"PulseNet: Under the Microscope"
Volume 2

Alternate DNA Stains – Results and Recommendations

All PulseNuts are well-acquainted with Ethidium Bromide (EtBr). It has been part of the standardized protocol since there was a standardized protocol! As such, lab folks are familiar with the precautions required to properly handle and dispose of this toxic chemical. Recently the Methods and Validation Lab, led by Kara Cooper, tested the effectiveness and safety of alternative DNA stains. Below are the findings from this study, including results comparing unwashed gels, the stain quality and degradation over time, cost analysis, and program recommendations. Please take a look at this summary report for valuable information when making decisions about what is in the best interest of your staff and the program. The recommendations will be reflected in the PulseNet Standardized 24-hr Protocol and added to the Important Documents Conference on CDCTeam. Feel free to contact Molly Freeman in the Methods and Validation Lab at evy7@cdc.gov with any questions.

Testing alternative DNA stains for use within the PulseNet Standardized Protocols

Background

Ethidium Bromide (EtBr) is the most widely used DNA stain in molecular biology and is the current stain of choice in the PulseNet standardized protocols. However, due to safety and health concerns associated with exposure to this chemical, there has been increased interest in the use of alternative DNA stains that are considered to be safer and can be disposed of down the sink rather than with hazardous waste disposal processes. When staining with EtBr, it is essential to de-stain the gel with several water washes to remove any non-specifically bound EtBr that may cause background fluorescence when capturing gel images. In contrast to EtBr, alternative DNA stains are claimed to not require a de-staining step, produce less background, and have more uniform staining of large and small DNA fragments. The the main drawback of these stains is that they are significantly more expensive than EtBr (Table 1). Adding to this expense are the vendor specifications suggesting that working solutions be made fresh every 24 hours, while EtBr can be re-used for several gels over many weeks. The CDC PulseNet laboratory conducted a series of experiments to evaluate the performance of various DNA stains compared to EtBr and to determine if these dyes could be implemented into PulseNet's PFGE protocols in a cost-effective manner.

Stains Tested:

1) Gel Red
2) SYBR® Safe
3) SYBR® Gold
4) Ethidium bromide (EtBr)

Characteristics evaluated:

1) Ability of the alternative DNA stains to generate a high quality image without a de-staining step.
2) Different exposure times to assess the amount of background fluorescence produced, if any.
3) Intensity of fluorescence across entire pattern/gel.
4) Stability of the staining solution for up to one week after it was prepared.

Table 1. Estimated cost per working solution of the stains tested.

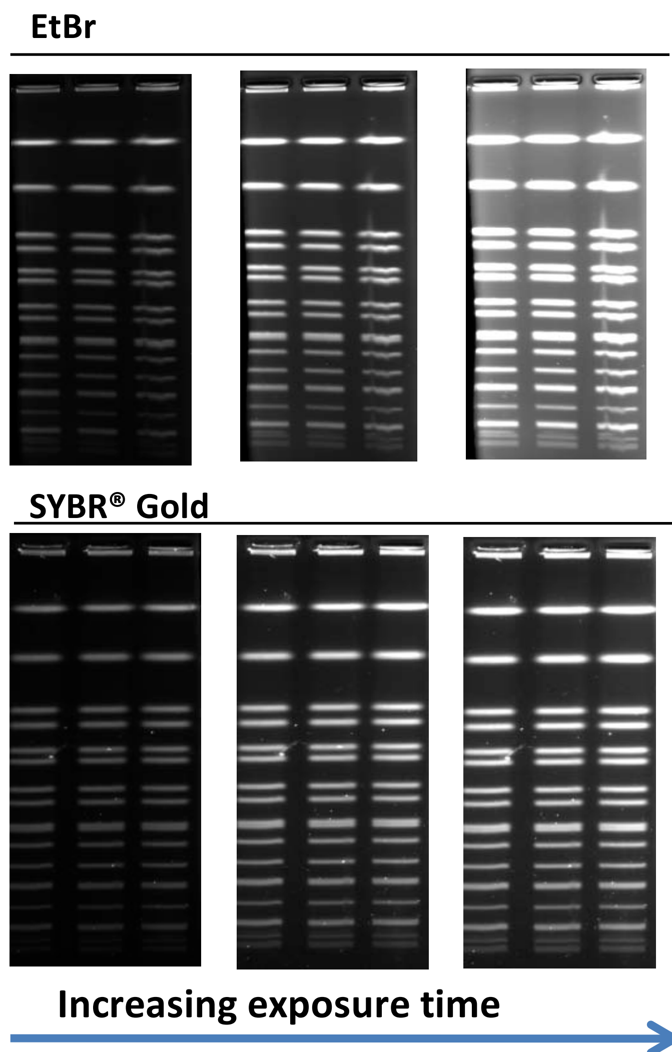
Stain Type	Stain Cost	Disposal Cost	Total Cost
SYBR® Safe (Invitrogen)	$5.88	$0.00	$5.88
SYBR® Gold (Invitrogen)	$11.70	$0.00	$11.70
GelRed™ (Biotium)	$9.50	$0.00	$9.50
EtBr (Sigma)	$0.23	$2.89	$3.12

Experiment 1

Set-up

A 15-well gel containing H9812 was run and subsequently cut into four parts and stained for 30 minutes with EtBr, GelRed™, SYBR® Safe, or SYBR® Gold. The portion stained with EtBr was de-stained before imaging while the others were imaged immediately (without destaining). Each gel portion was imaged with increasing exposure times to evaluate the image quality over a broad time range. The initial exposure time varied depending on the stain being used, however the range of time between the first and last image captured was the same. The stains were prepared following vendor recommendations and stored in light-protected containers.

Figure 1. Comparison of images obtained after staining with EtBr or SYBR® Gold using increasing exposure times.



Results

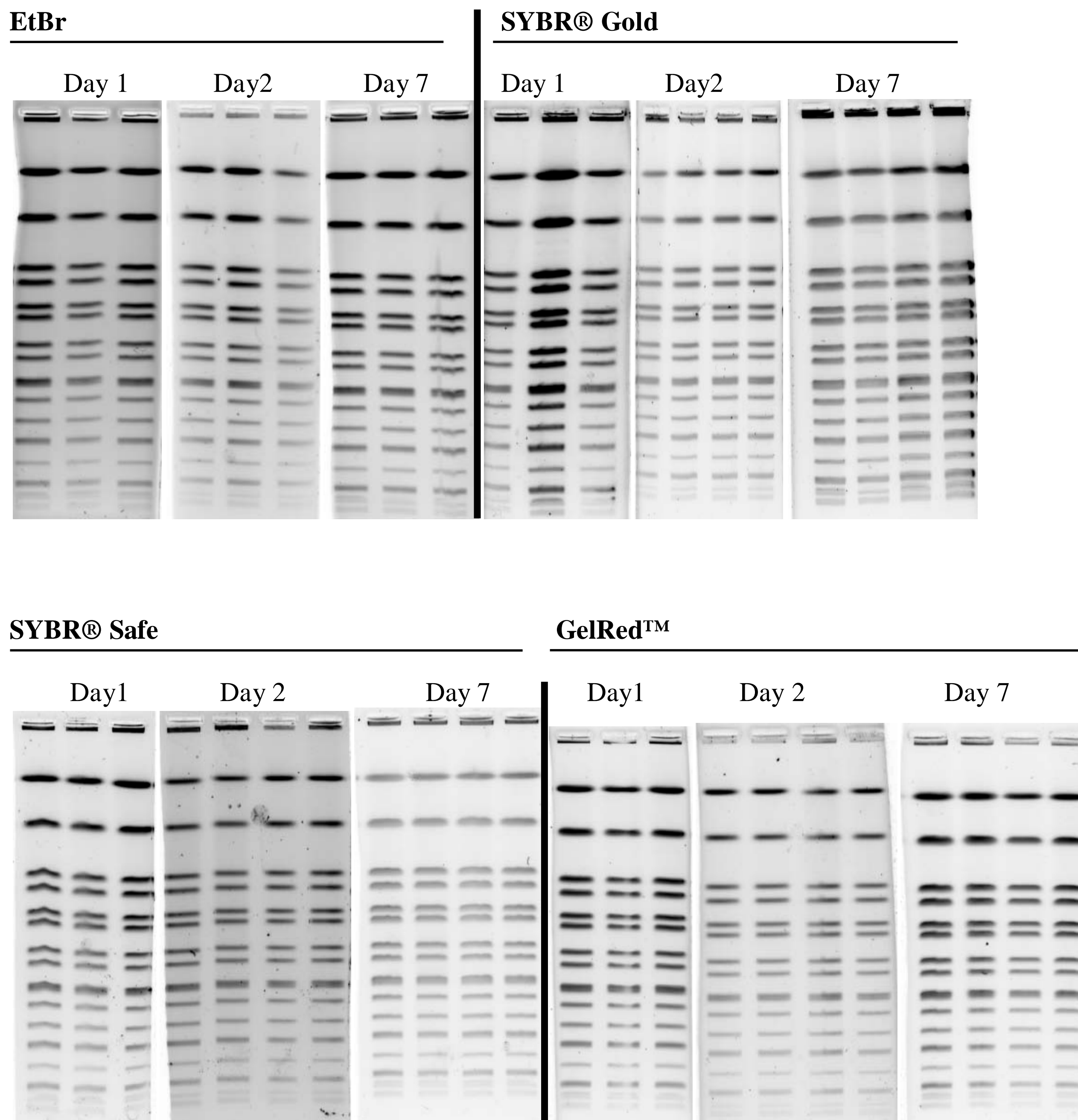
As the exposure time increased, additional background (gray) was observed on gels stained with EtBr. High background is a common problem during image acquisition and can complicate image analysis by reducing the contrast between the bands and the blank gel background. Additionally, the fluorescence within the bands increases significantly and they appear thicker and less sharp. Avoiding over-integration is particularly important when analyzing very complex patterns in which multiple bands migrate close together. In this situation, high background and thick bands may mask clues (white space between bands or shoulders) that indicate the presence of two bands. However, with the alternative DNA stains, as the exposure time increased, very little background was observed, the amount of fluorescence within the bands varied little and the bands remained distinct. This suggested that these stains can produce a relatively similar image over a broad range of exposure times. Due to this, laboratories may be less likely to over-expose their images.

Experiment 2

Set-up

A 15-well gel containing H9812 was run and subsequently cut into four parts and stained for 30 minutes with EtBr, GelRed™, SYBR® Safe, or SYBR® Gold. The gel portion stained with EtBr was de-stained before imaging while the others were imaged immediately. The stains were prepared following vendor recommendations and stored in light protected containers. The stability of the stains was evaluated by staining similar H9812 gel sections on Day 1, 2, and 7.

Figure 2. Comparison of dye stability over time.



Results

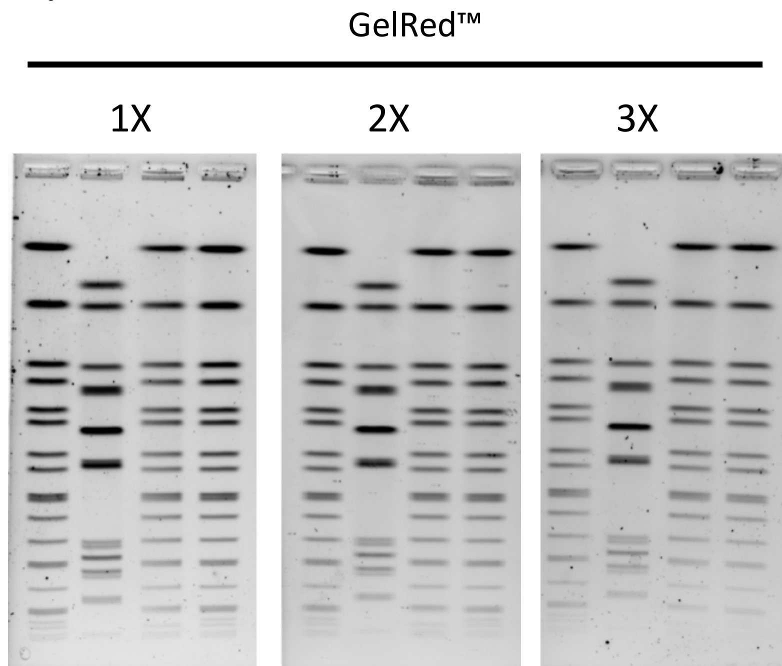
In general, very little difference in fluorescence intensity was observed between the gel stained on day one and those on subsequent days with each of the stains tested. However, with both SYBR® dyes a slight graying of the bands was seen by Day 7. This may be due to light exposure and stain degradation as containers were wrapped in aluminum foil and left out on the bench top. Consequently for later tests, these containers were stored in a cabinet and the gray appearance of the gels on Day 7 with the SYBR® dyes was not observed.

Experiment 3

Set-up

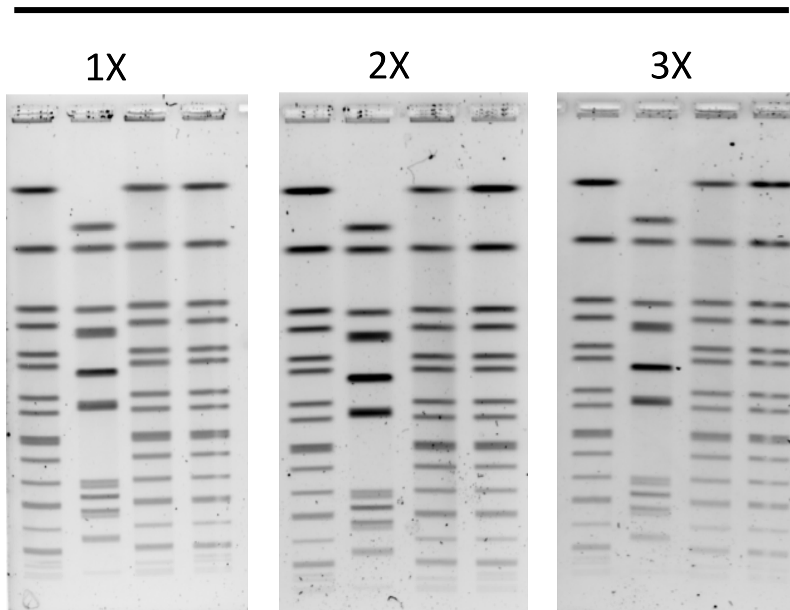
A 15-well gel containing two *Salmonella* isolates flanked on both sides with H9812 repeated 3 times was run. The gel was cut into three parts and stained for 30 minutes with GelRed™ at 1, 2, and 3× concentration. The stability of the GelRed™ at the various concentrations was evaluated by staining similar gel sections on Day 1, 3, and 7.

Day 1



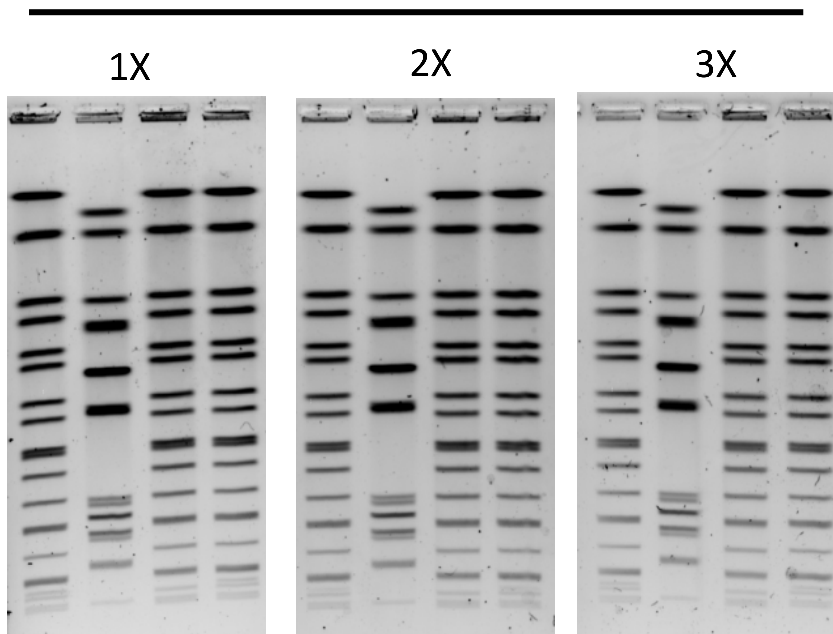
Day 3

GelRed™



Day 7

GelRed™



Results

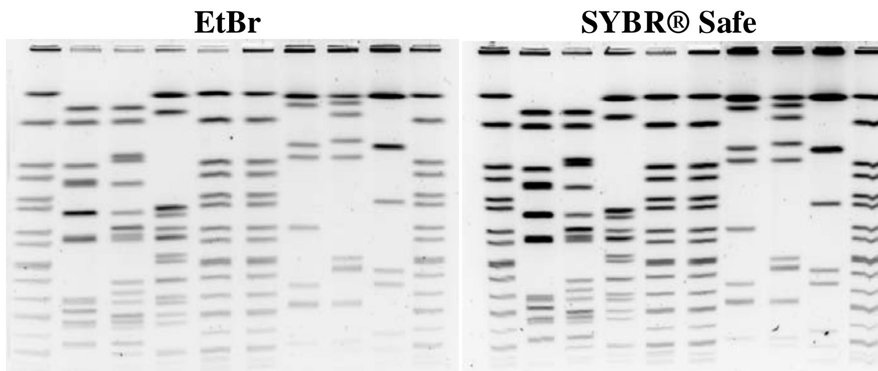
No variation in fluorescence intensity was observed between the different concentrations of GelRed™ stain. Additionally, the stains at each concentration were shown to be stable over 7 days.

Experiment 4

Set-up

Additional isolates were tested with the alternative DNA stains (GelRed™, SYBR® Safe, or SYBR® Gold) and the patterns were compared to those generated with EtBr.

Figure 4. Comparison of banding patterns generated with either EtBr or SYBR® Safe.



Results

No noticeable difference was observed in the appearance of the banding patterns when stained with either EtBr or any of the alternative DNA stains.

Summary and Conclusions

The alternative DNA stains successfully stained PFGE gels and generated patterns comparable to those generated with EtBr. One advantage of these stains is that de-staining is not necessary – gels can be directly imaged, saving time. Another advantage is that the resulting images have a limited amount of flourecscene over a wide range of exposure times. This is in contrast to EtBr which requires careful identification of the appropriate exposure time to avoid over-integration and background fluorescence which complicates image analysis. These advantages come with a relatively increased cost compared to EtBr. This cost increase would be even higher if laboratories need to make the working solutions fresh each day as recommended by the vendor. Because of this, we evaluated the appearance of gels stained with these alternative DNA stains during the course of a week. In general, they were found to generate similar looking images suggesting that these stains are stable for repeated use over the course of at least one week. We recommend storing these stain solutions in a light-protected box within a cabinet to decrease exposure to light and subsequent deterioration.

The PulseNet Method Development and Reference Laboratory would like to announce that laboratories wishing to begin implementing using these stains within their own laboratories to stain PulseNet PFGE gels are welcome to do so. The current PulseNet Standardized protocols will be edited to include these stains as options for use by PulseNet certified laboratories.