## Did you know:

- When preparing agarose for electrophoresis, it is best to sprinkle the agarose into room-temperature buffer, swirl, and let sit at least 1 min before microwaving. This allows the agarose to hydrate first, which minimizes foaming during heating.

- Electrophoresis buffer can affect the resolution of DNA. TAE (Tris-Acetate-EDTA) buffer provides better resolution of fragments >4 kb, while TBE (Tris-Borate-EDTA) buffer provides better resolution of 0.1– to 3–kb fragments. In addition, use TBE buffer when electrophoresing >150 V and use TAE buffer with supercoiled DNA for best results.

- Migration of DNA is retarded and band distortion can occur when too much buffer covers the gel. The slower migration results from a reduced voltage gradient across the gel.

- Loading DNA in the smallest volume possible will result in sharper bands.

- You can preserve DNA in agarose gels for long-term storage using 70% ethanol. [See Jacobs, D. and Neilan, B.A. (1995) *BioTechniques 19*, 892.]

- Electrophoresing a gel too "hot" can cause the DNA to denature in the gel. It can also cause the agarose gel to deform. Cool the gel with a small fan during the electrophoresis.

- For the Supercoiled DNA Ladder electrophoresed on <1% agarose gels, add 2 µg/ml ethidium bromide to the gel. Otherwise, smeared bands and extra bands will be seen because of different degrees of supercoiling. [See Longo, M.C. and Hartley, J.L. (1986) *Focus 8:3*, 3. (reprinted on page 63, this issue).]

- When glycerol-containing loading buffers are used in DNA samples electrophoresed through acrylamide gels, smiling bands may be accentuated especially in TBE.

- On a polyacrylamide gel, DNA fragments having AT-rich regions migrate slower than other DNA fragments of the same size. This anomalous migration is enhanced at lower temperatures and disappears at high temperatures. This anomalous migration is not observed on agarose gels. [See Stellwagen, N.C. (1983) *Biochemistry 22*, 6186.]

- The minimum amount of DNA detectable by ethidium bromide on a 3-mm-thick gel and a 5-mm-wide lane is 1 ng. Do not exceed 50 ng of DNA per band on a 3-mm-thick gel and 5-mm-wide lane.

*Shanta Dube*
*Technical Services*
*Life Technologies, Inc.*
*Rockville, Maryland 20849*

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# Troubleshooting DNA Agarose Gel Electrophoresis

*Shanta Dube*
*Technical Services*
*Life Technologies, Inc.*
*Rockville, Maryland 20849*

## *If you see faint or no bands on the gel:*

- There was insufficient quantity or concentration of DNA loaded on the gel. Increase the amount of DNA, but don't exceed 50 ng/band.
- The DNA was degraded. Avoid nuclease contamination.
- The DNA was electrophoresed off the gel. Electrophorese the gel for less time, use a lower voltage, or use a higher percent gel.
- Improper UV light source was used for visualization of ethidium bromide-stained DNA. Use a short-wavelength (254 nm) UV light for greater sensitivity. Note: For preparative gels, using a longer wavelength (312 nm) UV light will minimize DNA degradation.

## *If you see smeared DNA bands:*

- The DNA was degraded. Avoid nuclease contamination.
- Too much DNA was loaded on the gel. Decrease the amount of DNA.
- Improper electrophoresis conditions were used. Do not allow voltage to exceed ~20 V/cm. Maintain a temperature <30°C during electrophoresis. Check that the electrophoresis buffer used had sufficient buffer capacity. This is done by checking the pH in the anode and cathode chambers.
- There was too much salt in the DNA. Use ethanol precipitation to remove excess salts, prior to electrophoresis.
- The DNA was contaminated with protein. Use phenol extractions to remove protein prior to electrophoresis.
- Small DNA bands diffused during staining. Add the ethidium bromide during electrophoresis.

## *If you see anomalous DNA band migration:*

- Improper electrophoresis conditions were used. Do not allow voltage to exceed ~20 V/cm. Maintain a temperature <30°C during electrophoresis. Check that the electrophoresis buffer used had sufficient buffer capacity.
- The DNA was denatured. Do not heat standards [except for λDNA/*Hin*d III fragments (figure 1)] prior to electrophoresis. Dilute DNA standards in buffer with 20 mM NaCl.

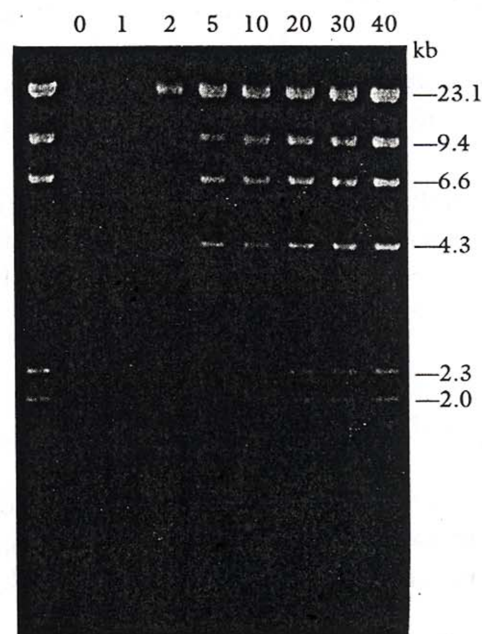


**FIGURE 1. The effect of ionic strength on the heat denaturation of λ DNA/*Hin*d III fragments.** 500 ng λ DNA/*Hin*d III fragments in NaCl concentrations of 0, 1, 2, 5, 10, 20, 30, and 40 mM were electrophoresed after heating at 65°C for 10 min.