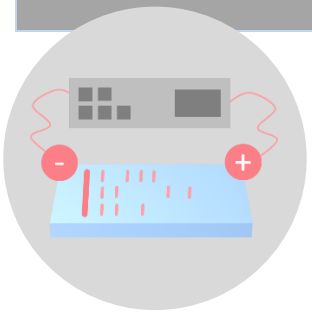


DNA and RNA Gel Electrophoresis

www.davids-bio.com (Custom Antibodies)
www.davids-science.de (Lab Material)

- 1 - Introduction

Gel electrophoresis is a fundamental technique in molecular biology that is used to separate and analyze macromolecules, such as DNA, RNA and proteins based on their size and charge. This technique makes use of the inherent mobility of charged molecules in an electric field, allowing for the separation and visualization of complex samples. Gel electrophoresis finds application in various research areas, including genetic analysis, protein characterization, and diagnostic procedures.

The principle of gel electrophoresis is grounded in the migration of charged molecules through a porous matrix or gel, driven by an applied electric field. The negatively charged DNA molecules migrate towards the positively charged electrode, with smaller fragments moving faster and traveling further through the gel.

Please keep in mind that working with Ethidium Bromide and DNA may be dangerous. Please always read the safety manuals and the guidelines from your institute before you work with hazardous materials.

Material	
Template	DNA or RNA samples of interest
Size Marker (Optional)	DNA ladder Control rRNA
DNA Loading Buffer	50% Glycerol (v/v) 0.3% Bromophenol blue (w/v) 0.3% Xylene cyanol (w/v) DNase-/RNase- free Water
RNA Loading Buffer, denaturing	95% Formamide (v/v) 0.3% Bromophenol blue (w/v) 0.3% Xylene cyanol (w/v) DNase-/RNase- free Water
Staining Solution	10 µl Ethidium bromide (10 mg/ml) ad 100 ml 1 x TAE Buffer
10 x TAE Buffer	400 mM Tris 20 mM Sodium acetate 10 mM EDTA DNase-/RNase- free Water

10 x MOPS	200 mM MOPS 50 mM Sodium acetate 10 mM EDTA DNase-/RNase- free Water	
DNA Agarose Gel	1 x TAE 1.5% agarose powder (DNA grade)	
RNA Agarose Gel	1 x MOPS 1.5% agarose powder 2.2% Formaldehyde add prior to pouring	Work under a fume hood when handling with Formaldehyde

- 2 - Method

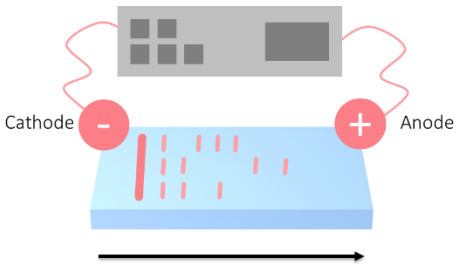
Agarose Gel Preparation

- Prepare a microwave-safe flask
- Prepare the DNA Agarose in the flask by adding agarose powder and 1 x TAE buffer (DNA Agarose Gel)
  For RNA: Use MOPS instead of TAE. Do not add Formaldehyde yet.
- Heat the mixture in a microwave until the agarose is completely dissolved
- Allow the agarose solution to cool to around 55°C
  For RNA: Add Formaldehyde stock solution to the agarose solution at a final concentration of 2.2%
- Pour the solution into the gel tray containing a comb
- Let the gel solidify for at least 30 minutes

Sample Preparation

- Mix each DNA sample with an appropriate amount of DNA Loading Buffer in a 1:1 ratio
  For RNA: Mix each RNA sample with RNA Loading Buffer in a 1:1 ratio
- Optional: Prepare a DNA Ladder according to the manual
- Carefully remove the comb from the gel tray

Electrophoresis



- Place the gel tray into the electrophoresis chamber
- Fill the chamber with 1x TAE Buffer until the gel is fully submerged
- Load the samples along with the DNA Ladder into the wells
- Connect the electrodes to the power supply (keep in mind, that the DNA runs to the positive electrode)
- Apply a voltage of 100 V
- Run the gel for 45 minutes until the dye front is about ¾ of the way down the gel

Visualization

- Wear appropriate protective gear, including nitrile gloves and safety goggles
- Prepare a fresh Staining Solution
- Submerge the gel in the Staining Solution for 30 minutes
- Place the stained gel on a UV transilluminator at a range of 300 – 360 nm
- Turn on the UV transilluminator and capture an image of the gel

Alternative Staining

Ethidium Bromide can be added to the gel after heating the TAE with the agarose. This may lead to a better visualization. Please ensure that the agarose gel solution is cooled down before adding ethidium bromide. In addition, always follow the instructions from your institute.

When the Ethidium Bromide is already added in the gel, the staining solution is not needed.

Please follow the institutions guidelines for proper ethidium bromide disposal

- 3 - Trouble Shooting

Reason	Solution
Weak Bands	Ensure proper sample preparation with adequate DNA/RNA concentration. Optimize nucleic acid extraction and purification methods. Double check the loading process to make sure samples are fully loaded. Adjust the running voltage in a range of 80 – 120 V while monitoring the temperature rise to avoid gel overheating. Ensure the gel is fully submerged in 1x TAE.
Distorted Bands	Use proper pipetting techniques and calibration to ensure accurate sample loading. Avoid overloading the wells with excessive volume. Make sure electrodes are properly connected and the voltage is evenly distributed across the gel.
Smear Bands	Use freshly prepared and high-grade DNA/RNA samples. Load appropriate amounts of sample in each well. Ensure gel, buffer equipment are properly cleaned to prevent contaminations.
Uneven Migration	Adjust the agarose concentration of the gel in a range of 0.5 – 1.5%. Ensure consistent distribution of loading dye in samples. Maintain proper buffer level in the electrophoresis chamber throughout the run.
No Separation	Choose an appropriate agarose concentration that suits the size range of the sample fragments. Ensure the gel is fully solidified before loading samples.
High Background	Use clean and sterile reagents, buffer and Nuclease-free water. Ensure proper staining during gel visualization.
Visualization	Consider adding ethidium bromide before the gel solidifies, to avoid long incubation times.