Sep 19, 2025

Agarose Gel Electrophoresis for DNA Analysis

DOI
https://dx.doi.org/10.17504/protocols.io.261geepeyg47/v1

Md Sahadat Ali[1], Fatima Tuz Zohora Mony[1], Jonathan D. Eisenback[1]

[1]Virginia Tech

Md Sahadat Ali
Virginia Tech

DOI: https://dx.doi.org/10.17504/protocols.io.261geepeyg47/v1

Protocol Citation: Md Sahadat Ali, Fatima Tuz Zohora Mony, Jonathan D. Eisenback 2025. Agarose Gel Electrophoresis for DNA Analysis. **protocols.io** https://dx.doi.org/10.17504/protocols.io.261geepeyg47/v1

Protocol status: Working
We use this protocol and it's working

Created: March 20, 2025

Last Modified: September 19, 2025

Protocol Integer ID: 124654

Keywords: Agarose gel electrophoresis, DNA separation, Ethidium bromide staining, DNA ladder, Gel casting, UV visualization, agarose gel electrophoresis for dna analysis, execution of agarose gel electrophoresi, agarose gel electrophoresi, dna analysis, electrophoresi, separate dna fragment, agarose gel matrix, ethidium bromide, electric field in an agarose gel matrix, known mutagen, gel

Disclaimer

This protocol follows standard laboratory safety practices. Ethidium bromide (EtBr) is hazardous, and proper safety measures must be followed. This protocol is intended for trained personnel following institutional biosafety guidelines. The authors are not responsible for misuse or procedural errors.

Abstract

This protocol describes the preparation and execution of agarose gel electrophoresis for DNA analysis. The method is used to separate DNA fragments based on size, using an electric field in an agarose gel matrix. The gel is stained with ethidium bromide (EtBr) for visualization under UV light. This protocol follows strict safety guidelines for handling EtBr, a known mutagen. Proper personal protective equipment (PPE), designated waste disposal, and workspace decontamination are required.

Guidelines

- EtBr is a mutagen—handle with gloves and avoid cross-contamination between contaminated and uncontaminated areas.
- All EtBr waste (gels, gloves, tips, and tubes) must be disposed of in a designated biohazard waste container.
- Ensure proper voltage selection based on the agarose gel concentration and DNA fragment size.
- Always confirm gel orientation—the wells must be on the negative (black) electrode side.
- Use a fresh pipette tip for each sample to prevent cross-contamination.
- Do not exceed recommended runtime to avoid running DNA off the gel.

Materials

Reagents & Solutions

- Agarose Powder – For gel matrix preparation
- 1X TAE Buffer – Running buffer for DNA migration
- Ethidium Bromide (EtBr) – DNA stain for visualization
- 6X DNA Loading Dye – Helps track migration and improves sample loading
- DNA Ladder (1 kb) – Molecular weight marker for DNA size estimation

Consumables & Equipment

- Gel Casting Tray – Mold for gel preparation
- Comb – To create sample wells
- Microwave – To dissolve agarose in TAE buffer
- Parafilm – For preparing loading dye mixtures
- Pipettes & Tips (P20, P200, P1000) – For precise liquid handling
- Gel Rig & Power Supply – For running electrophoresis
- UV Transilluminator – For DNA visualization
- Ethanol Spray & KimWipes® – For workspace decontamination
- Disposable Gloves – To prevent contamination and protect from EtBr exposure
- Dedicated EtBr Waste Bin – For proper disposal of contaminated materials

Safety warnings

- EtBr is hazardous—handle with gloves and work in designated areas.
- Avoid prolonged exposure to UV light, as it can damage DNA and cause skin burns.
- Dispose of EtBr-contaminated materials in a dedicated waste bin.
- Do not exceed recommended voltage or runtime to prevent overheating.

Ethics statement

This protocol involves agarose gel electrophoresis for DNA analysis and does not involve animal or human subjects. However, researchers must adhere to institutional biosafety regulations when handling potentially hazardous chemicals such as ethidium bromide (EtBr).
Proper safety precautions, waste disposal, and decontamination procedures must be followed in accordance with institutional and regulatory guidelines. Researchers are responsible for ensuring that the use of EtBr and other hazardous materials complies with relevant laboratory safety protocols and institutional policies.
If working with DNA from animal or human subjects, prior approval must be obtained from an Institutional Review Board (IRB) or an Institutional Animal Care and Use Committee (IACUC). Please include any relevant permit numbers and committee names when applicable.

Before start

- Prepare a clean and decontaminated workspace to minimize contamination.
- Ensure all materials are pre-labeled before starting the gel preparation.
- Wear PPE (lab coat, gloves, safety goggles) and handle EtBr-stained materials with caution.
- Confirm all power supply settings before starting electrophoresis to avoid overheating or excessive migration.
- Prepare an EtBr-free area for handling uncontaminated materials.
- Prepare ice for thawing samples.

Procedures

1 **Preparing DNA Samples for Loading**

1. Remove DNA samples from the fridge and thaw in an ice bath.

Note: Thawing too quickly may degrade DNA over time.

2. Prepare a strip of parafilm in the designated No EtBr area.
3. Aliquot 1 µL of 6X DNA loading dye onto the parafilm for each sample.
   - The exact amount does not need to be precise.
4. Mix DNA with loading dye at a ratio of 4 µL DNA per 1 µL loading dye.

Note: If using Taq polymerase with dye (e.g., Promega GoTaq Green), this step is not needed.

2 **Preparing Agarose Gel**

1. Weigh out the agarose powder (amount varies based on the desired concentration).
   - Agarose mass used: 0.6 g
   - Higher concentrations are used for smaller DNA fragments.
2. Dissolve agarose in 1X TAE buffer (volume based on gel size).
   - TAE buffer volume used: 75 mL
   - Final agarose concentration: 0.8%
3. Microwave the mixture for 1–1.5 minutes, stopping to mix intermittently.

Note: Watch closely to prevent boiling over.

3 **Casting the Gel**

1. Seal the gel casting tray using tape or clamps to prevent leaks.
   - Use two pieces of tape per side for a secure seal.
2. Add EtBr to the melted agarose and mix thoroughly.
   - Amount added: 2.5 µL for 75 mL of gel (1 µL per 30 mL).
3. Place the comb into the gel casting tray to create sample wells.
4. Let the agarose cool slightly, then slowly pour it into the tray to avoid air bubbles.
5. Allow the gel to solidify (~20 minutes).

4 **Running the Gel**

1. Carefully remove the tape and comb from the solidified gel.
2. Place the gel in the gel rig, ensuring the wells face the negative (black) electrode.
3. Add 1X TAE buffer to the gel rig until the gel is fully submerged.
4. Load 5 µL of the DNA ladder into the first and last lanes of the gel.
   - Ladder size: 1 kb

Best Practices to Avoid Bubbles: When loading DNA samples or the ladder into the gel lanes, exercise caution to prevent air bubbles, which can displace the sample and cause it to spill into the buffer.

- Ensure the pipette tip is completely filled with the sample, eliminating any air gaps at the tip.

- If air pockets are present, gently press the pipette plunger to expel the excess air before loading.
- Slowly and steadily dispense the sample into the well to ensure smooth loading.

5. Load prepared DNA samples into wells between the ladder lanes.
6. Attach the power supply and set the following parameters:

- Voltage: 100-150V
- Current: 100 mAmps
- Run time: ~35 minutes

7. Verify bubble formation at the negative electrode, confirming proper current flow.

5 **Gel Visualization**

1. Turn off the power supply and carefully remove the gel.
2. Visualize the gel under a UV transilluminator.

Note: Do not expose the gel to UV light longer than necessary, as excessive exposure may degrade DNA.

6 **Expected Results**

- Distinct bands corresponding to DNA fragments appear under UV light.
- Ladder bands allow estimation of fragment sizes.
- Properly run gels have clear, well-separated bands without smearing or excessive diffusion.
- Smearing or streaking may indicate degraded DNA or excessive voltage.

Protocol references

Lee, P. Y., Costumbrado, J., Hsu, C. Y., & Kim, Y. H. (2012). Agarose gel electrophoresis for the separation of DNA fragments. *Journal of visualized experiments : JoVE*, (62), 3923. https://doi.org/10.3791/3923

Acknowledgements

This protocol follows standard gel electrophoresis methods and has been optimized for routine DNA analysis in microbiology and molecular biology laboratories. Special thanks to Virginia Tech's Plant Pathology Laboratory for validation and implementation.