DNA Staining Using Fast Blast™ DNA Stain or SYBR® Safe Stain and Digital Analysis of Gel Images Using Logger *Pro* Software

SYBR® Safe DNA stain (Vernier Software & Technology) is an alternative to Fast Blast DNA stain (Bio-Rad Laboratories, Inc.). Both stains are convenient, safe, and nontoxic substitutes for ethidium bromide, a traditional DNA stain. However, they have different staining time requirements, sensitivity, visualization methods, and modes of operation. Gels stained with Fast Blast DNA stain do not require any equipment for visualization, unlike gels stained with SYBR® Safe stain. With both stains, Logger *Pro* software (Vernier) in combination with imaging systems from Bio-Rad or Vernier can document and analyze gel images. This guide is intended to help you decide which stain is best for your situation when sensitivity, equipment requirements, and time constraints are considered.

DNA fragments stained with Fast Blast DNA stain appear deep blue against a light blue background and are visible to the naked eye; they may be documented and analyzed manually as described in various curricula available as part of Biotechnology Explorer™ kits (Bio-Rad). Another option is to illuminate the gel with a White Light Transilluminator (Vernier), capture the image digitally with a gel imaging system such as the ProScope HR digital USB camera (Vernier), and analyze the stained gels with Logger *Pro* software.

Gels stained with SYBR® Safe stain require an external light source to view the bands. Bio-Rad and Vernier both offer imaging systems for this purpose. The Blue Digital Bioimaging system* (Vernier) uses the BlueView Transilluminator to excite the fluorescent stain. Digital images are captured with the ProScope HR digital USB camera and analyzed using Logger *Pro* software. Figure 1 shows images of DNA fingerprinting gels stained with Fast Blast and SYBR® Safe stains.

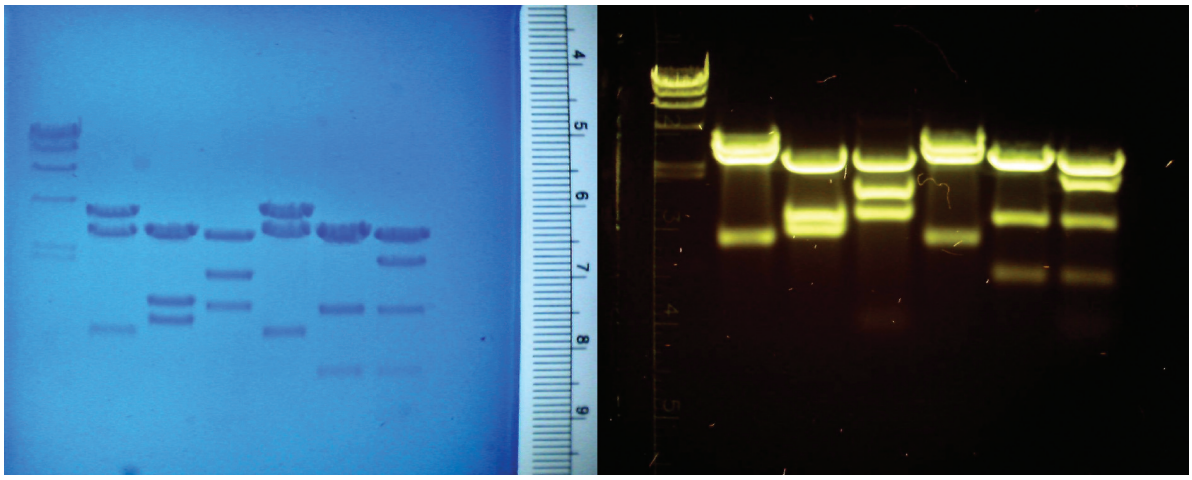


Fig. 1. DNA fingerprinting gels stained with Fast Blast DNA stain (left) and SYBR® Safe stain (right). The gel stained with Fast Blast stain was illuminated with the White Light Transilluminator; the gel on the right with the BlueView Transilluminator.

*The system includes a BlueView Transilluminator, imaging hood, ProScope HR digital USB camera, lens, and stand. Logger *Pro* is available separately.

BIO-RAD

The staining methods summarized in Table 1 are described in detail in the instruction manuals for the Biotechnology Explorer kits that use DNA gels.

Table 1. Stain selection.

Staining Options	Staining Procedure	Destaining Procedure	Sensitivity	How to Visualize	Mode of Operation
100x Fast Blast DNA stain for quick staining (total time, 12–15 min)	Stain for 2–3 min immediately after electrophoresis is complete.	Rinse for 10 sec, then wash twice for 5 min each; gel can be transferred to 1x Fast Blast DNA stain overnight.	50 ng	No special equipment is needed. Gel may be documented with White Light Transilluminator, Molecular Imager® VersaDoc™ MP imaging system (Bio-Rad), or Molecular Imager® Gel Doc™ XR+ imaging system (Bio-Rad). DNA stains deep blue in agarose gels against a light blue background.	Contains a cationic compound that binds to the negatively charged phosphate groups on DNA molecules. The proprietary dye formula stains DNA deep blue in agarose gels and provides vivid, consistent results.
1x Fast Blast DNA stain for overnight staining	Stain for 8 hr to overnight immediately after electrophoresis is complete; use of a rocking platform is recommended.	Not required			
SYBR® Safe stain before electrophoresis	Add stain (1 µl of 10,000x) to molten agarose before pouring gels.	Not required	0.5 ng	Use the Blue Digital Bioimaging system, VersaDoc MP imaging system, or Gel Doc XR+ imaging system. DNA stains orange against a black background.	The stain is intercalated between DNA base pairs and is not visible to the naked eye.
SYBR® Safe stain after electrophoresis	Stain for 30 min after electrophoresis is complete; use of a rocking platform is recommended.	Not required; a water rinse is adequate.			

Option 1: Use 100x Fast Blast DNA Stain for Quick Staining (total time 12–15 min)

Use this protocol if laboratory class time is sufficient to visualize the DNA bands after electrophoresis.

- Dilute the 500x concentrate to 100x by adding 24 ml of 500x concentrate to 96 ml of water to make 120 ml of 100x concentrate. We recommend using 120 ml of 100x Fast Blast stain to stain two 7 x 7 cm or 7 x 10 cm gels in individual staining trays (included in the kit); each tray can accommodate two gels
- Carefully remove the gels from their gel trays and slide them into the staining tray; add the 100x stain until the gels are completely submerged
- Stain for 2–3 min, maximum. Pour the 100x stain into a storage bottle and save for future use. The 100x stain may be reused several times
- Rinse the gels for 10 sec in 500–700 ml of clean, warm (40–55ºC) tap water
- Wash the gels for 5 min in 500–700 ml of clean, warm (40–55ºC) tap water. Repeat this step. The bands may appear fuzzy immediately after the second wash but will begin to become sharper within 5–15 min
- To obtain maximum contrast, additional washes in warm water may be necessary. Destain to the desired level, but do not leave the gel in water overnight. If complete destaining cannot be done in the allocated time, transfer the gel to a tray containing 1x Fast Blast stain overnight
- The gel is ready to be documented and analyzed manually or using an imaging system (White Light Transilluminator, Gel Doc XR+ system, or VersaDoc MP system)

Option 2: Use 1x Fast Blast DNA Stain for Overnight Staining

Use this method if there is not enough laboratory class time to allow for staining immediately after electrophoresis.

- Dilute the 500x concentrate to 1x by adding 0.24 ml of 500x concentrate to 120 ml of water to make ~120 ml of 1x concentrate. We recommend using 120 ml of 1x Fast Blast to stain two 7 x 7 cm or 7 x 10 cm gels in individual staining trays (included in the kit); each tray can accommodate two gels
- Carefully remove the gels from their gel trays and slide them into the staining tray; add the 1x stain until the gels are completely submerged
- Place the staining tray on a rocking platform and gently shake overnight. If a rocking platform is not available, swirl the solution and gel a few times during the staining period. This is crucial, because smaller fragments tend to diffuse without shaking. The bands will begin to develop after 2 hr, but at least 8 hr of staining is recommended for complete visibility
- The gel is ready to be documented and analyzed manually or using an imaging system (White Light Transilluminator, Gel Doc XR+ system, or VersaDoc MP system)

Option 3: Use SYBR® Safe DNA Gel Stain Before Electrophoresis

In contrast to the other three options, here concentrated SYBR® Safe stain is added directly to molten agarose prior to pouring the gels. Use this method for immediate visualization using a Blue Digital Bioimaging system.

- Follow the directions for preparing molten agarose; combine agarose powder and running buffer in appropriate volumes and heat until the agarose powder is dissolved. Allow to cool to 50ºC before adding the appropriate volume of SYBR® Safe stain using this ratio: 1 µl of SYBR® Safe stain 10,000x concentrate to 10 ml of agarose solution. Thus, for 40 ml of agarose, add 4 µl of SYBR® Safe stain. Mix by swirling the molten agarose; then pour the gels
- Store gels containing SYBR® Safe stain protected from light by covering them with aluminum foil at room temperature overnight
- Run prestained SYBR® Safe gels in the same buffer system and electrophoresis conditions as the gels that are not made with the SYBR® Safe stain (see Option 4)
- Prestained SYBR® Safe gels do not need to be stained or destained and can be viewed directly on an imaging system (Blue Digital Bioimaging system, Gel Doc XR+ system, or VersaDoc MP system)

Option 4: Use SYBR® Safe DNA Gel Stain (0.5x Concentration) After Electrophoresis

This protocol is most appropriate when a 50 min class period is available for staining the gels and analyzing them using Logger Pro software. This stain can be diluted from the 10,000x concentrate to 0.5x by adding 6 µl SYBR® Safe stain per 120 ml running buffer, or it can be purchased prediluted to a 0.5x concentration.

- After electrophoresis, carefully remove the gel from its gel tray and slide it into the staining tray; add enough stain to submerge the gels completely (about 120 ml of stain)
- Cover the staining tray with aluminum foil to protect the gel from light. Place the staining tray on a rocking platform with gentle shaking, and leave it for 30 min at room temperature. If a rocking platform is not available, periodically swirl the solution and gel a few times to obtain thorough and uniform staining patterns
- Rinse the gel with water and place it into the imaging system (Blue Digital Bioimaging system, Gel Doc XR+ system, VersaDoc MP system)

SYBR® Safe stain can be excited in the blue light range (in the high 400 nm range) and has an emission wavelength of 530 nm. Its fluorescence can be viewed using any of the imaging systems listed in Table 2. Use of a blue light transilluminator minimizes UV exposure and damage to samples, an important factor in applications such as cloning.

Table 2. Imaging systems and filter selection guide for use with SYBR® Safe stain.

Imaging System	Excitation Source	Emission Filter
VersaDoc MP imaging systems (Bio-Rad)	Broadband UV	530 nm LP
Molecular Imager® PharosFX™ systems (Bio-Rad)	488 nm	530 nm BP (#170-9459)
Gel Doc XR+ system (Bio-Rad)	302 nm	520DF30 (#170-8074)
Blue Digital Bioimaging system (Vernier)	Blue light, 480 nm	530 nm

Using Logger *Pro* Software to Document Gels or Import Gel Images

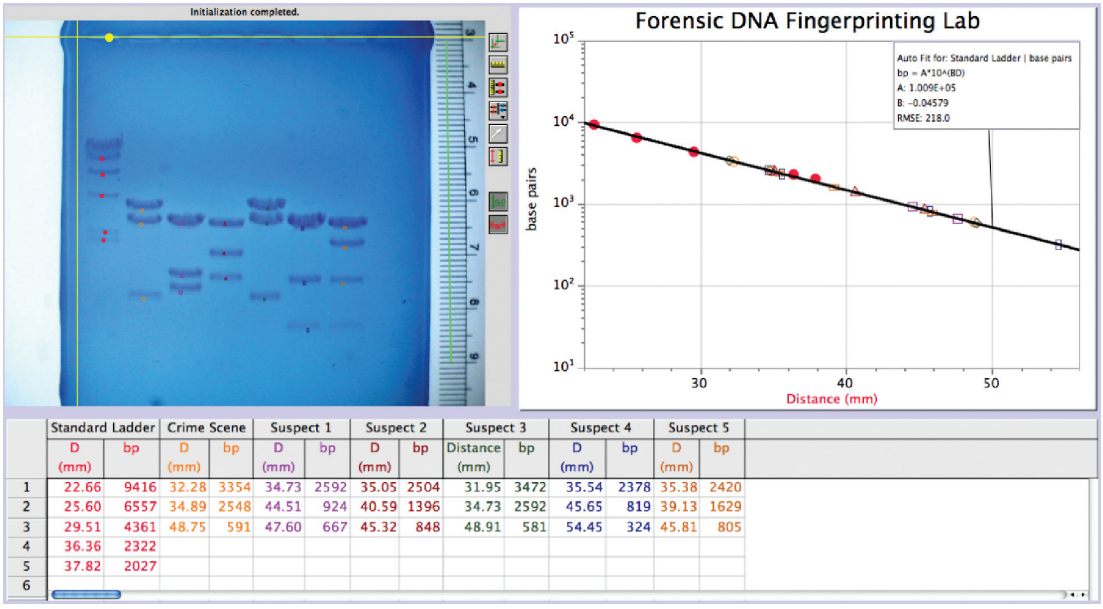
Logger *Pro* software can be used to analyze DNA band patterns and calculate base pairs in gels illuminated with the White Light Transilluminator or the BlueView Transilluminator.

The gel is imaged using the ProScope HR camera, which is a component of the Blue Digital Bioimaging system. To capture an image from within Logger *Pro* software, select **Insert>Gel Analysis>Take Photo**. The image brightness and contrast can be adjusted to provide the best image. Logger *Pro* also allows a gel image file from a digital camera to be imported in a similar manner by selecting **Insert>Gel Analysis>From File**. Complete operating instructions are available from Vernier Software & Technology.

Using Logger *Pro* Software to Analyze Gel Band Patterns

As an additional exercise or as an alternative to the paper and pencil exercise of measuring the gel band patterns, Logger *Pro* software will measure and graph a standard curve based on a standard ladder run in parallel to the samples and then automatically calculate the size of each band of DNA in the samples.

Figure 2 shows a forensic DNA fingerprinting gel that was stained with Fast Blast DNA stain, illuminated on a White Light Transilluminator, and documented and analyzed using Logger *Pro* software.



	Standard Ladder		Crime Scene		Suspect 1		Suspect 2		Suspect 3		Suspect 4		Suspect 5	
	D (mm)	bp	D (mm)	bp	D (mm)	bp	D (mm)	bp	Distance (mm)	bp	D (mm)	bp	D (mm)	bp
1	22.66	9416	32.28	3354	34.73	2592	35.05	2504	31.95	3472	35.54	2378	35.38	2420
2	25.60	6557	34.89	2548	44.51	924	40.59	1396	34.73	2592	45.65	819	39.13	1629
3	29.51	4361	48.75	591	47.60	667	45.32	848	48.91	581	54.45	324	45.81	805
4	36.36	2322												
5	37.82	2027												
6														

Fig. 2. Forensic DNA fingerprinting gel stained with Fast Blast DNA stain. The image was captured using the ProScope HR high resolution camera, and base pair determination and analysis were accomplished with Logger *Pro* software.

Ordering Information

Bio-Rad Laboratories
Toll free: 800-424-6723
Web: explorer.bio-rad.com
Email: lsg.orders.us@bio-rad.com

Vernier Software & Technology
Toll free: 888-837-6437
Web: store.vernier.com
Email: orders@vernier.com

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 4BIORAD* ***Australia*** *61 02 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 21 3237 9400* ***Canada*** *905 364 3435* ***China*** *86 21 6426 0808* ***Czech Republic*** *420 241 430 532* ***Denmark*** *44 52 10 00* ***Finland*** *09 804 22 00* ***France*** *01 47 95 69 65* ***Germany*** *089 318 84 0* ***Greece*** *30 210 777 4396* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 455 8800* ***India*** *91 124 4029300* ***Israel*** *03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *03 6361 7000* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *0318 540666* ***New Zealand*** *0508 805 500* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 495 721 14 04* ***Singapore*** *65 6415 3188* ***South Africa*** *27 861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *061 717 95 55* ***Taiwan*** *886 2 2578 7189* ***United Kingdom*** *020 8328 2000*

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