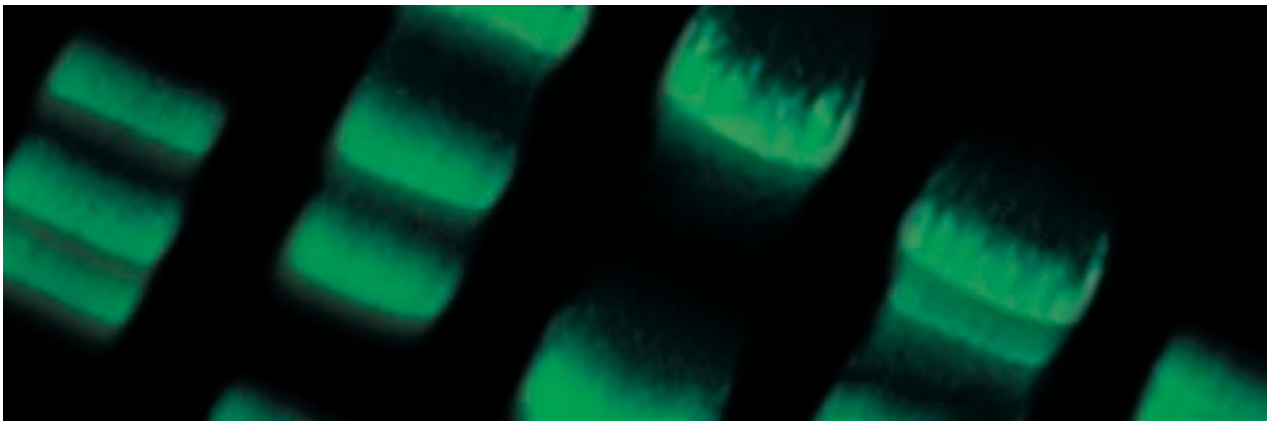


In This Section

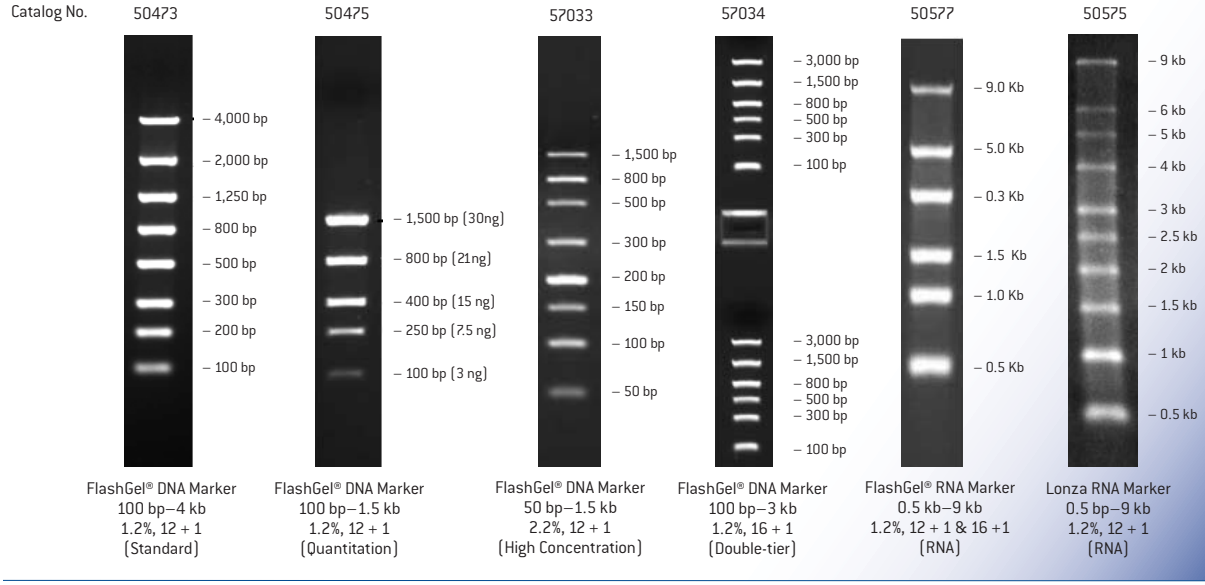
Size Range (Bold numbers indicate brighter bands)

	20 bp	20 bp Ext	100 bp	100 bp ext	Tandem	500 bp	Quant Ladder	Rev Quant Ladder	50 bp-1000 bp	50 bp-2500 bp	1 kb-10 kb
Standard Ladders											
(Cat. No.)	50330	50320	50321	50322	NA	50323	50334	50335	50461	50631	50471
SimplyLoad® Ladders											
(Cat. No.)	50331	50326	50327	50328	50333	50329	50336	50337	NA	NA	NA
Number of Fragments	25	50	10	30	21	16	5	5	9	13	9
Size Range	20 bp – 500 bp	20 bp – 1,000 bp	100 bp – 1,000 bp	10 bp – 3,000 bp	100 bp – 12 kb	500 bp – 8 kb	100 bp – 1,000 bp	100 bp – 1,000 bp	50 bp – 1,000 bp	50 bp – 2,500 bp	1 kb – 10 kb
	500 bp	1,000 bp	1,000 bp	**3,000 bp**	12 kb	8,000 bp	1,000 bp	1,000 bp	1,000 bp	2.5 kb	10 kb
	480 bp	980 bp	900 bp	2,900 bp	11 kb	7,500 bp	700 bp	700 bp	700 bp	2 kb	7 kb
	460 bp	960 bp	800 bp	2,800 bp	10 kb	7,000 bp	500 bp	500 bp	525 bp	1.5 kb	5 kb
	440 bp	940 bp	700 bp	2,700 bp	9 kb	6,500 bp	200 bp	200 bp	500 bp	1250 bp	4 kb
	420 bp	920 bp	600 bp	2,600 bp	8 kb	6,000 bp	100 bp	100 bp	400 bp	**1 kb**	3 kb
	400 bp	900 bp	500 bp	2,500 bp	7 kb	5,500 bp			300 bp	700 bp	2.5 kb
	380 bp	880 bp	400 bp	2,400 bp	6 kb	**5,000 bp**			200 bp	525 bp	2 kb
	360 bp	860 bp	300 bp	2,300 bp	**5 kb**	4,500 bp			100 bp	500 bp	1.5 kb
	340 bp	840 bp	200 bp	2,200 bp	4 kb	4,000 bp			50 bp	400 bp	1 kb
	320 bp	820 bp	100 bp	2,100 bp	3 kb	3,500 bp				300 bp	
	300 bp	800 bp		2,000 bp	2 kb	3,000 bp				200 bp	
	280 bp	780 bp		1,900 bp	**1 kb**	2,500 bp				100 bp	
	260 bp	760 bp		1,800 bp	900 bp	2,000 bp				50 bp	
	240 bp	740 bp		1,700 bp	800 bp	1,500 bp					
	220 bp	720 bp		1,600 bp	700 bp	1,000 bp					
	200 bp	700 bp		1,500 bp	600 bp	500 bp					
	180 bp	680 bp		1,400 bp	500 bp						
	160 bp	660 bp		1,300 bp	400 bp						
	140 bp	640 bp		1,200 bp	300 bp						
	120 bp	620 bp		1,100 bp	200 bp						
	100 bp	600 bp		1,000 bp	100 bp						
	80 bp	580 bp		900 bp							
	60 bp	560 bp		800 bp							
	40 bp	540 bp		700 bp							
	20 bp	520 bp		600 bp							
		500 bp		500 bp							
		480 bp		400 bp							
		460 bp		300 bp							
		440 bp		200 bp							
		420 bp		100 bp							
		400 bp									
		380 bp									
		360 bp									
		340 bp									
		320 bp									
		300 bp									
		280 bp									
		260 bp									
		240 bp									
		220 bp									
		200 bp									
		180 bp									
		160 bp									
		140 bp									
		120 bp									
		100 bp									
		80 bp									
		60 bp									
		40 bp									
		20 bp									

FlashGel® DNA and RNA Markers

Size Range	0.5 Kb - 9.0 kb	0.5 Kb - 9.0 kb	100 bp - 3 kb	100 bp - 4 kb	100 bp - 1.5 kb	50 bp - 1.5 kb
Ladder/Marker	RNA Marker	RNA Marker	DNA Marker	DNA Marker	QuantLadder	DNA Marker
Cat. No.	50575	50577	57034	50473	50475	57033
Number of Bands	10	6	5	7	5	8
	9.0 kb	9.0 kb	3,000 bp	4,000 bp	1,500 bp	1,500 bp
	6.0 kb	5.0 kb	1,500 bp	2,000 bp	800 bp	800 bp
	5.0 kb	3.0 kb	800 bp	1,250 bp	400 bp	500 bp
	4.0 kb	1.5 kb	500 bp	800 bp	250 bp	300 bp
	3.0 kb	1.0 kb	100 bp	300 bp	100 bp	200 bp
	2.5 kb	0.5 kb		200 bp		150 bp
	2.0 kb			100 bp		100 bp
	1.5 kb					50 bp
	1.0 kb					
	0.5 kb					

FlashGel® System Markers and Ladders



Introduction

GelStar® and SYBR® Green Nucleic Acid Gel Stains are highly sensitive fluorescent stains for detecting nucleic acids in agarose gels. Unlike ethidium bromide, these stains fluoresce only upon binding to nucleic acids. Background staining is minimal and destaining of gels is not required. GelStar® Stain gives high detection sensitivity for double-stranded or single-stranded DNA and RNA. SYBR® Green I Stain exhibits a preferential affinity for double-stranded nucleic acids and SYBR® Green II Stain is most sensitive for single-stranded nucleic acids.

Advantages

High sensitivity – 25 - 100 times more sensitive than ethidium bromide

Flexible – Add GelStar® Stain directly to the agarose solution or post-stain your gel with GelStar® or SYBR® Green Stains

Fast – No destaining required

The table below compares the detection sensitivities of several commonly used staining methods. Samples detected with SYBR® Green Stains were post-stained with the dye. Samples detected with Ethidium Bromide or GelStar® Stain were detected by in-gel staining. Detection limits were determined by using DNA samples that were serially diluted and by recording the lowest amount that gave a visible band on photographs. Photographic conditions were varied as needed to use the longest exposure time possible without increasing gel background to an unacceptable level.

Stain and Method	ssDNA	dsDNA
GelStar® Stain – in gel	25 pg	20 pg
Ethidium bromide, no destain	1.25 ng	350 pg
Ethidium bromide, destain	350 pg	100 pg
SYBR® Green I or II Stain	60 pg	20 - 30 pg

Materials

- Clear polypropylene container (e.g., Rubbermaid® Recycling #5 Plastics)
- GelStar® Photographic Filter (Wratten® #9 equivalent) or SYBR® Green Photographic Filter (Wratten® #15 equivalent)
- Microcentrifuge
- UV transilluminator, Dark Reader® Transilluminator (Clare Chemical Research, Inc.) or CCD imaging system

Reagents

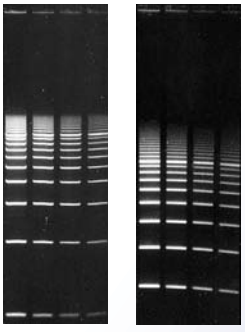
- Buffer between pH 7.5 - 8.5 (TAE, TBE or TE)
- GelStar® or SYBR® Green I or II Stain stock solution

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.

The photographs below demonstrate the detection sensitivity of GelStar® Nucleic Acid Gel Stain when used in-gel or when gels have been post-stained.

GelStar® Stain

In-Gel | Post-Stained



Lonza's 500 bp DNA Ladder was separated on 1% SeaKem® GTG® Agarose gels 20 cm long, 4 mm thick, run in 1X TBE Buffer (Prepared from Lonza's AccuGENE® 10X TBE Buffer) at 6 V/cm for 3 hours. GelStar® Stain was diluted 1:10,000 and added directly to the agarose or the gel was post stained for 30 minutes in a 1:10,000 dilution of GelStar® Stain in buffer. Lane 1: 10 ng DNA/band; Lane 2: 5 ng DNA/band; Lane 3: 2.5 ng DNA/band; Lane 4: 1.25 ng DNA/band.

The FlashGel® System includes gel cassettes pre-stained with a similar high sensitivity stain. Refer to page 18-25.

Tips for staining gels with GelStar® or SYBR® Green I Stain

Follow the guidelines below to increase the detection sensitivity of GelStar® or SYBR® Green Stains

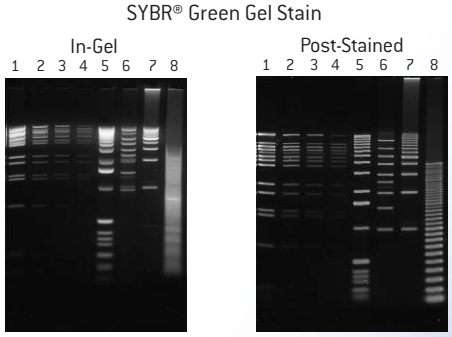
- New clear polypropylene containers (e.g., Rubbermaid® Recycling #5 Plastics) should be obtained for use with GelStar® and SYBR® Green Stains; when stored in the dark in polypropylene containers, the diluted stain can be used for up to 24 hours and will stain 2 to 3 gels with little decrease in sensitivity; the containers should be rinsed with distilled water (do not use detergents) after each use and dedicated to GelStar® or SYBR® Green Stain use only
- These stains bind to glass and some non-polypropylene (polystyrene) plastics resulting in reduced or no signal from the nucleic acid
- A 1X working solution of GelStar® or SYBR® Green Stain should be prepared just prior to use from the 10,000X stock solution by diluting in a pH 7.5 to 8.5 buffer (e.g., TAE, TBE or TE)
- Agarose gels should be cast no thicker than 4 mm. As gel thickness increases, diffusion of the stain into the gel is decreased, lowering the efficiency of DNA detection
- Optimal sensitivity for GelStar® and SYBR® Green Stains is obtained by using the appropriate photographic filters for each stain
- GelStar® Stain: Wratten® or Tiffen® #9 Filter
- SYBR® Green Stains: Wratten® or Tiffen® #15 Filter
- We do not recommend photographing gels with a 254 nm transilluminator; an outline of the UV light source may appear in photographs; a filter that will allow a 525 nm transmission and exclude infrared light is required

Procedure for staining DNA with GelStar® or SYBR® Green Stains

For optimal resolution, sharpest bands and lowest background, stain the gel with GelStar® or SYBR® Green Stain following electrophoresis. Alternatively, GelStar® Stain can be included in the agarose gel.

It is not recommended to include SYBR® Green Stains in the agarose gel. When the dye is incorporated into the agarose, the gel is more sensitive to DNA overloading, and the electrophoretic separation of DNA may not be identical to that achieved with ethidium bromide.

The photograph below demonstrates the effect of adding SYBR® Green I Stain to the agarose.



DNA markers were separated on 1% SeaKem® Gold Agarose gels 10-cm-long, 3 mm thick, run in 1X TBE Buffer for 1 - 1.5 hours. SYBR® Green I Stain was diluted 1:10,000 and added directly to the agarose or the gel was post stained for 30 minutes in a 1:10,000 dilution of SYBR® Green Stain in Buffer. Lanes 1 - 4: BstE II digest of lambda DNA; 1:2 dilutions with an initial loading of 25 ng/lane. Lane 5: 1 kb DNA ladder (Invitrogen, Inc.); 200 ng/lane (SYBR® Green I stained gel); 1 µg/lane (Ethidium bromide stained gel). Lane 6: Lonza's 1-10 kb DNA Ladder; 100 ng/lane. Lane 7: Gensura's 1 kb DNA Ladder; 100 ng/lane. Lane 8: Lonza's 100 bp DNA Ladder; 100 ng/lane.

Follow the steps below to stain DNA after electrophoresis

1. Remove the concentrated stock solution of GelStar® or SYBR® Green Stain from the freezer and allow the solution to thaw at room temperature.
2. Spin the solution in a microcentrifuge to collect the dye at the bottom of the tube.
3. Dilute the 10,000X concentrate to a 1X working solution (1 µl per 10 ml), in a pH 7.5 - 8.5 buffer, in a clear plastic polypropylene container. Prepare enough staining solution to just cover the top of the gel.
4. Remove the gel from the electrophoresis chamber.
5. Place the gel in staining solution.
6. Gently agitate the gel at room temperature.
7. Stain the gel for 15 - 30 minutes.
   The optimal staining time depends on the thickness of the gel, concentration of the agarose, and the fragment size to be detected. Longer staining times are required as gel thickness and agarose concentration increase.
8. Remove the gel from the staining solution and view with a 300 nm UV transilluminator, CCD camera or Dark Reader® Transilluminator (Clare Chemical Research, Inc.). GelStar® and SYBR® Green Stained Gels do not require destaining. The dyes fluorescence yield is much greater when bound to DNA than when in solution.

Follow this procedure when including GelStar® Stain in the agarose gel.

1. Remove the concentrated stock solution of GelStar® Stain from the freezer and allow the solution to thaw.
2. Spin the solution in a microcentrifuge tube.
3. Prepare the agarose solution (see pages 83-84).
4. Once the agarose solution has cooled to 70°C, add the stain by diluting the stock 1:10,000 into the gel solution prior to pouring the gel (1 µl per 10 ml).
5. Slowly swirl the solution.
6. Pour the gel into the casting tray (see page 85).
7. Load your DNA onto the gel.
8. Run the gel.
9. Remove the gel from the electrophoresis chamber.
10. View with a 300 nm UV transilluminator, CCD camera or Dark Reader® Transilluminator (Clare Chemical Research, Inc.). GelStar® Stained gels do not require destaining. The dye's fluorescence yield is much greater when bound to DNA than when in solution.

Staining vertical gels with GelStar® and SYBR® Green Stains

Incorporating GelStar® and SYBR® Green Stains into the gel or prestaining the DNA for use in a vertical format is not recommended. The dye binds to glass or plastic plates and DNA may show little to no signal. Gels should be post-stained as described in the previous section.
Follow this procedure when staining vertical gels with GelStar® or SYBR® Green Stain

1. Remove the concentrated stock solution of GelStar® or SYBR® Green Stain from the freezer and allow the solution to thaw at room temperature.
2. Spin the solution in a microcentrifuge to collect the dye at the bottom of the tube.
3. Dilute the 10,000X concentrate to a 1X working solution, in a pH 7.5 - 8.5 buffer, in a clear plastic polypropylene container. Prepare enough staining solution to just cover the top of the gel.
4. Remove the gel from the electrophoresis chamber.
5. Open the cassette and leave the gel in place on one plate.
6. Place the plate, gel side up in a staining container.
7. Gently pour the stain over the surface of the gel.
8. Stain the gel for 5 - 15 minutes.
9. View with a 300 nm UV transilluminator, CCD camera or Dark Reader® Transilluminator (Clare Chemical Research, Inc.). GelStar® or SYBR® Green Stained gels do not require destaining. The dye's fluorescence yield is much greater when bound to DNA than when in solution.

Visualization by Photography

Gels stained with GelStar® and SYBR® Green Stains exhibit negligible background fluorescence, allowing long film exposures when detecting small amounts of DNA. Use the appropriate photographic filter for the stain you are using.

The table below provides suggested film types and photographic conditions

Polaroid® Film	f-stop	Exposure time
Type 57 or 667	4.5	0.5 - 2 seconds
Type 55	4.5	15 - 45 seconds

Visualization by image capture system

For the best results and optimal sensitivity, visualize GelStar® Stained Gels on The Dark Reader® Transilluminator (Clare Chemical Research, Inc.) GelStar® and SYBR® Green Stains are compatible with most CCD and video imaging systems. Due to variations in the filters for these systems, you may need to purchase a new filter. Lonza does not sell filters for this type of camera. Contact your systems manufacturer and using the excitation and emission information listed, they can guide you to an appropriate filter.

Stain	Emission (nm)	Excitation (nm)
GelStar® Stain	527	493
SYBR® Green I Stain	521	494
SYBR® Green II Stain	513	497

Application notes

— The fluorescent characteristics of GelStar® and SYBR® Green Stains make them compatible with argon ion lasers.

— These stains are removed from double-stranded DNA by standard procedures for ethanol precipitation of nucleic acids.

— Gels previously stained with ethidium bromide can subsequently be stained with GelStar® or SYBR® Green Stain following the standard protocol for post-staining. There will be some decrease in sensitivity when compared to a gel stained with only GelStar® or SYBR® Green Stain.

— The inclusion of GelStar® and SYBR® Green Stains in cesium chloride density gradient plasmid preparations is not recommended. The effect of the dye on the buoyant density of DNA is unknown.

— These stains do not appear to interfere with enzymatic reactions.

— We recommend the addition of 0.1% to 0.3% SDS in the prehybridization and hybridization solutions when performing Southern blots on gels stained with these dyes.

— Double-stranded DNA-bound GelStar® or SYBR® Green Stain fluoresces green under UV transillumination. Gels that contain DNA with single-stranded regions may fluoresce orange rather than green.

Decontamination

Staining solutions should be disposed of by passing through activated charcoal followed by incineration of the charcoal. For absorption on activated charcoal, consult Sambrook, ***et al.***, pp. 6.16 - 6.19, (1989). Follow state and local guidelines for decontamination and disposal of Nucleic Acid staining solutions

Introduction

Ethidium bromide is a fluorescent dye which detects both single- and double-stranded DNA. However, the affinity for single-stranded DNA is relatively low compared to double-stranded DNA. Ethidium bromide contains a planar group which intercalates between the bases of DNA and, when bound to DNA, results in an increase in fluorescence yield. Ethidium bromide-stained DNA is detected by ultraviolet radiation. At 254 nm, UV light is absorbed by the DNA and transmitted to the dye; at 302 nm, and 366 nm, UV light is absorbed by the bound dye itself. In both cases, the energy is re-emitted at 590 nm in the red-orange region of the visible spectrum.

Procedure

For optimal resolution, sharpest bands and lowest background, stain the gel with ethidium bromide following electrophoresis.

Ethidium bromide can also be included in the gel and electrophoresis buffer (0.5 µg/ml) with only a minor loss of resolution. The electrophoretic mobility of DNA will be reduced by approximately 15%.

Follow the steps below to stain DNA after electrophoresis

1. Prepare enough working solution of ethidium bromide. (0.5 - 1 µg/ml of ethidium bromide in distilled water or gel buffer) to cover the surface of the agarose gel.
2. Remove the gel from the electrophoresis chamber.
3. Submerge the gel for 20 minutes in the ethidium bromide solution.
4. Remove the gel from the solution.
5. Submerge the gel for 20 minutes in a new container filled with distilled water.
6. Repeat in fresh distilled water.
7. Gels can be viewed with a hand-held or tabletop UV light. For gel concentrations of 4% or greater, these times may need to be doubled. If after destaining the background is still too high, continue to destain.

Materials

- Staining vessel larger than gel
- UV transilluminator
- Magnetic stir plate
- Magnetic stir bar

Reagents

- Ethidium bromide stock solution (10 mg/ml)
  - 1.0 g ethidium bromide
  - 100 ml distilled water
  - Stir on magnetic stirrer for several hours
  - Transfer the solution to a dark bottle
  - Store at room temperature
- Electrophoresis buffer or distilled water

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.

Follow the steps below when including ethidium bromide in the agarose gel

1. Prepare agarose solution (see Section II).
2. While the agarose solution is cooling, add ethidium bromide to a final concentration of 0.1 to 0.5 µg/ml to the solution.
3. Gently swirl the solution.
4. Pour the gel into the casting tray.
5. Add ethidium bromide to the running buffer to a final concentration of 0.5 µg/ml.
6. Load and run the gel (see Section III).
7. Destain the gel by submerging the gel in distilled water for 20 minutes.
8. Repeat in fresh distilled water.
9. Gels can be viewed with a hand-held or tabletop UV light during or after electrophoresis.

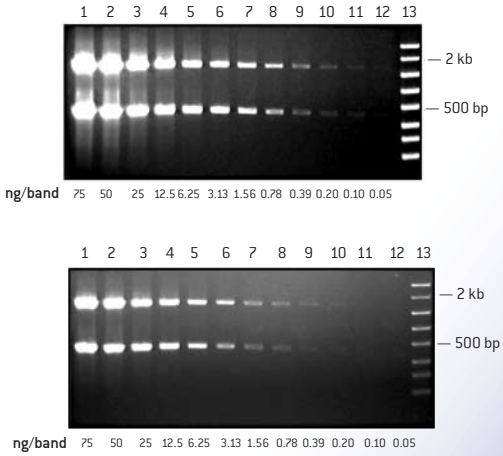
Decontamination of ethidium bromide solutions

Decontamination of ethidium bromide solutions is described in Sambrook, *et al.*, pp. 6.16 - 6.17 (1989). Follow local guidelines and regulations for ethidium bromide decontamination and disposal.

Introduction

The FlashGel® System uses a proprietary stain that is 5-20 times more sensitive than ethidium bromide stain. Samples prepared at DNA concentrations one-fifth of the concentration typically required for ethidium bromide stained gels will clearly resolve on FlashGel® Cassettes. DNA levels of 5 ng per band or more are visible on the lighted FlashGel® Dock under most ambient light conditions. DNA levels as low as 0.1 ng per band can be detected on gel images and photos. DNA levels can be adjusted to provide best performance depending upon the image analysis system used (Figure 1).

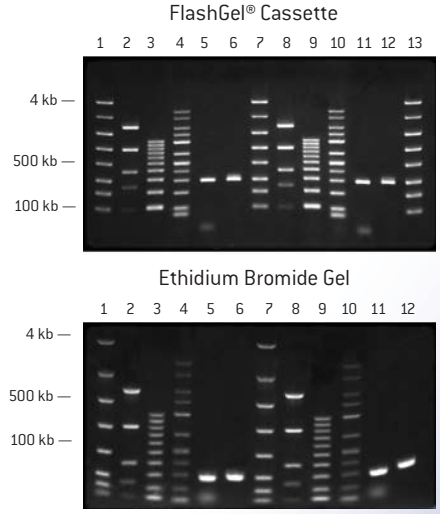
Figure1:
DNA concentration detectable with the FlashGel® System



1.2% FlashGel® Cassettes, 12+1 well format. 275 volts for 7 minutes. DNA concentrations are ng/band Lanes 1-12: Sample dilution series of 400 bp & 1,500 bp purified fragments (BioVentures) Lane 13: FlashGel® DNA Marker. Photographed on UV Transilluminator or Dark Reader® Transilluminator. Dark Reader® Transilluminator Gel illuminated on the Dark Reader® with Dark Reader® orange filter in place. Photographed with CCD imager (EtBr filter in place) at 2 second exposure. UV transilluminator: Gel illuminated on UV transilluminator. Photographed with CCD imager (EtBr filter in place) at 2 second exposure.

Because the system is so sensitive, a load volume of 5 µl or less is recommended for best performance. Samples may be diluted with FlashGel® Loading Dye (for best results), or with water or other common buffers (e.g., TE Buffer) before adding a loading dye. See the table in Figure 2 for examples of sample and marker dilutions in a FlashGel® Cassette compared to an ethidium bromide stained agarose gel. The ethidium stained gel required at least 4 times more sample than the FlashGel® Cassette. One-tenth the concentration of the FlashGel® DNA Marker and QuantLadder, and one-twentieth the concentration of the PCR products were required for good sensitivity of detection on FlashGel® Cassettes compared to ethidium bromide gel.

Figure2:
FlashGel® Cassette vs. Ethidium Bromide Gel



FlashGel® Loading Dye was added to all samples prior to loading.

Lanes 1&7: FlashGel® DNA Marker; Lanes 2&8: FlashGel® QuantLadder; Lanes 3&9: Lonza 100 bp Ladder; Lanes 4&10: Lonza 50-2,500 bp Marker; Lanes 5&11: 285 bp β-Actin PCR; Lanes 6&12: 294 bp Ambion control PCR. Samples diluted with 1X FlashGel® Loading Dye prior to loading. Dilutions and load volumes optimized for each sample in each gel system. See table below.

Lane Nos.	Sample Type	Load volumes and dilutions	
		FlashGel® Cassette	Ethidium Bromide Gel
Lanes 1 & 7	FlashGel® DNA Marker	5 µl 1:5 dilution	10 µl undiluted
Lanes 2 & 8	FlashGel® QuantLadder	5 µl 1:5 dilution	10 µl undiluted
Lanes 3 & 9	Lonza 100 bp Ladder	3 µl 1:15 dilution	12 µl 1:15 dilution
Lanes 4 & 10	Lonza 50-2500 bp Marker	3 µl 1:5 dilution	12 µl 1:5 dilution
Lanes 5 & 11	285 bp β-Actin PCR	5 µl 1:50 dilution	2 µl undiluted PCR rxn
Lanes 6 & 12	294 bp Control PCR (Ambion)	5 µl 1:50 dilution	2 µl undiluted PCR rxn
Lane 13	FlashGel® DNA Marker	5 µl 1:5 dilution	

High Sensitivity Detection using The FlashGel® System — continued

Refer to The FlashGel® System Protocol for complete user instruction, safety and environmental precautions.

References

Carmichael, G.G. and McMaster, *G.K. Meth. Enzymol. Academic Press, Inc.* 1980.
Flores, N., *et al., BioTechniques* **13(2)**: 203 - 225, 1992.
FMC BioProducts, *Resolutions* **11(2)**, 1995.
McMaster G.K. and Carmichael, G.G., *Proc. Natl. Acad. Sci.* **74**: 4835 - 4848, 1977.
Peats, S., *Anal. Biochem.* **140**: 178 - 182, 1984.
Sambrook, J., *et al., Molecular Cloning: A Laboratory Manual,* 2nd edition, Cold Spring Harbor Laboratory Press, 1989.
Schneeberger, C., *et al., PCR Methods & App.* **4**: 234 - 238, 1995.