Experiment 7: Agarose Gel Electrophoresis of DNA
Department of Biochemistry
By :Abbas Hamza Khudair
Academic Year 2025-2026

Experiment 7: Agarose Gel Electrophoresis of DNA

Objective

To separate DNA fragments based on molecular size using agarose gel electrophoresis and to visualize the resulting band patterns for qualitative analysis.

Introduction

Agarose gel electrophoresis is an essential technique in molecular biology for resolving DNA fragments ranging from tens of base pairs to several kilobases. DNA's negatively charged phosphate backbone allows it to migrate toward the anode when an electric field is applied. The agarose matrix acts as a molecular sieve, enabling smaller fragments to migrate faster than larger ones. This method is routinely used for assessing genomic DNA integrity, PCR products, restriction digests, and cloning experiments.

Principle

DNA samples are loaded into wells in an agarose gel submerged in an ionic buffer such as TAE or TBE. When voltage is applied, DNA migrates through the gel according to fragment size. After electrophoresis, DNA is stained using intercalating dyes such as ethidium bromide or SYBR Safe and visualized under UV or blue-light illumination.

Materials and Equipment

Material / Equipment	**Purpose**
Agarose powder	Forms gel matrix for DNA separation
10× TAE or 10× TBE	Running buffer and pH maintenance
DNA ladder	Molecular-size reference
DNA samples	Material for separation
Loading dye (6×)	Density and tracking dyes
Gel casting tray and combs	Formation of wells
Microwave or hot plate	Dissolving agarose
Electrophoresis chamber and power supply	Generates electric field
DNA stain (EtBr or SYBR Safe)	Visualization of DNA bands
UV or blue-light transilluminator	Imaging of DNA
Gloves and goggles	Protection from chemicals and UV light

Procedure

1. Prepare 1% agarose by dissolving 1 g agarose in 100 mL of 1× TAE or TBE buffer.
2. Heat until fully dissolved; cool to 50–60°C.
3. Add stain if pre-casting (recommended for SYBR Safe).
4. Pour into the casting tray and insert combs; allow gel to solidify.
5. Place gel in electrophoresis chamber and cover with running buffer.
6. Mix each sample with loading dye.
7. Load DNA ladder and samples into wells.
8. Run electrophoresis at 80–120 V until adequate separation is reached.
9. Visualize DNA bands under UV or blue light.
10. Capture and document the gel image.

References

1. Sambrook J, Russell DW. *Molecular Cloning: A Laboratory Manual*.
2. Green MR, Sambrook J. *Introduction to Gel Electrophoresis*. Cold Spring Harbor Protocols.
3. Watson JD et al. *Molecular Biology of the Gene.*
4. Nelson DL, Cox MM. *Lehninger Principles of Biochemistry*.
5. Stryer L et al. *Biochemistry*.

Short Questions (No Answers)

1. How does agarose concentration influence the resolution of DNA fragments?
2. What are the principal differences between TAE and TBE buffers?
3. What conditions can lead to smearing of DNA bands?