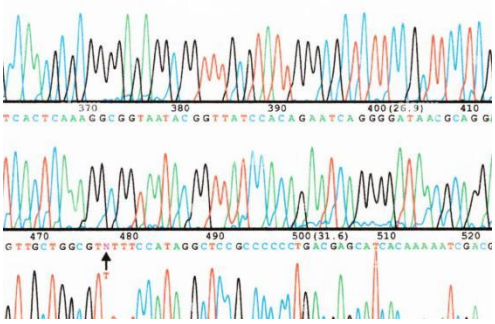
- Gene identification and annotation.
- Comparative genomics and evolutionary studies.
- Protein structure and function prediction.
- Drug discovery and personalized medicine.
- Large-scale data analysis in transcriptomics and proteomics.

LAB 11: DNA Sequencing

Introduction: DNA sequencing is the process of determining the exact order of nucleotides (A, T, G, C) in DNA. It is fundamental for understanding genetic information, diagnosing diseases, and studying evolution.

Principles

- DNA sequencing relies on DNA polymerase to synthesize new strands.
- Incorporation of labeled nucleotides allows detection of sequence order.
- Sequencing technologies differ in scale, accuracy, and speed.

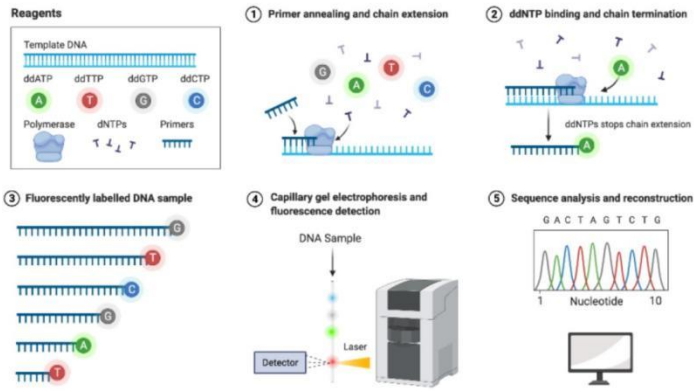


Methods of DNA Sequencing

- **Sanger Sequencing (Chain Termination Method):**
  - Uses dideoxynucleotides (ddNTPs) to stop DNA synthesis.
  - Produces fragments of varying lengths.
  - Fragments are separated by capillary electrophoresis.
  - Fluorescent labels allow automated reading.
  - Best for small DNA fragments (up to ~1000 bp).
- **Next-Generation Sequencing (NGS):**
  - Massively parallel sequencing of millions of fragments.
  - Platforms: Illumina, Ion Torrent.
  - Produces short reads (50–300 bp) but very high throughput.
  - Used for whole genomes, transcriptomes, and metagenomics.

Detailed Protocol (Sanger Sequencing)

1. Prepare DNA template.
2. Add primer, DNA polymerase, dNTPs, and fluorescent ddNTPs.
3. Perform PCR amplification.
4. Separate fragments by capillary electrophoresis.
5. Detect fluorescence peaks to read sequence.

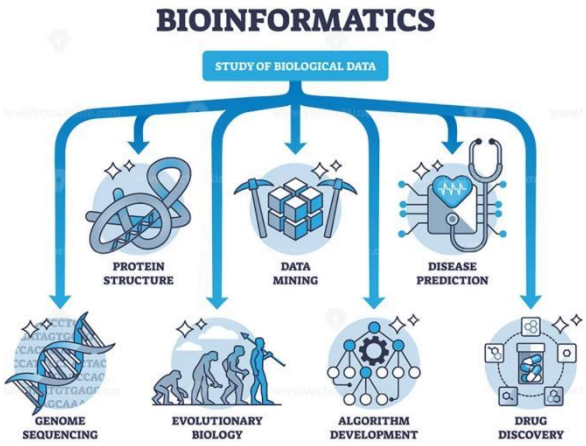


Result Interpretation

- Chromatogram**:** Peaks represent nucleotides.
- Clear peaks = accurate sequence.
- Overlapping peaks = sequencing errors.
- NGS output = millions of short reads requiring computational assembly.

LAB 12: Applications of Bioinformatics and DNA Sequencing

Introduction: The combination of bioinformatics and DNA sequencing has transformed biology. Sequencing provides raw genetic data, while bioinformatics enables analysis, annotation, and interpretation.



Principles

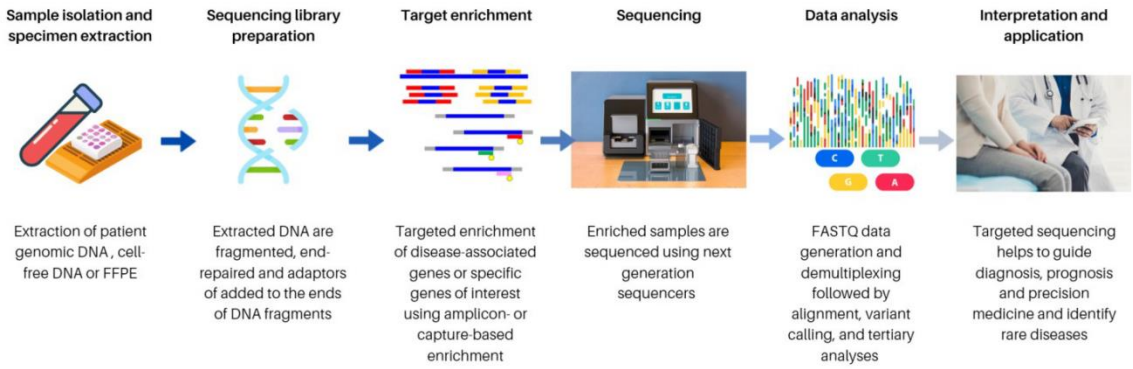
- Sequencing generates DNA or RNA reads.
- Bioinformatics tools align, assemble, and annotate sequences.
- Results are interpreted in biological, medical, and evolutionary contexts.

Applications

- **Genomics:** Whole genome sequencing, gene annotation, mutation detection.
- **Transcriptomics:** RNA-seq for gene expression profiling.
- **Proteomics:** Predict protein coding regions, motifs, and structures.
- **Medical Diagnostics:** Identify mutations, pathogens, cancer biomarkers.
- **Evolutionary Biology:** Phylogenetic analysis, comparative genomics.
- **Personalized Medicine:** Tailor treatments based on patient's genetic profile.

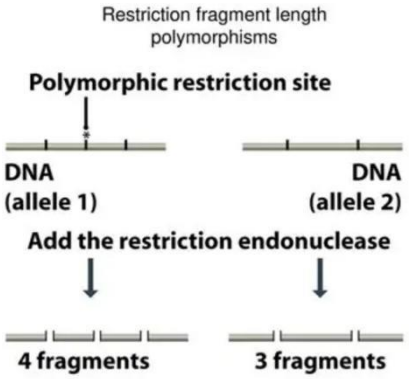
Troubleshooting

- **Poor sequencing quality:** Contaminated template, low DNA concentration.
- **Bioinformatics errors:** Incorrect alignment parameters, outdated databases.
- **Interpretation issues:** Misannotation, lack of reference genome.



LAB 13: Restriction Fragment Length Polymorphism (RFLP) Technique

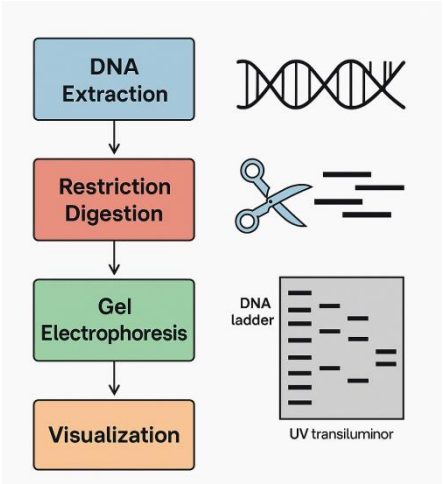
1. Introduction: Restriction Fragment Length Polymorphism (RFLP) is a molecular technique used to detect variations in DNA sequences. It relies on the fact that restriction enzymes cut DNA at specific recognition sites. If mutations or polymorphisms alter these sites, the resulting DNA fragments will differ in length. RFLP was one of the earliest DNA fingerprinting methods and remains important in genetic mapping, disease diagnosis, forensic analysis, and plant/animal breeding programs.



2. Principle of Work: The Restriction Fragment Length Polymorphism (RFLP) technique works by cutting DNA with restriction enzymes that recognize specific sequences. If mutations or polymorphisms change these recognition sites, the enzyme will cut differently, producing fragments of varying lengths. These fragments are then separated by agarose gel electrophoresis, where differences in banding patterns reveal genetic variation between individuals. In this way, RFLP detects DNA sequence differences by showing how changes in restriction sites alter fragment sizes.

3. Materials & Equipment

- Genomic DNA samples (from blood, tissue, or any living organsim).
- Restriction enzymes (EcoRI, HindIII, BamHI, etc.) and Restriction digestion buffer specific to enzyme
- Agarose powder, electrophoresis buffer (TAE/TBE), Gel casting tray, combs, and electrophoresis chamber.
- DNA loading dye.
- DNA ladder (molecular size marker).
- Ethidium bromide or SYBR Green for DNA visualization.
- UV transilluminator or gel documentation system.



4. Procedure (General RFLP Workflow)

Step 1: DNA Extraction

- Isolate high-quality genomic DNA.
- Check purity (A260/A280 ratio ~1.8).
- Ensure DNA is intact (no smearing on gel).

Step 2: Restriction Digestion

- Mix DNA with restriction enzyme and buffer.
- Incubate at optimal temperature (usually 37 °C).
- Allow complete digestion (1–2 hours).
- Stop reaction by heat inactivation (if enzyme allows).

Step 3: Gel Electrophoresis

- Prepare agarose gel (0.8–1% for large fragments).
- Load digested DNA with loading dye.
- Include DNA ladder for size comparison.
- Run electrophoresis at 80–100 V until fragments are separated.

Step 4: Visualization

- Stain gel with ethidium bromide or SYBR Green.
- Place gel under UV transilluminator.
- Observe banding patterns.
- Photograph gel for record and analysis

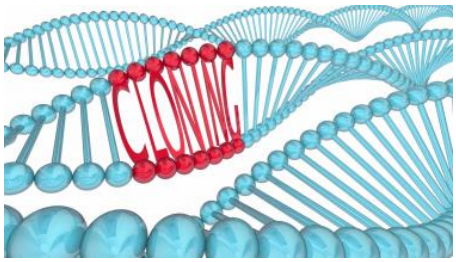
5. Result Interpretation

- **Same fragment pattern:** Indicates identical restriction sites (no polymorphism).
- **Different fragment lengths:** Indicates polymorphism due to mutation, insertion, or deletion.
- **Band intensity:** Reflects DNA quantity.
- **Polymorphic bands:** Used as genetic markers in mapping or identification.

6. Applications

- **Genetic mapping:** Identify linkage between markers and traits.
- **Disease diagnosis:** Detect mutations associated with genetic disorders.
- **Forensic science:** DNA fingerprinting for identity testing.
- **Plant and animal breeding:** Marker-assisted selection.
- **Evolutionary studies:** Compare genetic variation among populations.

LAB 14 & 15: Molecular Cloning Techniques



1. Introduction: Molecular cloning is a fundamental technique in molecular biology that enables scientists to isolate, replicate, and manipulate specific DNA fragments. By inserting a gene of interest into a carrier DNA molecule (vector) and introducing it into host cells, researchers can produce multiple identical copies of the gene. This technique is widely used in gene function studies, protein production, genetic engineering, and biotechnology.

2. Principle: Molecular cloning is based on the principle of cutting and joining DNA fragments and then using the natural replication machinery of host cells to amplify them. Restriction enzymes cut DNA at specific recognition sites, creating fragments with defined ends. The target DNA fragment is inserted into a vector that has been cut with the same enzyme to generate compatible ends. DNA ligase seals the fragment into the vector, forming recombinant DNA. When this recombinant DNA is introduced into a host cell, usually *E. coli*, the cell replicates the plasmid during its normal growth cycle, producing many identical copies of the inserted gene.

3. Essential Components

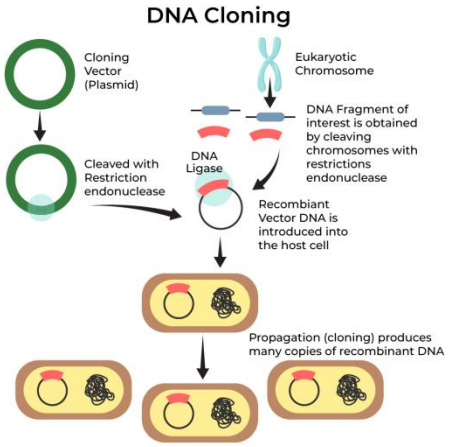
- **DNA fragment (insert):** The gene or sequence to be cloned.
- **Restriction enzymes:** Recognize and cut DNA at specific sites.
- **Vectors:** Plasmids, bacteriophages, or cosmids that carry DNA fragments into host cells.
- **DNA ligase:** Joins DNA fragments together.
- **Host cells:** Usually bacteria (*E. coli*) used to replicate recombinant DNA.
- **Selectable markers:** Genes such as antibiotic resistance used to identify successful clones.
- **Reporter genes:** Optional markers (e.g., lacZ) to distinguish recombinant from non-recombinant clones.

4. Techniques in Molecular Cloning

- **Restriction enzyme-based cloning:** Traditional method using restriction enzymes and ligase.
- **TA cloning:** Uses PCR products with A-overhangs and T-vectors for quick cloning.
- **Directional cloning:** Employs two different restriction sites to ensure correct orientation of the insert.
- **Gateway cloning:** Uses recombination instead of restriction enzymes for efficient DNA transfer.
- **Expression cloning:** Uses vectors with promoters and tags to produce proteins from cloned genes.

- **Site-directed mutagenesis:** Introduces specific mutations into cloned DNA for functional studies.

5. Procedure



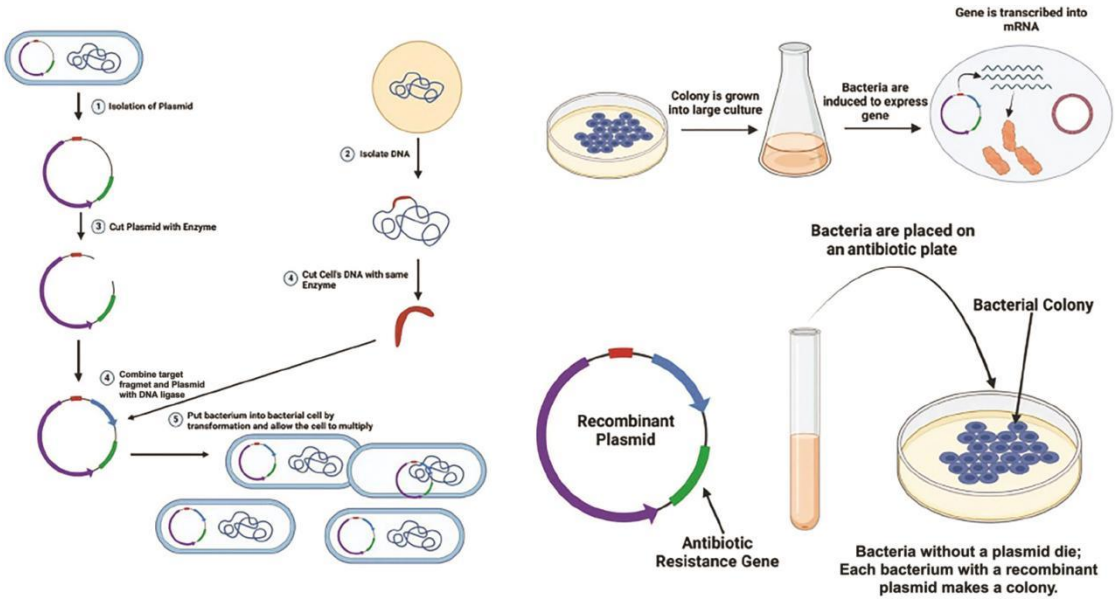
1. DNA Isolation
   - Extract genomic DNA or amplify the gene of interest using PCR.
   - Purify DNA to remove proteins, salts, and contaminants.
   - Check DNA quality by spectrophotometry (A260/A280 ratio ~1.8) and agarose gel electrophoresis.
2. Preparation of Vector
   - Select a suitable plasmid vector (e.g., pUC19, pBR322, or expression vector).
   - Digest the vector with restriction enzymes to create compatible ends.
   - Confirm digestion by running vector on agarose gel.
3. Preparation of Insert DNA
   - Digest the DNA fragment with the same restriction enzymes used for the vector.
   - Purify the digested fragment using gel extraction or column purification.
   - Quantify DNA concentration to ensure optimal ligation ratios.
4. Ligation
   - Mix digested vector and insert DNA in appropriate molar ratios.
   - Add DNA ligase and buffer to join the DNA ends.
   - Incubate at suitable temperature (often 16 °C overnight or room temperature for shorter times).
   - Confirm ligation efficiency by running a small aliquot on agarose gel.
5. Transformation
   - Prepare competent host cells (commonly *E. coli*).
   - Introduce recombinant plasmid into cells using:
     - **Heat shock method:** Expose cells briefly to high temperature to allow DNA uptake.
     - **Electroporation method:** Apply electrical pulse to create temporary pores in the cell membrane.
   - Recover cells in non-selective medium for 30–60 minutes to allow expression of antibiotic resistance genes.

6. Selection of Transformants

- Plate transformed cells on Luria-Bertani (LB) agar containing the appropriate antibiotic (e.g., ampicillin).
- Only cells with recombinant plasmids will grow.

6. Applications

- Gene function studies.
- Protein production (enzymes, hormones, vaccines).
- Genetic engineering in agriculture and medicine.
- Medical research (disease genes, diagnostics, gene therapy).
- Synthetic biology (designing new genetic circuits).
- Evolutionary studies (comparing gene sequences across species).



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