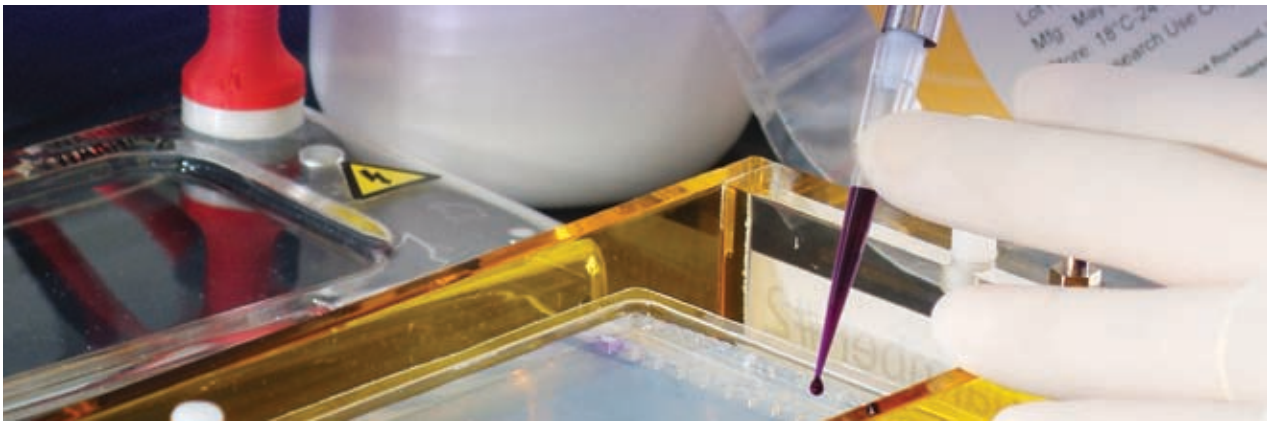


In This Section

Introduction

The amount of DNA to load per well is variable. Most important are the quantities of DNA in the bands of interest.

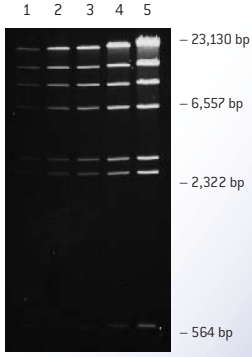
Optimal DNA loading amount

The amount of DNA that may be loaded on a gel depends on several factors:

- Well volume
- Fragment size: The capacity of the gel drops sharply as the fragment size increases, especially over a few kilobases
- Distribution of fragment sizes
- Voltage gradient: Higher voltage gradients are better suited to DNA fragments <1 kb, lower voltages are better suited to fragments >1 kb
- Detection Method: The least amount of dsDNA in a single band that can be reliably detected with ethidium bromide is approximately 10 ng, with GelStar® Nucleic Acid Gel Stain is approximately 20 pg and with SYBR® Green I Stain is 60 pg

Overloaded DNA results in trailing and smearing, a problem that will become more severe as the size of the DNA increases.

The photograph below shows the effect of overloaded and underloaded DNA on an agarose gel. Where samples are loaded in excess (0.5 µg/lane), you can see band broadening and smearing of the larger molecular weight fragments. Where samples are underloaded, you lose the small molecular weight fragments. The optimum loading level for the marker used in the photograph is 0.1 µg/lane.



Separation of DNA markers in a 1% SeaKem® GTG® Agarose gel prepared and run in 1X TBE Buffer. *Hin*d III digested lambda DNA (Boehringer Mannheim) was loaded from left to right at 0.025, 0.05, 0.1, 0.2 and 0.5 µg/lane. 20 cm long gels were run at 6 V/cm for 2 hours, 5 minutes.

The optimal amount of DNA to load in the well may be calculated by the fraction of the total DNA which is in the band of interest, represented by the following:

$$\frac{\text{Fragment of interest (kbp)}}{\text{Size of DNA sample (kbp)}} \times 100 = \%\ \text{DNA in band of interest}$$

NOTE: The most DNA compatible with a clean sharp band is approximately 100 ng.

For example:
The size of your DNA sample is 48.5 kbp and when run on the gel 8 fragments are separated. Your fragment of interest is 2.3 kbp.

Calculation:

$$\frac{2.3\ \text{kbp}}{48.5\ \text{kbp}} \times 100 = 4.7\%\ \text{DNA in fragment of interest}$$

If you load 1 µg of DNA, then 4.7% of the 1 µg of loaded sample will appear in your fragment of interest (47 ng).

Introduction

Gel loading buffers serve three purposes in DNA electrophoresis:

— Increase the density of the sample: This ensures that the DNA will drop evenly into the well

— Add color to the sample: Simplifies loading

— Add mobility dyes: The dyes migrate in an electric field towards the anode at predictable rates. This enables one to monitor the electrophoretic process

Loading buffers

At least five loading buffers are commonly used for agarose gel electrophoresis. They are prepared as six-fold concentrated solutions. If needed, 10X solutions of each buffer can also be prepared. Alkaline loading buffer is used when performing alkaline gel electrophoresis.

Loading Buffer	6X Recipe	Storage Temperature
Sucrose Based	40% (w/v) Sucrose 0.25% Bromophenol Blue 0.25% Xylene cyanol FF	4°C
Glycerol Based	30% Glycerol in distilled water 0.25% Bromophenol Blue 0.25% Xylene cyanol FF	4°C
Ficoll® Based	15% Ficoll® (Type 400) Polymer in distilled water 0.25% Bromophenol Blue 0.25% Xylene cyanol FF	room temperature
Alkaline	300 mN NaOH 6 mM EDTA 18% Ficoll® (Type 400) Polymer in distilled water 0.15% Bromocresol Green 0.25% Xylene cyanol FF	4°C

Lonza offers ready-to-use DNA loading buffers. Refer to page 41 for information.

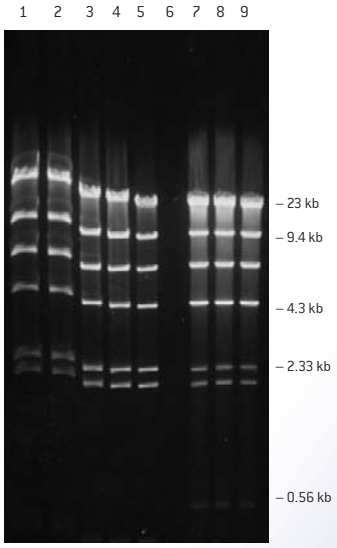
Ficoll® based loading buffers

To increase the sharpness of DNA bands, use Ficoll® (Type 400) Polymer as a sinking agent instead of glycerol. The use of the lower molecular weight glycerol in the loading buffer allows DNA to stream up the sides of the well before electrophoresis has begun and can result in a U-shaped band. In TBE gels, glycerol also interacts with borate which can alter the local pH.

Sample preparation

Loading buffer that is too high in ionic strength causes bands to be fuzzy and migrate through the gel at unpredictable rates. Ideally, the DNA sample should be resuspended in the same solution as the running buffer. If this is not possible, use a sample buffer with a lower ionic strength than the running buffer.

The photograph below shows the effect of high salt concentrations in loading buffers on DNA resolution.



Separation of *Hin*d III digested lambda DNA (Invitrogen, Inc.) marker (5 µg/lane) in a 1% SeaKem® GTG® Agarose gel prepared and run in 1X TAE Buffer. 20 cm long gels were run at 6 V/cm for 2 hours. The sample buffer was mixed with varying amounts of NaCl to obtain different final salt concentrations.
Lane 1: 4 M NaCl, **Lane 2:** 3 M NaCl,
Lane 3: 1 M NaCl, **Lane 4:** 0.5 M NaCl,
Lane 5: 0.25 M NaCl and **Lanes 7 - 9:** No addition of NaCl.

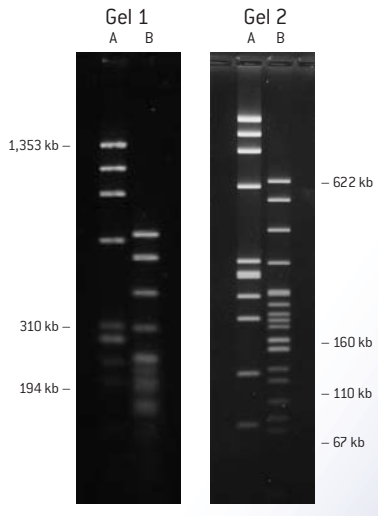
Optimal voltage

The distance used to determine voltage gradients is the distance between the electrodes, not the gel length. If the voltage is too high, band streaking, especially for DNA ≥12 kb - 15 kb, may result. When the voltage is too low, the mobility of small (≤1 kb) DNA is reduced and band broadening will occur due to dispersion and diffusion.

The photographs below show the effect of voltage on small DNA. The small fragments on the gel run at 1 V/cm show severe band broadening and fuzziness.

The gel run under 5 V/cm has sharp bands both in the small fragments and the larger fragments. Buffer also plays a role in band sharpness.

The photographs below show the effect of voltage and buffer on large DNA. When large DNA is subjected to very high voltage, smearing occurs.



Separation of DNA markers in 3% NuSieve® 3:1 Agarose Gels. Lane A: *Hae* III digested ϕX174 DNA (New England Biolabs); 0.5 µg/lane. Lane B: *Msp* I digested pBR322 DNA (New England Biolabs); 0.5 µg/lane. Gel 1: 21 cm long gel run at 1 V/cm in 1X TAE Buffer (recirculating) for 16 hours. Gel 2: 20 cm long gel run at 5 V/cm in 1X TBE Buffer for 2 hours, 10 minutes.

Voltage table

The table below provides a quick reference for optimal voltage for DNA electrophoresis.

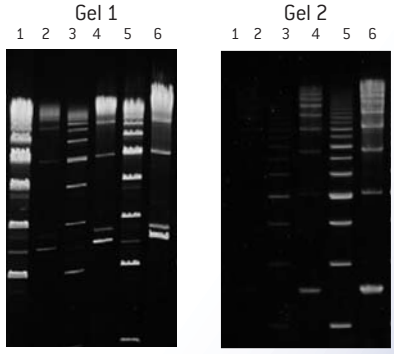
Recommended Voltages and Buffers Related to DNA Size and Application

		Buffer	
Size	Voltage	Recovery	Analytical
≤1 kb	5 V/cm	TAE	TBE
1 kb to 12 kb	4 - 10 V/cm	TAE	TAE/TBE
>12 kb	1 - 2 V/cm	TAE	TAE

NOTE: MetaPhor® Agarose can also be run at very high voltages to achieve 1% - 2% resolution. See "Fast Running Protocols for High Resolution in MetaPhor® Agarose Gels," this chapter.

Optimal electrophoretic time

The gel should be run until the band of interest has migrated 40% - 60% down the length of the gel (see the Dye Mobility Table). Band broadening resulting from dispersion and diffusion results in a decrease in resolution in the lower third of the gel. Resolution may also be decreased in smaller gels, since longer electrophoretic runs result in greater separation between two fragments.



Separation of Gensura's 5 kb and 2 kb DNA ladders in 0.5% SeaKem® LE Agarose gels. Odd numbered lanes are 5 kb ladders; even numbered lanes are 2 kb ladders. Lane 1: 5 ng/band; Lane 2: 5 ng/band; Lane 3: 20 ng/band; Lane 4: 20 ng/band; Lane 5: 60 ng/band; Lane 6: 60 ng/band. Gel 1: 20 cm long gel run at 8 V/cm in 1X TBE Buffer without recirculation. Gel 2: 21 cm long gel run at 1 V/cm in 1X TAE Buffer with recirculation.

The FlashGel® System (pages 18-25) enables very fast (5 minute) high voltage separation of DNA fragments 10 bp to 10 kb.

Introduction

The protocols in the following section describe how to increase resolution to a 1% size difference with DNA between 100 bp and 500 bp, and decrease your electrophoretic time to 1.5 hours.

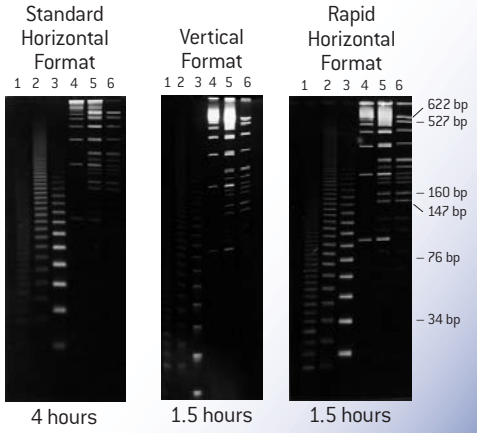
A standard horizontal submarine gel apparatus can be used to achieve resolution which is comparable to polyacrylamide gels at ≤8%. MetaPhor® Agarose can also be used to achieve similar resolution in a standard vertical gel electrophoretic system.

The photographs below show the resolution achieved in 4% MetaPhor® Agarose gels, using the same markers in three different formats.

Electrophoretic conditions

	Vertical Format	Rapid Horizontal Format
Resolution	1% (≤500 bp)	1% (≤500 bp)
Run Time	1 - 1.5 hours	1.5 hours
Gel Concentration	3% - 4%	3% - 4%
Gel Length	16 cm - 26 cm	20 cm
Gel Thickness	1 mm	3 mm
Gel Buffer	1X TAE or 1X TBE	1X TAE or 1X TBE
Running Buffer	1X TAE or 1X TBE	0.5X TAE or 0.5X TBE
TBE		
Voltage*	17 V/cm	17 V/cm
Temperature	ambient	15°C**

*V/cm is determined by the total voltage divided by the interelectrode distance in cm.
***Circulate electrophoresis buffer with a recirculator-chiller water bath.*



Lane 1: 4 bp linker ladder; Lane 2: 8 bp linker ladder; Lane 3: 12 bp linker ladder; Lane 4: 600 ng of 100 bp ladder; Lane 5: 400 ng of pBR322/*Msp* I digest + 600 ng of 100 bp ladder; Lane 6: 400 ng of pBR322/*Msp* I digest.

Materials

- Horizontal electrophoresis chamber to accommodate a 20 cm long gel or vertical electrophoresis chamber
- Power supply
- Recirculator-chiller water bath

Reagents

- Electrophoresis buffer (TAE or TBE)
- MetaPhor® Agarose
- GelStar® or SYBR® Green Nucleic Acid Gel Stains or ethidium bromide solution

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

Fast running protocol for horizontal gels

NOTE: This protocol cannot be used with only a peristaltic pump in the cold room; the gel will melt.

1. Prepare a 3 - 4% MetaPhor® Agarose gel in 1X electrophoresis buffer.
2. Cast a 3 mm thick, 20 cm long agarose gel.
3. Allow the gel to solidify at room temperature.
4. Place at 4°C for 30 minutes.
5. Place the gel in the electrophoresis chamber.
6. Use 0.5X TBE or 1X TAE Running Buffer in the electrophoresis chamber.
7. Add the running buffer to a depth of 3 mm over the surface of the gel.
8. Load 20 ng - 50 ng of DNA. If this cannot be estimated, load varying amounts of the sample. The sharpest bands are seen with small (5 µl - 10 µl) loading volumes.
9. Run the gel at 17 V/cm (interelectrode distance).
10. When the sample has left the well and moved into the gel, begin recirculating the electrophoresis buffer.
11. Chill and circulate the electrophoresis buffer with a recirculator-chiller water bath.
12. Run the gel for approximately 1.5 hours.

Troubleshooting

The fast running protocol will not work if the buffer is not chilled or recirculated. The photograph to the right shows the effect of DNA resolution on running the gel in a cold room (4°C), without buffer recirculation.

Fast running protocol for vertical gels

1. Cast a vertical agarose gel in 1X electrophoresis buffer following the Vertical Gel Casting Instructions on page 86.
2. Carefully flush the wells with running buffer.
3. Load 20 ng - 50 ng of DNA in the band of interest. If this cannot be estimated, load varying amounts of the sample. The sharpest bands are seen with a small (5 µl - 10 µl) loading volume.
4. Run the gel at 17 V/cm interelectrode distance.
5. Run the gel approximately 1 - 1.5 hours.

Msp I digest of pBR322 (New England Biolabs), 400 ng/lane and Lonza's 100 bp DNA Ladder, 600 ng/lane run on a 4% MetaPhor® Agarose gel prepared in 1X TAE Buffer. The gel was run at 17 V/cm in 0.5X TAE Buffer for 90 minutes in the cold room without buffer recirculation.

NOTE: The gel melted and was allowed to resolidify prior to staining.

References

FMC BioProducts, *Resolutions 9(1),* 1992.
Rickwood, *et al., Nucleic Acid Electrophoresis: A Practical Approach*, 2nd edition, Oxford University Press, 1990.
Sambrook J., *et al., Molecular Cloning: A Laboratory Manual,* 2nd edition, Cold Spring Harbor Laboratory Press, 1989.
Vandenplas S., *et al., J. Medical Genetics 21:* 164 - 172, 1984.