Imaging Nucleic Acid Gels on the Odyssey® Fc Imager

Developed for:

Odyssey Fc Imaging System

LI-COR®
Experience Excellence

Published September 2011. The most recent version of this protocol is posted at: *http://biosupport.licor.com*

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I. Introduction

The Odyssey Fc Imager, with 600 channel capabilities, can image agarose gels stained with popular DNA stains, such as ethidium bromide and SYBR Safe DNA stain, with sub-nanogram sensitivity. The Odyssey Fc Imager contains a 532 nm diffuse source with an excitation maximum of 520 nm and a detection maximum of 600 nm. These instrument parameters are within the range of the excitation and emission wavelengths of ethidium bromide (Ex/Em = 302 & 518/605 nm[1,2]) and other visible fluorescent nucleic acid stains and provide a sensitive gel documentation option.

SYBR Safe DNA stain (Ex/Em = 502/530 nm) has also been tested on the Odyssey Fc Imager (using the 600 channel) with sensitivities exceeding ethidium bromide detection. The maximum fluorescence emission wavelength of SYBR Safe is very close to the maximum excitation wavelength. However, the Odyssey Fc 600 channel collects excitation light at a wavelength 50 nm higher than the maximum excitation wavelength of SYBR Safe. These instrument properties decrease the background and improve the signal-to-noise ratio for nucleic acid detection.

Specific instructions are given in this technical note for ethidium bromide and SYBR Safe use. There are a variety of commercial DNA stains that may be appropriate for fluorescent imaging with the Odyssey Fc 600 channel. SYBR Green I (Life Technologies), GelStar (FMC), Gel Red™ (Biotium), Gel Green™ (Biotium) and Nancy-520 (Sigma) stains have also been tested at LI-COR (see example images on page 7). Other nucleic acid binding stains may also be compatible with the Odyssey Fc Imager. Please check the excitation and emission spectra of each stain.

The Odyssey Fc Imager is also equipped with two infrared channels (700 and 800) and a chemiluminescent detection channel. Nucleic acid detection in the 700 channel is achieved with Syto® 60 stain, a cell-permeant cyanine dye. A detailed protocol is available for the use of Syto 60 with the Odyssey and Aerius family of imagers (LI-COR, *Syto 60 Staining of Nucleic Acids in Gels*).

Note: *Any questions regarding specific properties of the DNA binding stains should be directed to the representative vendors listed in this technical guide.*

II. DNA Separation and Detection on Agarose Gels

A. Suggested Materials

This section is intended as a guideline; other materials may be substituted.

- High Grade or Molecular Biology Grade agarose
  (Low melting-point agarose may increase the degree of speckling on the digital image.) ***OR***
- E-Gel® Pre-cast agarose gels from Life Technologies (Ethidium Bromide, SYBR® Safe, or Clear gel types)
- 1X TAE or TBE buffer
- Ethidium Bromide (EtBr, 10 mg/mL solution) ***OR***
- SYBR Safe DNA stain (10,000X concentrate in DMSO)
- Gel tank and casting tray for running submersion gels
- Power supply

Note: *Dispose of all gel and buffer solutions in accordance with the regulations of your facility.*

B. In-Gel Pre-Staining Protocol

Gel Preparation

1. Prepare desired agarose (0.8%, 1.0%, 1.2%, etc.) in 1X TAE or 1X TBE buffer.
2. Heat to dissolve agarose.
3. Cool solution until warm to the touch (60°F) prior to adding DNA stain.
   - **Ethidium Bromide**— Stock solutions are typically 10 mg/mL. Add ethidium bromide to give a final concentration of 0.5 µg/mL.
   - **SYBR Safe**— Stock solutions are typically 10,000X. Add SYBR Safe to a final concentration of 1X.
4. Pour molten agarose solution into casting tray and set comb into place. Allow gel to solidify.
5. Remove comb and place gel in buffer tank.

   Note: *Both Ethidium Bromide and SYBR Safe are positively charged stains and will migrate in the opposite direction of the DNA. If the stain is included only in the gel, but not the buffer, there will be an area of high background indicating the stain has not migrated out of the gel.*

Buffer Preparation

6. Prepare enough buffer (1X TAE or 1X TBE) to fill the apparatus.
7. Add DNA stain to buffer.
   - **Ethidium Bromide**— Add 5 µL of 10 mg/mL EtBr stock solution to 100 mL of buffer (final concentration: 0.5 µg/mL).

- **SYBR® Safe—** Add 10 µL of 10,000X SYBR Safe stock solution to 100 mL of buffer (final concentration: 1X).

Electrophoresis and Destaining

8. Prepare samples with loading buffer and load in gel.
9. Electrophorese samples at 5-8 V/cm.
10. (Optional) Destain in water for 20 minutes. Repeat as necessary to remove background from the gel.
11. Image on the Odyssey Fc Imager using the 600 channel. Refer to *III. Image Acquisition on the Odyssey Fc Imager* for more information.

C. Post-Electrophoresis Staining Protocol

Gel Preparation

1. Prepare desired agarose (0.8%, 1.0%, 1.2%, etc.) in 1X TAE or 1X TBE buffer.
2. Heat to dissolve agarose.
3. Cool solution until warm to the touch (60°F) prior to pouring in casting tray.
4. Pour molten agarose solution into casting tray and set comb into place. Allow gel to solidify.
5. Remove comb and place gel in buffer tank.

Buffer Preparation

6. Prepare enough buffer (1X TAE or 1X TBE) to fill the apparatus.

Electrophoresis and Destaining

7. Prepare samples with loading buffer and load in gel.
8. Electrophorese samples at 5-8 V/cm.

Staining Procedure

9. Prepare enough solution of 1X TAE or water to cover the agarose gel.
   - **Ethidium Bromide—** Add 5 µL of 10 mg/mL EtBr stock solution to 100 mL of buffer (final concentration: 0.5 µg/mL).
   - **SYBR Safe—** Add 10 µL of 10,000X SYBR Safe stock solution to 100 mL of buffer (final concentration: 1X).
10. Soak gel for 20 minutes in the prepared solution.
11. Destain in water for 20 minutes. Repeat as necessary to remove background from the gel.
12. Image on the Odyssey Fc Imager using the 600 channel. Refer to *III. Image Acquisition on the Odyssey Fc Imager* for more information.

D. E-Gel® Pre-Cast Agarose Gels

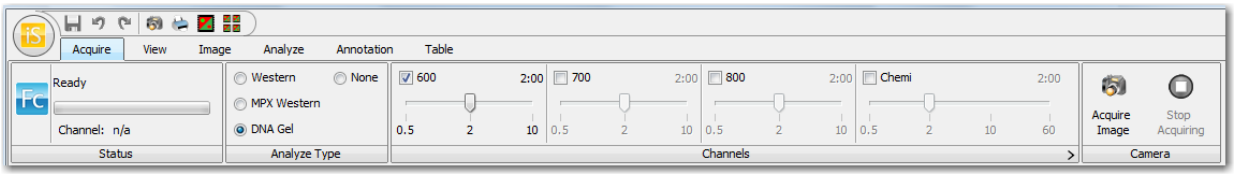
The E-Gel pre-cast agarose gels containing Ethidium Bromide or SYBR Safe are compatible with digital imaging on the Odyssey Fc Imager using the 600 channel. The clear versions of the E-Gel gels allow for post-staining with a DNA binding stain of your choice. Follow the manufacturer's protocols for sample preparation and gel electrophoresis parameters.

III. Image Acquisition on the Odyssey Fc Imager

1. Place gel face-up on an Odyssey Fc Imaging Tray.

   Note: *E-Gel® gel cassettes can be placed directly on the tray without removing the gel. The cassette has low background in the sample imaging area.*

2. Open the imaging drawer by pressing the imaging drawer open/close button.
3. Place the Odyssey Fc Imaging Tray containing the gel in the imaging drawer. Close the drawer by pressing the imaging drawer open/close button again.
4. Open Image Studio software and connect to the Odyssey Fc Imager.
5. Click on the **Acquire** tab to show the Acquire ribbon.



6. In the Analyze Type group, select **DNA Gel** for automatic analysis or select **None**.
7. In the Channels group, select the **600** channel and deselect the other channels.
8. Select the acquisition time by dragging the slider in the 600 box. Typical acquisition times for agarose gels are from 0.5 to 2 minutes.
9. Once the parameters have been set, click on **Acquire Image** to start the acquisition. The Status group provides information on the imaging process.

   Note: *To end an acquisition before it is completed, click on* ***Stop Acquiring****. All existing and pending channel images will be discarded.*

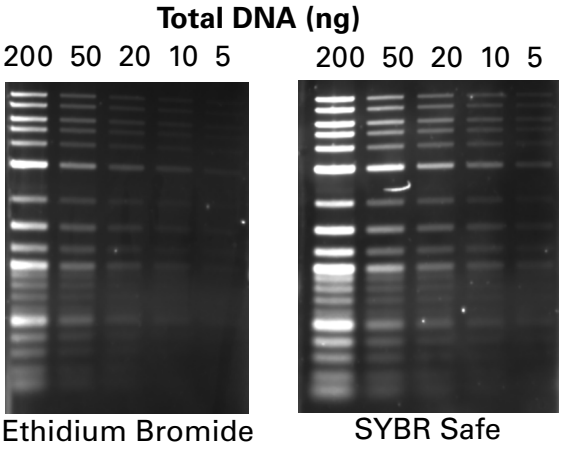
10. Adjust the Lookup Table for the 600 channel to optimize the image display. Refer to *How to Adjust the Lookup Tables in Image Studio for an Optimal Image Display* (LI-COR Biosciences) for more information.

To excise a DNA band from the gel, carefully lift or slide the prepared gel onto an ultraviolet transilluminator (if using ethidium bromide), or a blue light transilluminator (if using SYBR® Safe). If using an E-Gel pre-cast agarose gel cassette, first remove the gel by opening the cassette with the E-Gel Opener.

IV. Results — Ethidium Bromide and SYBR Safe

A. Sensitivity of the Odyssey Fc Imager, 600 Channel

The images in Figure 1 were prepared following the post-electrophoresis staining protocol on page 4 with Ethidium Bromide and SYBR® Safe DNA stains. These images show the sensitivity of the Odyssey Fc Imager.



Sensitivity of the Odyssey Fc Imager

Figure 1. Dilutions (200 - 5 ng) of a 2-log DNA ladder (0.1 - 1kb; New England Biolabs) were loaded on a 1% agarose gel. Gels were post-stained with 0.5 µg/mL ethidium bromide or 1X SYBR Safe DNA stain in 1X TAE buffer. Images were collected on the Odyssey Fc Imager (600 channel) using a 2 minute acquisition time.

B. DNA Samples — Plasmid Digests and PCR Products

DNA samples were loaded on 1.2% E-Gel® gels (Ethidium Bromide and SYBR Safe), electrophoresed for 30 minutes, and imaged on the Odyssey Fc Imager (2 minutes) in E-Gel cassettes.

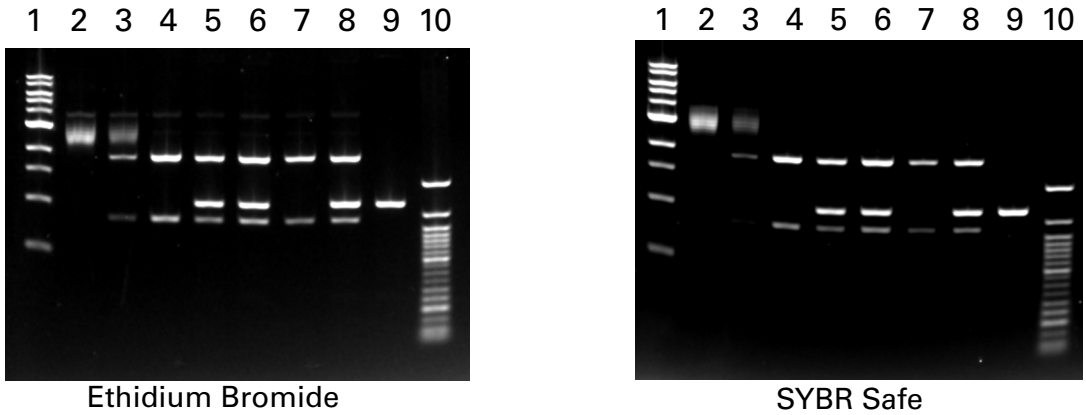


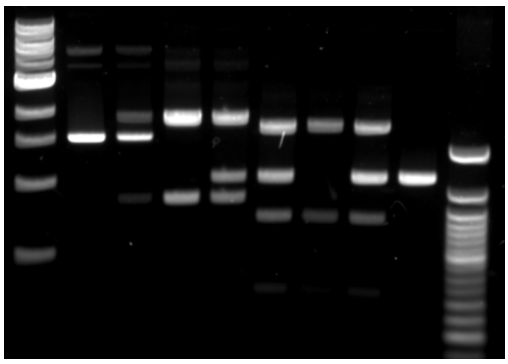
Figure 2. 1.2% E-Gel gels (Ethidium Bromide and SYBR Safe) run for 30 minutes and then imaged on the Odyssey Fc Imager (2 minutes) in E-Gel cassettes. Lane contents shown in the following guide.

Lane 1: 380 ng 1 kb Ladder
Lane 2: 100 ng pUC19
Lane 3: 500 ng pUC19 + pUC19/XmnI/HindIII
Lane 4: 150 ng pUC19/XmnI/HindIII
Lane 5: 380 ng pUC19/XmnI/HindIII + 50 ng PCR product
Lane 6: 380 ng pUC19/XmnI/HindIII + 75 ng PCR product
Lane 7: 80 ng pUC19/XmnI/HindIII
Lane 8: 100 ng pUC19/XmnI/HindIII + 100 ng PCR product
Lane 9: 125 ng PCR product
Lane 10: 400 ng 50 bp Ladder

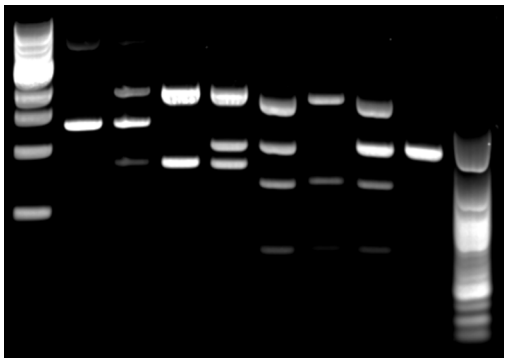
C. Examples of Other DNA Stains

The same DNA samples from Figure 2 were loaded on 1.2% agarose gels pre-stained with the DNA stains as specified. Images were acquired on the Odyssey Fc Imager using the 600 channel.

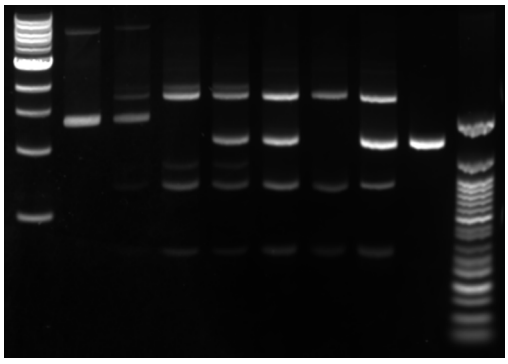
Nancy-520



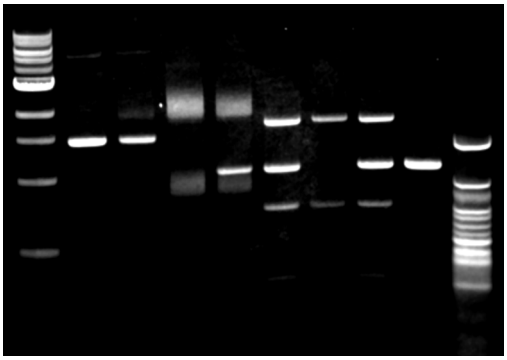
GelStar



GelGreen™



GelRed™



SYBR® Green I

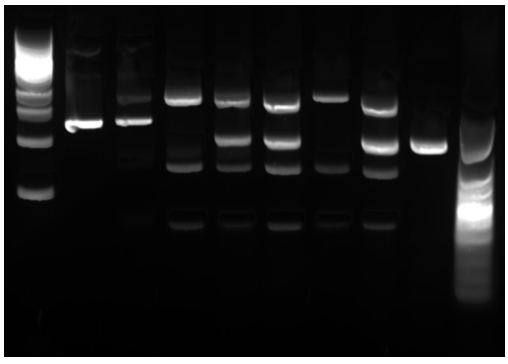


Figure 3: Gels were pre-stained using these DNA stains according to the manufacturers' recommendations. Images were acquired on the Odyssey Fc Imager in the 600 channel (2 minutes). Lane contents are identical to those described in Figure 2.

V. References

1. Waring MJ., (1965) *J Mol Biol.* 13(1):269-82 *Complex formation between ethidium bromide and nucleic acids*

2. LePecq JB, Paoletti C., (1967) *J Mol Biol.* 27(1):87-106 *A fluorescent complex between ethidium bromide and nucleic acids. Physical-chemical characterization*

LI-COR Biosciences, (2010) *Syto 60 Staining of Nucleic Acids in Gels*

LI-COR Biosciences, (2011) *How to Adjust the Lookup Tables in Image Studio for an Optimal Image Display*

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988-12443 09/11