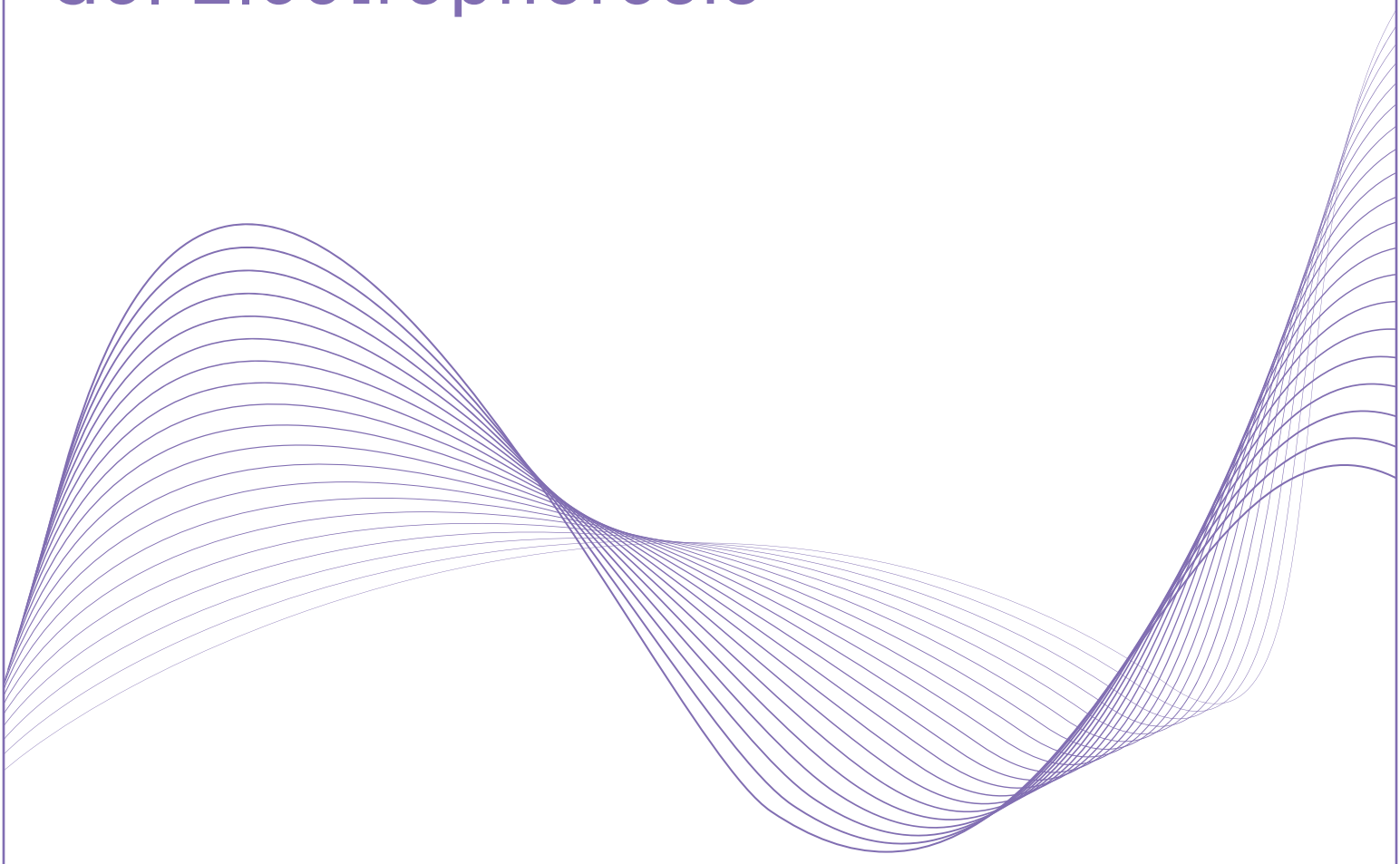
GUIDE

Volume 4: Sanger Quick Tips for Running an Informative Gel for PCR Visualization by Gel Electrophoresis



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Accurate assessment of PCR success depends on a well-resolved gel image with sufficient band separation and definition. There are several simple and effective steps you can take to get the most information out of your gels.

Running Buffer

When choosing a running buffer, it is key to consider downstream applications. Common electrophoresis buffers include:

- **TBE:** Tris-borate-EDTA (TBE) is the recommended electrophoresis running buffer for fragments less than 1.5 kb. TBE is a good choice for standard gel visualization and can be run at moderate voltages (no more than 10V/cm gel box length, cathode to anode). A note of caution about TBE buffer: borate can impede enzymatic reactions, so if you aim to recover the DNA from your gel for downstream use, a buffer lacking borate is recommended (e.g., TAE). TBE is normally used at a 0.5X working concentration.
- **TAE:** Tris-acetate-EDTA (TAE) is another common electrophoresis running buffer. TAE has less buffering capacity than TBE, so gels must be run for a slightly longer time at lower voltage, and the buffer should be replaced after every usage for best results. However, DNA isolated from a TAE gel has the advantage of being suitable for downstream enzymatic reactions. Thus, TAE is the buffer of choice for DNA recovery. TAE is also better for larger fragments (>1.5 kb), as well as for genomic DNA and supercoiled DNA. TAE is normally used at a 0.5X-1X working concentration. NOTE: The same supercoiled DNA sample would run differently in TBE and TAE buffers, and this should be taken into account when comparing results between the two buffering systems.
- **Alternatives to TAE/TBE:** Over the last several years, bench scientists have determined several alternatives to the classic TBE/TAE electrophoresis buffers, namely Sodium Borate (SB) and Lithium Borate (LB). They are viable for quick visualization of DNA molecules. Gels in these buffers can be run at higher voltages (typically 10-50 V/cm) without overheating, thus decreasing the time needed for sufficient band separation. Like TBE, both SB and LB contain borate, therefore they are not suitable for band extraction and downstream enzymatic reactions with recovered DNA.

Tips for Success

Always make sure the agarose is dissolved in the same buffer used in the electrophoresis chamber.

1. AGAROSE GEL CONCENTRATION

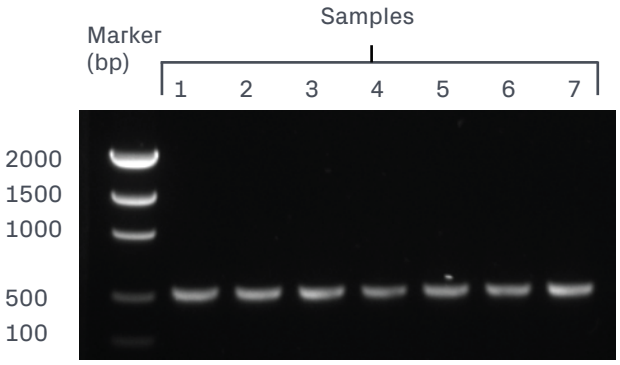
Agarose concentration affects gel porosity, which in turn determines its resolving power. The concentration of agarose in the prepared gel should be based on the size of DNA molecules to be resolved (Table 1). Note that gel rigs often come with two types of combs; thick combs create higher-volume wells that are appropriate for preparative work and thin combs create lower-volume wells for analytical work.

DNA Band Size (bp)	% Agarose in Gel (w/v)
<250	Not Recommended
250 - 1,500	2.00
300 - 4,000	1.50
400 - 7,000	1.20
500 - 9,000	1.00
800 - 11,000	0.75
1,000 - 30,000	0.50

Table 1. Agarose Gel Percentage Guidelines by DNA Size

2. LADDER

Make sure you include at least one lane of DNA standards, known as a ladder, on each gel. Ladders are available in many sizes and configurations; the best way to select the appropriate ladder is to identify the size range of DNA molecules you wish to resolve. A 100-bp ladder typically contains bands of 100-bp increments ranging from about 100 bp to 1500 bp. A 1-kb ladder contains 1,000-bp increments covering a range from about 500 bp to 10 kb. A 2-Log ladder is also a popular option, as it spans both the lower size range (from 100 bp onwards) through to the larger 10 kb range. Many labs stock both the 100 bp and the 1 kb ladder. Note that for gel consistency and size accuracy, it is best to use the same loading buffer for both your ladder and samples.



3. AMOUNT OF TEMPLATE TO LOAD

The mass of DNA required to visualize your band on an agarose gel tends to be in the nanogram range. The minimum recommended amount per band is about 10 ng; anything less than that will be very difficult to detect on a gel. For crisp bands with no distortion (e.g., “smiley faces”) or smearing, ~150 ng per band should be the highest mass loaded into a lane. The appropriate amount of sample volume to load depends on the volume of the well in the gel; at a minimum, the sample should occupy at least a third of the well’s capacity.

4. LOADING BUFFER SELECTION

There are a variety of different sample loading buffers available. The easiest approach is to use the same loading buffer as that used for the ladder. Loading buffers can also be prepared from reagents normally stocked in a standard molecular biology lab. You can either obtain commercially available loading buffers from several vendors or prepare one from reagents normally stocked in a standard molecular biology lab. They usually

contain a high density compound, such as Ficoll® or glycerol, and one or more dyes, such as Orange G, xylene cyanol, and bromophenol blue. These buffers are normally prepared at a 6X concentration and run at a 1/6 final concentration (or 1X) in your sample (Table 2).

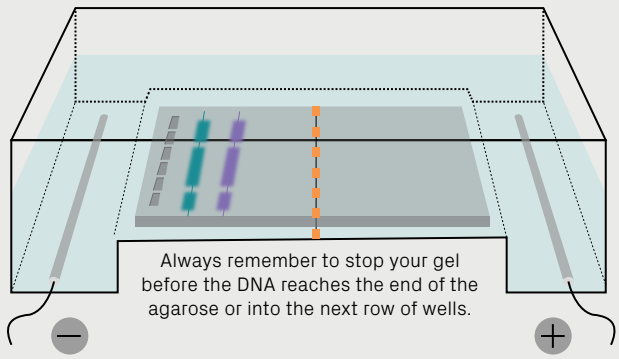
Recipe (6X)	High-Density Compound
40% (w/v) Sucrose 0.25% Bromophenol Blue 0.25% Xylene cyanol FF	Sucrose
30% Glycerol in distilled water 0.25% Bromophenol Blue 0.25% Xylene cyanol FF	Glycerol
15% Ficoll® 0.25% Bromophenol Blue 0.25% Xylene cyanol FF	Ficoll

Table 2. Common Loading Buffers

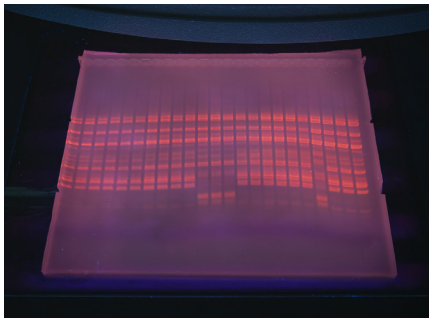
5. VOLTAGE AND RUNNING TIME

In general, gel electrophoresis should be run with sufficient time to clearly separate your bands of interest as well as those of the ladder. Under such conditions, you can accurately determine the number of DNA molecules present in your sample and estimate their size. In general, the minimum running distance for the gel should be about 5 cm, but it can be as long as needed for your specific purposes. The recommended voltage depends on the type of running buffer and agarose being used. For example, standard agarose gels with TBE buffer are usually run at 5-10V/cm (distance measured between electrodes). Using low-melt agarose or less stable buffers may need a lower voltage and longer running time for optimal results. Solutions with high buffering capacities can be run at a higher voltage, and this requires less time for sufficient band separation. Be sure to check the recommendations of your agarose supplier for more specific guidance on running conditions. The loading buffer dye front will enable you to easily visualize the progress of electrophoresis; keep in mind that some dye fronts may actually migrate behind your band of interest, so check the electrophoretic properties of your dye (i.e., the size of DNA which co-migrates with the dye).

Tips for Success



6. CHOOSING AN APPROPRIATE GEL STAIN



Ethidium bromide (EtBr) is a widely used stain for DNA detection. EtBr is a long-lasting stain that can be mixed directly into your agarose gel before it is poured, obviating the need for a subsequent gel staining step. It is cost effective and has good sensitivity for band visualization. The major drawback of EtBr is toxicity - it is a mutagen. Therefore, many labs choose safer alternatives, such as SYBR® Gold and SYBR® Safe. Compared to EtBr, the SYBR® series is less stable and more expensive but has equal or better sensitivity. Due to their instability, these types of dyes can require loading directly into the sample, or post-staining prior to visualization. Follow the recommendations of the manufacturer in order to determine optimal stain application techniques for your gel.

Gel Interpretation and Troubleshooting Tips

1. BAND DISTORTION

A common problem encountered when running gels is band distortion, often observed as a U-shape or “smiley face”. These banding patterns usually occur when there is too much sample loaded on your gel and/or the gel is run at too high a voltage; most of the time if you reduce the amount loaded on the gel and reduce the voltage, you can eliminate this issue. If this does not resolve the problem, high salt or contamination with organic compounds could be the problem. An extra precipitation and wash step might help.

2. OVERHEATING

Overheating can cause bands in the middle lanes of the gel to run faster than those on the edge. Under these conditions, it can be difficult to estimate the molecular size of the band or make comparisons between samples on the gel. If this occurs, you should reduce the voltage or, alternatively, run the gels in a cold room or chill the electrophoresis tank with cold packs.

3. SMEARING

If you are expecting a nice, clean band but end up with a disseminated smear on your gel, check for the following causes:

- High voltage
- Concentration and/or type of buffer used in the gel is different than that in the chamber
- Heterogeneous gel, caused by incomplete dissolution of the agarose prior to casting
- High salt or organic compound concentration in the template
- Suboptimal PCR - have these primers worked in the past?

Refer to the Sanger QuickTips guide Volume 1: Tips for a Robust, Single-band PCR product for troubleshooting and optimization ideas.

4. BLURRY BANDS

In addition to the issues discussed above, blurry or fuzzy bands can be caused by a poorly focused image. To obtain sharpness, manually adjust the focus on your gel imaging system (or use autofocus, if available) every time you capture an image. Note that an image can become out-of-focus when changing the zoom of the lens, so adjust the zoom first, then focus.

5. EXTREMELY BRIGHT OR FAINT BANDS

To capture a good gel image, the exposure must be adjusted properly. Too long an exposure can result in blown-out bands, which may cause two nearby bands to appear as one. If the exposure is too short, you may fail to detect fainter bands. It is often helpful to try a series of exposures to make sure the gel image is informative and accurate.

6. MULTIPLE BANDS

The presence of multiple bands indicates that your primer set amplified more than one unique product. Take a peek at our Tip Sheet section on PCR tips for a high-intensity single-band product.

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7. PRIMER DIMER

Primer self-annealing often shows up as a small smear at the front edge of the gel (around 50 bp in length). This indicates that the primer set is annealing to itself and needs to be redesigned. There are online tools available to check the propensity for primers to form homo- or heterodimers; Azenta scientists recommend that you use these tools before ordering any newly-designed primer set.

8. INSUFFICIENT BAND SEPARATION

If you fail to achieve good resolution even after using the full length of the gel, follow the following suggestions:

- Adjust the agarose percentage.
- Use a longer gel.
- Run the gel at a lower voltage for a longer length of time; this can help increase band resolution. The gel rig can even be set up in a cold room or refrigerator to maximize the crispness of your bands.
- Make sure buffer composition and concentration is the same in the gel and in the buffer chamber.
- Investigate possible sources of contamination (e.g., salt, organic compounds) in your template.

9. MISSING BANDS

Before panic sets in, set up the PCR again and make sure all reaction components are added correctly. Always include a positive and negative control, if available. It helps to keep a checklist in your lab notebook and physically check off each component as it is added. If, after a gel re-run, all the bands are still missing, you can investigate the following possibilities:

- Did the bands migrate off the gel? (i.e., the gel was run too long). Look for the position of the dye front and the ladder to confirm.
- Did the bands migrate down into another gel space (if you have a multi-row gel set up)?
- Check PCR design - have these primers worked in the past?

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