

Agarose gel electrophoresis

Background information

Agarose gel electrophoresis of DNA is used to determine the presence and distinguish the type of nucleic acids obtained after extraction and to analyze restriction digestion products. Desired DNA fragments can be physically isolated for various purposes such as sequencing, probe preparation, or for cloning fragments into other vectors.

Both agarose and polyacrylamide gels are used for DNA analysis. Agarose gels are usually run to size larger fragments (greater than 200 bp) and polyacrylamide gels are run to size fragments less than 200 bp. Typically agarose gels are used for most purposes and polyacrylamide gels are used when small fragments, such as digests of 16S rRNA genes, are being distinguished. There are also specialty agaroses made by FMC (e.g., Metaphor) for separating small fragments. Regular agarose gels may range in concentration from 0.6 to 3.0%. Pouring gels at less or greater than these percentages presents handling problems (e.g., 0.4% agarose for genomic DNA partial digests requires a layer of supporting 0.8% gel). For normal samples make agarose gels at 0.7%. The chart below illustrates the optimal concentrations for fragment size separation.

% Agarose	Range of resolution Linear dsDNA (kbp)	% Acrylamide	Range of resolution	
			dsDNA (bp)	ssDNA (nt)
0.5	30 – 1.0	3.5	100 – 1000	750 – 2000
0.7	12 – 0.8	5.0	75 – 500	200 – 1000
1.0	10 – 0.5	8.0	50 – 400	50 – 400
1.2	7 – 0.4	12.0	35 – 250	
1.5	3 – 0.2	15.0	20 – 150	
		20.0	5 – 100	

The values listed are approximate and can vary depending on the reference that is used. If you do not know your fragment sizes then the best approach is to start with a 0.7% gel and change subsequently if the desired separation is not achieved.

Nucleic acids must be stained prior to visualization. Most laboratories use ethidium bromide but other stains (e.g., SYBR green, GelStar) are available. Gels can be stained either before or after electrophoresis. To stain prior to electrophoresis, add stain to the dissolved agarose just before pouring the gel and swirl to mix. Ethidium bromide is the most commonly used nucleic acid stain, however if you are going to cut out bands for cloning do not add ethidium bromide to the agarose because it will cause your DNA to nick. Instead stain the gel after electrophoresis. The ethidium bromide stain runs in the opposite

direction of your DNA therefore the upper and lower portions of the gel appear differentially stained especially when gels are electrophoresed at high voltages for a short time. If you desire even staining, include ethidium bromide in the electrophoresis buffer. The stain usually evens out when gels are run at low voltages overnight.

Dispose of all ethidium bromide-containing material in appropriately labeled containers. **Caution: ethidium bromide is a potent mutagen. Wear gloves and do not mouth pipette.**

DNA possesses a consistent charge to mass ratio, therefore the major factor influencing migration through a gel matrix is size (and occasionally secondary structure). Fragment sizes can be determined by comparison to standard DNA size markers. These standards are usually plasmids or other DNA of known sequence, cleaved with a restriction enzyme(s) that yields a series of fragments within a wide size range. There are also a number of commercially synthesized size ladders that can be purchased. After the completion of electrophoresis the gel should be photographed with a ruler lined up along the length of the gel (with 0.0 mm at the well edge). Using a semi-log plot of mobility (cm migrated) versus size (in bp, of the known DNA marker fragments) standard curves can be constructed. The X axis is the log scale (fragment size) and the y axis is a linear scale (distance migrated). The size of a fragment from other restriction digests on the same gel can be determined from this standard curve by determining the distance migrated from the photograph containing the ruler.

If you want to determine fragment concentration(s), you can estimate the amount by one of two means:

- (a) Run uncut, known DNA in a series of concentrations on the same gel (concentration predetermined by other means, e.g., A_{260} reading) and visually compare the density of the fragment bands of unknown concentration with the known series, or,
- (b) Compare fragment(s) of unknown concentrations with lambda *HindIII* or other cut DNA marker, by calculating DNA concentration of each different sized standard fragment as follows:

Lambda/*HindIII* DNA fragments: total genome length = 48,502 bp

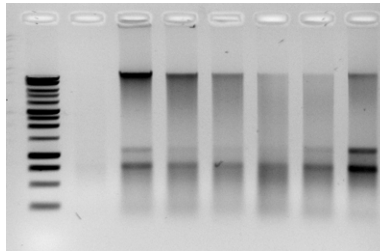
Size (bp = base pairs)	% of genome	ng/0.5 μ g	ng/1.0 μ g
23,130	47.7	239	477
9,416	19.4	97	194
6,557	13.5	68	135
4,361	9.0	45	90
2,322	4.8	24	48
2,027	4.2	21	42
564	1.2	6	12

Materials

Buffer (usually 1X TAE or TBE)
Agarose (electrophoresis grade)
Ethidium Bromide (10 mgml⁻¹ stock)

The protocol in brief

You will pour, load and run an agarose gel to visualize DNA that you have either (i) isolated from your sample, or (ii) generated via PCR.



DNA visualized on an agarose gel following ethidium bromide staining. The first lane is a DNA size marker containing defined DNA fragments of known size.

A. Casting a gel

1. Combine an appropriate volume of 1X Tris-acetate-EDTA (TAE) buffer with an appropriate amount of agarose (these values are determined based on the gel dimensions and the desired percentage of agarose). Swirl the flask to evenly distribute the agarose. If an exact agarose concentration is required weigh the flask and its content at this time.
2. Heat the solution in the microwave oven for 1 minute. Wear protective gloves and be extremely careful, remove the flask from the microwave oven (before it boils over), swirl, and reheat while keeping constant watch to be sure it does not boil over. When it starts to boil, stop and swirl again repeating the process until all of the agarose goes into solution. **Caution: even when the agarose is not boiling, when you remove it from the microwave oven it can boil over and burn you.** Undissolved agarose (hold flask up to the light) will appear as flecks in an otherwise clear solution.
3. Place the flask in a 55-65° C water bath or on a stir-plate to cool. The gel should be poured when the temperature of the solution is 55-65° C. If poured too hot the gel trays will warp. Re-weigh the flask and its content at this time and add water until the original weight is achieved.
4. Prepare the gel apparatus for casting the gel while the agarose is cooling.

There are several different types of gel boxes and these preparations will depend on the particular one you are using.

5. Just prior to pouring the gel, add ethidium bromide to the dissolved agarose and swirl to mix. If you are going to cut out bands for cloning do not add ethidium bromide to the agarose because it will cause your DNA to nick. Instead stain the gel after electrophoresis.

Ethidium Bromide Concentrations to be used:

stock solution: 10 mg ml⁻¹
working concentration: 0.5 µg ml⁻¹ for staining solution
working concentration: 0.3 µg ml⁻¹ for gels

Dispose of all ethidium bromide-containing material in appropriately labeled containers. CAUTION: ETHIDIUM BROMIDE IS A POTENT MUTAGEN. WEAR GLOVES AND DO NOT MOUTH PIPETTE.

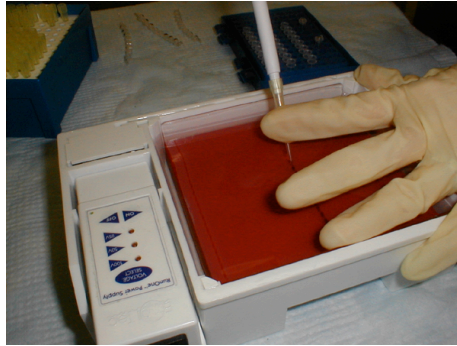
7. Pour the gel into the casting tray and adjust the comb to keep the wells perpendicular. If using an independent gel tray press the trays down if air bubbles form between the gel and pouring tray. Floating trays often result in the comb teeth penetrating to the tray and your samples will leak out of the bottom when you load them. Allow the gel to cool and harden (20-30 minutes) prior to use.

B. Preparing the gel for electrophoresis

8. Add a few ml of 1X TEA buffer to the well area of the gel and carefully remove the comb by pulling straight up. Avoid breaking the walls of the wells.
9. Fill the electrophoresis tank with buffer solution (1X TAE) and place the gel (in the casting tray) on the tank platform. The buffer must cover the gel by 1-2 mm.

C. Prepare samples for loading/running the gel

10. Add an appropriate volume of loading dye (6X or 10X) to the sample (1 µl of 6X sample dye for every 5 µl of sample, i.e. 5 µl of dye will be added to your 25 µl PCR reaction).
11. Load the gel using a 1-10 µl pipette. Stick the tip below the surface of the buffer, but above the well bottom being careful not to poke a hole in the buffer well, and dispense the sample slowly. The sample will sink (due to the glycerol in the loading buffer) through the buffer and settle in the well. Load the prepared size marker.



Loading of an agarose gel. Be careful not to poke the pipette tip through the bottom of the well.

12. After the gel has been loaded, gently place the cover on the apparatus and hook up the power leads. DNA is negatively charged and will migrate toward the positive (red lead and jack in power supply) electrode. Adjust the power to between 50 and 100 volts (constant voltage). Run the gel until the first dye front (bromophenol blue) has migrated about two-thirds the length of the gel and the second dye front (xylene cyanol) has migrated approximately one-third of the length of the gel. Do not touch the buffer when the power is on.



The loading dye is colored blue and is heavier than the electrophoresis buffer. Therefore, you can efficiently load the sample into the well and monitor the progress of the electrophoresis.

13. Turn off power before removing the gel for photographing.
14. Place the gel on the UV transilluminator to visualize the DNA. Remember to always wear eye protection if you are not using an automated image analysis system to view your gel.