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Protocol

Agarose Gel Electrophoresis

Michael R. Green and Joseph Sambrook

This protocol describes steps for the preparation and running of agarose gels and for staining and visualization of DNA in gels using three dyes: ethidium bromide, SYBR Gold, and SYBR Green 1.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Agarose solutions (see Tables 1 and 2 in Introduction: **Analysis of DNA by Agarose Gel Electrophoresis** [Green and Sambrook 2019])

Agarose gels are cast by melting the agarose in the presence of the desired buffer until a clear, transparent solution is achieved. The melted solution is then poured into a mold and allowed to harden. Upon hardening, the agarose forms a matrix, the density of which is determined by the concentration of the agarose. For rapid analysis of DNA samples, the use of a minigel is recommended (see Box 1).

DNA samples

DNA size standards

Samples of DNAs of known size are typically generated by restriction enzyme digestion of a plasmid or bacteriophage DNA of known sequence. Alternatively, they are produced by ligating a monomer DNA fragment of known size into a ladder of polymeric forms. Size standards for both agarose and polyacrylamide gel electrophoresis may be purchased from commercial sources, or they can be prepared easily in the laboratory. It is a good idea to have two size ranges of standards, including a high-molecular-weight range from 1 to >20 kb and a low-molecular-weight range from 100 to 1000 bp. A stock solution of size standards can be prepared by dilution with a gel-loading buffer and then used as needed in individual electrophoresis experiments.

Electrophoresis buffer (usually 1× TAE <R> or 0.5× TBE <R>)

Gel-loading buffer I <R>, II <R>, III <R>, or IV <R> (6×)

Modified polysaccharides (optional; see Step 3)

Staining solution (one of the following, prepared in the electrophoresis buffer used to prepare and run the gel):

SYBR Gold stock solution, diluted 1:10,000 in electrophoresis buffer

SYBR Green I stock solution, diluted 1:10,000 in electrophoresis buffer

Ethidium bromide solution: 0.5 µg/mL in electrophoresis buffer

From the Molecular Cloning collection, edited by Michael R. Green and Joseph Sambrook.

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BOX 1. ELECTROPHORESIS THROUGH MINIGELS

Most routine electrophoretic analyses of DNA can be performed very rapidly using agarose minigels. Minigels are particularly useful when a rapid answer is required before the next step in a cloning protocol can be undertaken.

Because the wells are smaller and the gels are thinner, less DNA than normal is required for visualization. In addition, because the gels can be prepared in advance and run rapidly and because they require smaller amounts of reagents, there are considerable savings in both time and money. Many investigators prepare one gel at the beginning of the week and use it over and over again through the course of an experiment. Thus, a particular set of samples may be loaded, run out onto the gel, and visualized. The gel then may be "erased" by running the samples off the gel into the buffer. Note that minigels are best suited for the analysis of small DNA fragments (<3 kb). Larger fragments resolve poorly because of the high voltages that are generally used and the comparatively short length of the gel.

Several types of miniature electrophoresis tanks are manufactured commercially, typically as smaller versions of the companies' larger electrophoresis models. Each gel slot holds 3–12 µL of sample, depending on the thickness of the gel and the width of the teeth of the comb. Usually, 10–100 ng of DNA in the gel-loading buffer of choice is applied to a slot. The gel is then run for 30–60 min at high voltage (5–20 V/cm) until the bromophenol blue and xylene cyanol FF have migrated the appropriate distance. An image of the gel under UV illumination can be captured on a CCD camera.

Equipment

Disposable micropipette, automatic micropipettor, or drawn-out Pasteur pipette or glass capillary tube
Equipment for agarose gel electrophoresis (clean, dry horizontal electrophoresis apparatus with chamber and comb, or clean, dry glass plates with appropriate comb)
Gel-sealing tape

Common types of laboratory tape, such as Time Tape or VWR Laboratory Tape, are appropriate for sealing the ends of the agarose gel during pouring.

Glass bottle with cap or Erlenmeyer flask
Image capturing system (e.g., a CCD camera and a UV illuminator)
Insulated gloves or tongs
KimWipes
Microwave oven or boiling water bath
Plastic wrap (e.g., Saran Wrap)
Power supply device capable of up to 500 V and 200 mA
Shallow plastic tray, large enough to accommodate the glass plate
Water bath preset to 55°C

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METHOD

1. Seal the edges of a clean, dry glass plate (or the open ends of the plastic tray supplied with the electrophoresis apparatus) with tape to form a mold (Fig. 1). Set the mold on a horizontal section of the bench.
2. Prepare sufficient electrophoresis buffer (usually 1× TAE or 0.5× TBE) to fill the electrophoresis tank and to cast the gel.

   It is important to use the same batch of electrophoresis buffer in both the electrophoresis tank and the gel. Small differences in ionic strength or pH create fronts in the gel that can greatly affect the mobility of DNA fragments. When measuring the sizes of unknown DNAs, ensure that all samples are applied to the gel in the same buffer. The high concentrations of salt in certain restriction enzyme buffers (e.g., BamHI and EcoRI) retard the migration of DNA and distort the electrophoresis of DNA in the adjacent wells.

3. Prepare a solution of agarose in electrophoresis buffer at a concentration appropriate for separating the particular size fragments expected in the DNA sample(s): Add the correct amount of

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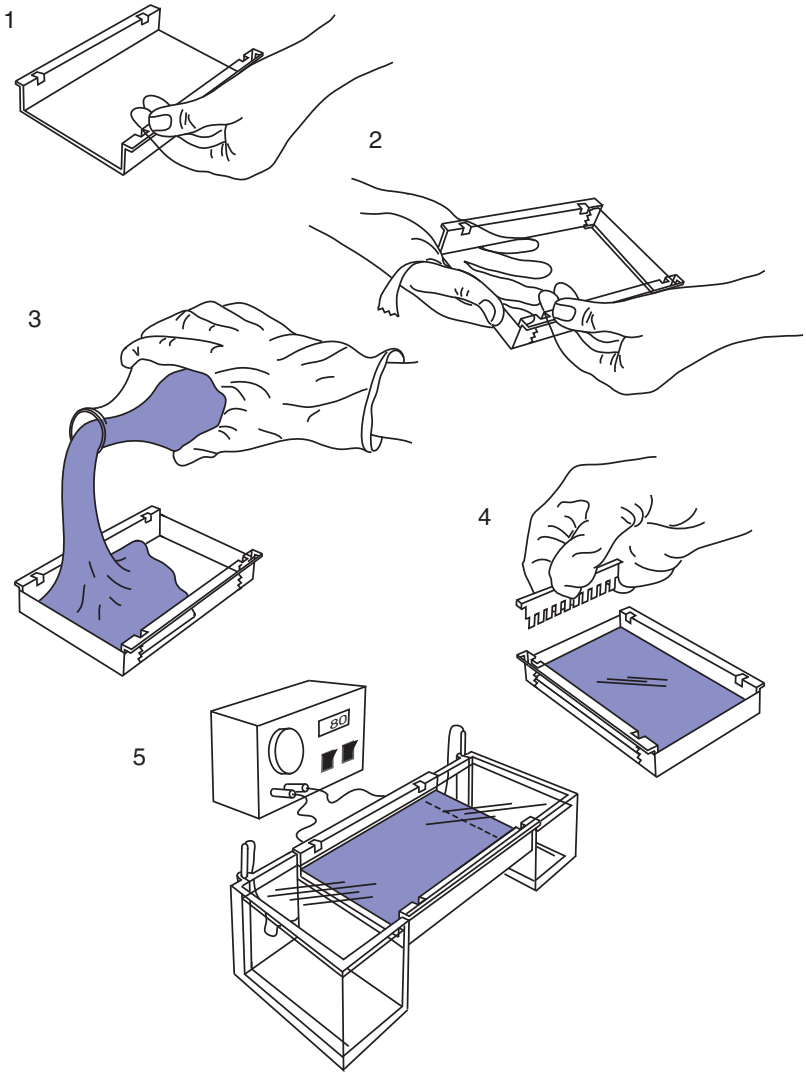

FIGURE 1. Pouring a horizontal agarose gel.

powdered agarose (see Table 1) to a measured quantity of electrophoresis buffer in an Erlenmeyer flask or a glass bottle.

The buffer should occupy <50% of the volume of the flask or bottle.

The concentrations of agarose required to separate DNAs in different size ranges is given in Table 1. DNAs differing in size by only a few base pairs can be separated when certain high-resolution agaroses (e.g.,

TABLE 1. Range of separation in gels containing different amounts of standard low-EEO agarose

Agarose concentration in gel (% [w/v])	Range of separation of linear DNA molecules (kb)
0.3	5–60
0.6	1–20
0.7	0.8–10
0.9	0.5–7
1.2	0.4–6
1.5	0.2–3
2.0	0.1–2

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MetaPhor Agarose; Lonza Walkersville) are used to cast the gel. Alternatively, modified polysaccharides (commercially available) can be added to regular agarose to enhance separation. This substance, used at a concentration of 0.5%–2.0% (w/v), together with agarose, increases resolution, renders the cast gel more clear, and increases the strength of the gel.

4. Loosely plug the neck of the Erlenmeyer flask with KimWipes. If using a glass bottle, make certain the cap is loose. Heat the slurry in a boiling water bath or a microwave oven until the agarose dissolves.

   The agarose solution can become superheated and may boil violently if it is heated for too long in the microwave oven.

   Heat the slurry for the minimum time required to allow all of the grains of agarose to dissolve. Undissolved agarose appears as small "lenses" or translucent chips floating in the solution. Wear an oven mitt, and carefully swirl the bottle or flask from time to time to make sure that any unmelted grains of agarose sticking to the walls enter the solution. Longer heating times are required to dissolve higher concentrations of agarose completely. Check that the volume of the solution has not been decreased by evaporation during boiling; replenish with H_2O if necessary.

5. Use insulated gloves or tongs to transfer the flask/bottle into a water bath at 55°C. When the molten gel has cooled, add ethidium bromide to a final concentration of 0.5 µg/mL (if using ethidium bromide). Mix the gel solution thoroughly by gentle swirling.

   SYBR dyes should not be added to the molten gel solution (see Step 14).

   When preparing gels in plastic (lucite) trays, it is important to cool the melted agarose solution to <60°C before casting the gel. Hotter solutions warp and craze the trays. At one time, solutions containing high concentrations of agarose (2% or above) were stored at 70°C to prevent premature gelling. However, this treatment has become unnecessary because of improvements in the methods used to purify and prepare standard agaroses.

6. While the agarose solution is cooling, choose an appropriate comb for forming the sample slots in the gel. Position the comb 0.5–1.0 mm above the plate so that a complete well is formed when the agarose is added to the mold.

   Most apparatuses have side walls or outside "legs" that allow appropriate placement of the comb. If this is not the case and if the comb is too close to the glass plate, the base of the well may tear when the comb is withdrawn, causing samples to leak between the gel and the glass plate. This problem is more common when low concentrations of agarose (<0.6%) or low-gelling-temperature agarose are used.

7. Pour the warm agarose solution into the mold.

   The gel should be between 3 and 5 mm thick. Check that no air bubbles are under or between the teeth of the comb. Air bubbles present in the molten gel can be removed easily by poking them with the corner of a KimWipe.

   When preparing gels that contain low concentrations of agarose (<0.5%), first pour a supporting gel (1% agarose) without wells. Allow this gel to harden at room temperature on the glass plate or plastic tray, and then pour the lower-percentage gel directly on top of the supporting gel. Stacking the gels in this way reduces the chance that the lower-percentage gel will fracture during subsequent manipulations (e.g., photography and processing for Southern hybridization). Make sure that both gels are made from the same batch of buffer and contain the same concentration of ethidium bromide. Gels cast with low-melting-temperature agarose and gels that contain <0.5% agarose can also be chilled to 4°C and run in a cold room to reduce the chance of fracture.

8. Allow the gel to set completely (30–45 min at room temperature), then pour a small amount of electrophoresis buffer on the top of the gel, and carefully remove the comb. Pour off the electrophoresis buffer, and carefully remove the tape. Mount the gel in the electrophoresis tank.

9. Add just enough electrophoresis buffer to cover the gel to a depth of ~1 mm.

   It is not necessary to prerun an agarose gel before the samples are loaded.

10. Mix the samples of DNA with 0.20 volume of the desired 6× gel-loading buffer.

    The maximum amount of DNA that can be applied to a slot depends on the number of fragments in the sample and their sizes. If there is more than 500 ng of DNA in a band of 0.5 cm, the slot will be overloaded, resulting in trailing, smiling, and smearing—problems that become more severe as the size of the DNA increases. When simple populations of DNA molecules (e.g., bacteriophage λ or plasmid DNAs) are to be analyzed, 100–500 ng of DNA should be loaded per 0.5-cm slot. When the sample consists of a very large

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number of DNA fragments of different sizes (e.g., restriction digests of mammalian DNA), however, it is possible to load 20–30 µg of DNA per slot without significant loss of resolution.

The maximum volume of solution that can be loaded is determined by the dimensions of the slot. (A typical slot [0.5 × 0.5 × 0.15 cm] will hold ~40 µL.) Do not overfill a slot with a DNA sample solution. To reduce the possibility of contaminating neighboring samples, it is best to make the gel a little thicker or to concentrate the DNA by ethanol precipitation rather than to fill the slot completely.

11. Slowly load the sample mixture into the slots of the submerged gel using a disposable micropipette, an automatic micropipettor, or a drawn-out Pasteur pipette or glass capillary tube. Load size standards into slots on both the right and left sides of the gel.

    For many purposes, it is not necessary to use a fresh pipette tip for every sample as long as the tip is thoroughly washed with buffer from the anodic chamber between samples. However, if the gel is to be analyzed by Southern hybridization or if bands of DNA are to be recovered from the gel, it is sensible to use a separate pipette tip for every sample.

12. Close the lid of the gel tank, and attach the electrical leads so that the DNA will migrate toward the positive anode (red lead). Apply a voltage of 1–5 V/cm (measured as the distance between the positive and negative electrodes). If the leads have been attached correctly, bubbles should be generated at the anode and cathode (due to electrolysis), and within a few minutes, the bromophenol blue should migrate from the wells into the body of the gel. Run the gel until the bromophenol blue and xylene cyanol FF have migrated an appropriate distance through the gel.

    The presence of ethidium bromide allows the gel to be examined by UV illumination at any stage during electrophoresis. The gel tray may be removed and placed directly on a transilluminator. Alternatively, the gel may be examined using a handheld source of UV light. In either case, turn off the power supply before examining the gel!

    During electrophoresis, the ethidium bromide migrates toward the cathode (in the direction opposite to that of the DNA). Electrophoresis for protracted periods of time can result in the loss of significant amounts of ethidium bromide from the gel, making detection of small fragments difficult. In this case, restain the gel by soaking it for 30–45 min in a solution of ethidium bromide (0.5 µg/mL).

13. When the DNA samples or dyes have migrated a sufficient distance through the gel, turn off the electric current and remove the leads and lid from the gel tank. If ethidium bromide is present in the gel and electrophoresis buffer, skip to Step 15.

14. Gently submerge the gel into the desired staining solution in the plastic tray. Use just enough staining solution to cover the gel completely, and stain the gel for 30–45 min (with ethidium bromide) or for 30 min (with SYBR dyes) at room temperature.

15. Remove the gel from the staining solution, using the glass plate as a support. Rinse the gel with water, and carefully blot excess liquid from the surface of the gel with a pad of KimWipes.

16. Cover the gel with a piece of plastic wrap. Smooth out any air bubbles or folds in the plastic wrap with the broad end of a slot comb or a crumpled KimWipe.

17. Place a piece of plastic wrap on the working surface of a UV illuminator. Invert the gel and place it on the plastic wrap; then remove the glass plate.

18. Capture an image of the gel under UV transillumination on a CCD camera equipped with the appropriate screens and emission filters (Oatey 2007).

    Considerations for photography and for any desired following documentation are described in the Discussion.

DISCUSSION

Agarose is a linear polymer composed of alternating residues of D-galactose and L-galactose joined by α-(1 → 3) and β-(1 → 4) glycosidic linkages. Chains of agarose form helical fibers that aggregate into supercoiled structures with a radius of 20–30 nm. Gelation of agarose results in a three-dimensional (3D) mesh of channels whose diameters range from 50 to >200 nm (Norton et al. 1986; for review, see Kirkpatrick 1990).

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Commercially prepared agarose polymers are believed to contain about 800 galactose residues per chain. However, agarose is not homogeneous: The average length of the polysaccharide chains varies from batch to batch and from manufacturer to manufacturer (for further details, see Introduction: **Analysis of DNA by Agarose Gel Electrophoresis** [Green and Sambrook 2019]). In addition, lower grades of agarose may be contaminated with other polysaccharides, as well as salts and proteins. This variability can affect the gelling/melting temperature of agarose solutions, the sieving of DNA, and the ability of the DNA recovered from the gel to serve as a substrate in enzymatic reactions. These potential problems can be minimized by using special grades of agarose that are screened for the presence of inhibitors and nucleases and for minimal background fluorescence after staining with ethidium bromide.

Photography of DNA in Gels and Gel Documentation Systems

Photographs of gels stained with ethidium bromide and other dyes may be made using transmitted or incident UV light. Most commercially available devices (transilluminators) emit UV light at 302 nm. The fluorescent yield of ethidium bromide–DNA complexes is considerably greater at this wavelength than at 366 nm but slightly less than at short-wavelength (254 nm) light. However, the amount of nicking of the DNA is much less at 302 nm than at 254 nm (Brunk and Simpson 1977).

Images of stained gels may be captured by integrated systems containing light sources, fixed-focus digital cameras, and thermal printers. The CCD cameras of these systems use a wide-angle zoom lens ($f = 75$ mm) that allows the detection of very small amounts of ethidium bromide-stained DNA (0.01–0.5 ng is claimed). These systems are generally fitted with a UV filter and have safety switches to turn off the UV light when the door is opened. Files can be saved in a variety of formats for transfer to computer systems for storage and analysis.

In the more advanced gel documentation systems, gel images are directly transmitted to a computer and visualized in real time. A variety of images can be captured from agarose and other fluorescent gels, colorimetric gels, autoradiography film, and blotting membranes. The images can be manipulated on screen with respect to field, focus, and cumulative exposure time before printing. Individual images can be printed, saved, and stored electronically in several file formats and further manipulated with image analysis software programs. The average file size for a stained agarose gel image is ~0.3 MB; thus, extensive archiving requires large-capacity storage systems. Vendors that sell gel-documentation systems include Bio-Rad, FOTODYNE, Kodak, and DNR Bio-Imaging Systems.

Although the results obtained with these documentation systems are entirely satisfactory for immediate analysis, the printed images fade during storage and are devoid of aesthetic appeal. More pleasing and durable results are obtained from highly sensitive Polaroid film Type 57 or 667 (ASA 3000). With an efficient UV light source (>2500 mW/cm^2), a Wratten 22A (red/orange) filter, and a good lens ($f = 135$ mm), an exposure of a few seconds is sufficient to obtain images of bands containing as little as 10 ng of DNA. With a long exposure time and a strong UV light source, the fluorescence emitted by as little as 1 ng of ethidium bromide-stained DNA can be recorded on film. For detection of extremely faint DNA bands stained with this dye, a lens with a shorter focal length ($f = 75$ mm) should be used in combination with a conventional wet-process film (e.g., Kodak no. 4155). This setup allows the lens to be moved closer to the gel, concentrates the image on a smaller area of film, and allows for flexibility in developing and printing the image.

SYBR Gold and SYBR Green I

When excited by standard transillumination at 300 nm, SYBR Gold and SYBR Green give rise to bright fluorescent signals that can be captured on conventional black-and-white Polaroid film or on charge-coupled device (CCD)–based image detection systems. Gels are stained with SYBR dyes after electrophoresis is complete.

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Images of gels stained with SYBR Gold may be captured by CCD camera under UV illumination with a blue light converter screen and an SG emission filter (Oatey 2007).

The level of background fluorescence is so low that no destaining is required. The stained nucleic acid can be transferred directly to membranes for northern or Southern hybridization. SYBR Gold and SYBR Green I may be removed from nucleic acids recovered from gels by ethanol precipitation.

RECIPES

6× Gel-Loading Buffer I

0.25% (w/v) xylene cyanol FF
40% (w/v) sucrose in H_2O
0.25% (w/v) bromophenol blue

Store at 4°C.

6× Gel-Loading Buffer II

15% (w/v) Ficoll (Type 400; Pharmacia) in H_2O
0.25% (w/v) bromophenol blue
0.25% (w/v) xylene cyanol FF

Store at room temperature.

6× Gel-Loading Buffer III

30% (v/v) glycerol in H_2O
0.25% (w/v) bromophenol blue
0.25% (w/v) xylene cyanol FF

Store at 4°C.

6× Gel-Loading Buffer IV

0.25% (w/v) bromophenol blue
40% (w/v) sucrose in H_2O

Store at 4°C.

TAE

Prepare a 50× stock solution in 1 L of H_2O:
242 g of Tris base
57.1 mL of acetic acid (glacial)
100 mL of 0.5 M EDTA (pH 8.0)

The 1× working solution is 40 mM Tris-acetate/1 mM EDTA.

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TBE Buffer

Prepare a 5× stock solution in 1 L of H_2O:
54 g of Tris base
27.5 g of boric acid
20 mL of 0.5 M EDTA (pH 8.0)

The 0.5× working solution is 45 mM Tris-borate/1 mM EDTA.

TBE is usually made and stored as a 5× or 10× stock solution. The pH of the concentrated stock buffer should be ~8.3. Dilute the concentrated stock buffer just before use and make the gel solution and the electrophoresis buffer from the same concentrated stock solution. Some investigators prefer to use more concentrated stock solutions of TBE (10× as opposed to 5×). However, 5× stock solution is more stable because the solutes do not precipitate during storage. Passing the 5× or 10× buffer stocks through a 0.22-µm filter can prevent or delay formation of precipitates.

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