

Agarose Gel Electrophoresis

Principle, Procedure, Applications — Study Notes

1. Introduction

Agarose gel electrophoresis is a widely used molecular biology technique for separating nucleic acids (DNA or RNA) according to their size and charge. Agarose, a linear polysaccharide extracted from seaweed, forms a porous gel matrix when dissolved in buffer and cooled. When an electric field is applied across the gel, negatively charged nucleic acid molecules migrate through the pores towards the positive electrode (anode), with smaller fragments moving faster and farther than larger ones.

2. Principle

The phosphate backbone of DNA/RNA carries a uniform negative charge, so nucleic acid fragments migrate toward the anode under an applied electric field. The agarose matrix behaves like a molecular sieve: smaller molecules pass through the pores more easily and travel faster, while larger molecules are retarded. Migration distance is inversely proportional to the logarithm of molecular size, which allows fragment sizes to be estimated by comparison with a DNA ladder (a set of fragments of known size) run in a parallel lane.

3. Apparatus and Set-up

The apparatus consists of a horizontal electrophoresis tank filled with buffer (commonly TAE or TBE), a casting tray holding the solidified agarose gel with sample wells at one end, platinum electrodes at each end of the tank, and a DC power supply that maintains a constant voltage or current.

Agarose Gel Electrophoresis — Apparatus Set-up

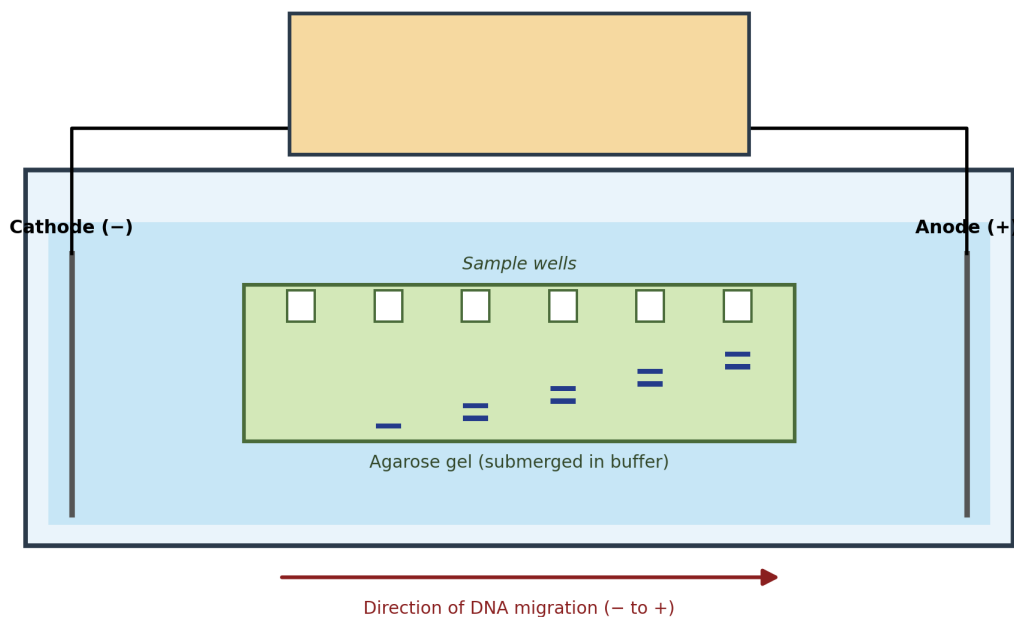


Fig. 1 — Schematic of a horizontal agarose gel electrophoresis set-up, showing the gel tray submerged in buffer between the cathode and anode.

4. Materials Required

- Agarose powder (0.5–2% w/v depending on fragment size range)
- Electrophoresis buffer (1X TAE or 0.5X TBE)
- Casting tray, well comb, and horizontal gel tank with lid
- DC power supply
- DNA sample mixed with loading dye (contains glycerol/sucrose and tracking dyes)
- DNA molecular weight marker (ladder)
- Intercalating dye for visualization (ethidium bromide or a safer alternative such as SYBR Safe)
- UV transilluminator or gel documentation system

5. Procedure

1. **Prepare the gel:** Dissolve the required amount of agarose in buffer by heating until clear; cool to ~55–60°C before pouring to avoid warping the tray.
2. **Cast the gel:** Pour the molten agarose into the casting tray with a comb in place to form wells; allow it to solidify at room temperature (20–30 minutes).
3. **Set up the tank:** Place the solidified gel (comb removed) into the tank and submerge it completely in running buffer.
4. **Load the samples:** Mix DNA samples with loading dye and carefully pipette into the wells; load a DNA ladder in a separate lane for size reference.
5. **Run the gel:** Connect the electrodes to the power supply (DNA runs toward the anode/red terminal) and apply a constant voltage (typically 5–10 V/cm) until the dye front has migrated an adequate distance.
6. **Stain and visualize:** If not pre-stained, soak the gel in an intercalating dye solution, then view and photograph the bands under UV or blue-light illumination.
7. **Analyze:** Compare migration distances of sample bands with the ladder to estimate fragment sizes.

6. Interpreting the Gel

After electrophoresis, the gel shows a series of bands in each lane. Distance migrated decreases with increasing fragment size, so the largest fragments remain closest to the wells and the smallest travel farthest toward the anode. Plotting the log of fragment size (from the ladder) against migration distance gives an approximately linear relationship, which is used to estimate the sizes of unknown fragments.

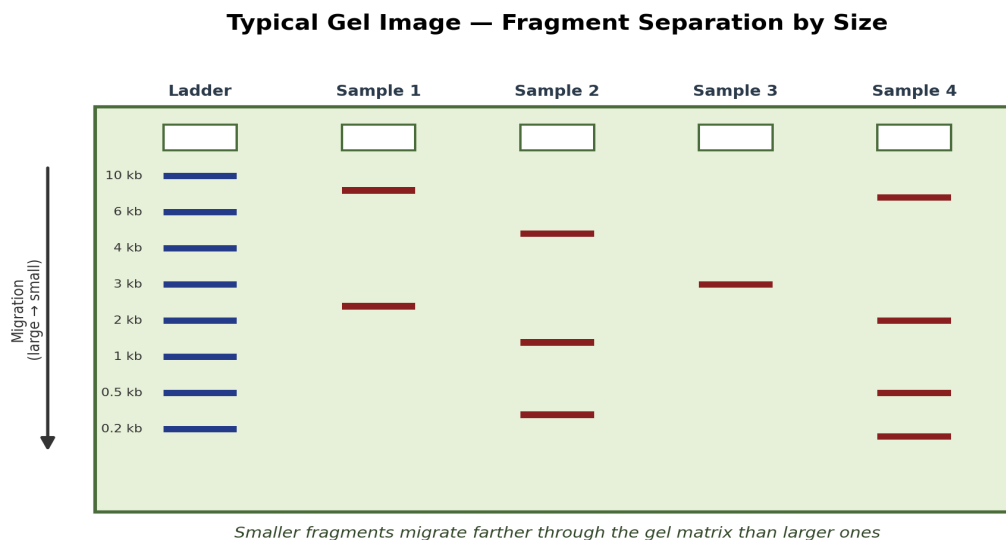


Fig. 2 — Representative gel image: a DNA ladder (left lane) alongside sample lanes. Band position indicates fragment size — smaller fragments run farther.

7. Factors Affecting DNA Migration

Factor	Effect on Migration
Agarose concentration	Higher % → smaller pores → better resolution of small fragments; lower % → resolves larger fragments
Voltage applied	Higher voltage increases speed but reduces resolution and can cause band smearing
DNA conformation	Supercoiled, circular, and linear forms of the same size migrate at different rates (supercoiled generally fastest)
Buffer composition/ionic strength	Affects conductivity and heat generation; too low buffer can cause slow migration, too high can overheat the gel
Ethidium bromide (if pre-added)	Intercalation slightly reduces DNA mobility (~15%)

8. Applications

- Estimating the size and quantity of DNA/RNA fragments
- Analyzing PCR products and confirming amplification success
- Restriction digestion mapping and analysis of cloned DNA
- DNA fingerprinting and forensic analysis
- Separating fragments prior to Southern/Northern blotting
- Quality checking genomic DNA or RNA integrity before downstream applications

Note: Ethidium bromide is a mutagen and suspected carcinogen — always wear gloves and handle gels under proper safety precautions; UV exposure also requires eye and skin protection.

9. Quick Summary

Parameter	Typical Value/Range
Agarose concentration	0.5% (large fragments, >10 kb) to 2% (small fragments, <0.5 kb)
Common buffers	TAE (1X) or TBE (0.5X–1X)
Applied voltage	5–10 V/cm gel length
Direction of DNA movement	Cathode (–) → Anode (+)
Visualization	UV/blue-light transillumination after staining with EtBr or SYBR dyes