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Longevity Of SYBR® Safe Signal In Agarose Gel Electrophoresis

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Abstract

SYBR ® Safe is utilized as a safer alternative to ethidium bromide in nucleic acid electrophoresis. The utilization of ethidium bromide has been well characterized in a number of scenarios where there has been a delayed use of electrophoresis gels. The longevity of the SYBR® Safe signal determines the potential for a gel to be poured on one day and electrophoresed on another. It also determines the potential for capturing data of an electrophoresed gel at a later date. This study also investigated if a gel can be re-used by electrophoresing the gel again, utilizing previously unused lanes. This study offers supplementary information for the use of SYBR® Safe in smaller teaching and research molecular biology laboratories.

Keywords: SYBR® Safe, electrophoresis, teaching

1. Introduction

SYBR® Safe is used as an alternative to ethidium bromide for the detection of DNA. Ethidium bromide, the previously conventional way of staining and back-staining electrophoresed DNA, inserts itself between nucleotide bases of DNA making it a potential mutagen and a hazard in teaching laboratories. Additionally, ethidium bromide is difficult to dispose of safely.[1] The endurance of SYBR® Safe is of special interest to teaching laboratories, because it dictates the ability to use or re-use a gel at a later date. This study investigates the utilization of SYBR® Safe in scenarios typical of those encountered in small research and teaching laboratories at the undergraduate level.

2. Methodology

Agarose gel electrophoresis followed standard preparation and running protocol using Lambda Hind III ladder or a DNA ladder measuring from 100 to 1200 base pairs.[2] Experiments were conducted under light and dark conditions and in room temperature and cold conditions as specified in each experiment type. Agarose minigels (0.7%) were prepared with 0.35 g of agarose powder and 50mL 1x TAE buffer. When molten, 5 µL of (10,000x concentration) SYBR® Safe was added to the molten TAE/agarose mixture. Gels were allowed to solidify for approximately 1 hour and then were utilized for the varying experimental protocols described below. Agarose gels were typically electrophoresed at 100 V. DNA bands were documented using the Bio-Rad gel documentation system. In each experiment, all agarose gels were stored at approximately 4º C in the dark between experiments.

In order to investigate the effect of temperature on band intensity and clarity, gels were poured as described above and allowed to solidify in either a cold room (4ºC) or at room temperature (approximately 24ºC).

To investigate whether gels could be poured one day and electrophoresed with DNA loaded at a later date, 0.7% agarose gels were poured with SYBR® Safe, loaded and electrophoresed after the designated number of days, and examined under UV light to detect fluorescent DNA bands.

To investigate the longevity of the SYBR® Safe signal over time, 0.7% agarose gels were poured with SYBR® Safe, loaded and electrophoresed, and examined under UV light to detect fluorescent DNA bands. A photograph was taken each subsequent day to document the number of DNA bands apparent by visual inspection.

In order to investigate the ability to reuse a gel, open lanes on gels that were electrophoresed at least one month prior were loaded with DNA and the gel was electrophoresed again. The gel was back-stained with 10 µL of SYBR® Safe in 50 mL 1x TAE buffer for 30 minutes on a shaker at 50 rpm, then DNA bands were examined under UV light.

3. Results

Table 1 summarizes general qualitative observations for all three experiments described. Gels poured at 4º C had DNA bands that were tighter but displayed lower brightness than gels poured at room temperature where DNA bands were diffuse but displayed higher brightness.

Table 1. effect of temperature on quality of DNA banding patterns

Temperatures Poured and Set at	DNA Bands	Visualized Brightness Under UV Light
4°C	Tight	Dimmer
24°C	Diffuse	Brighter

Table 2 shows that whether gels were poured under either ambient light or in the dark, all DNA bands were visible for at least three days as long as gels were stored in the dark immediately after being prepared.

Table 2. effect of light on preparation conditions of electrophoresis gels

Condition Under Which Gel Was Poured and Set	Length of Time All Bands Detected Under UV light after Gel Poured
Light	At Least up to 3 days after poured
Dark	At Least up to 4 days after poured

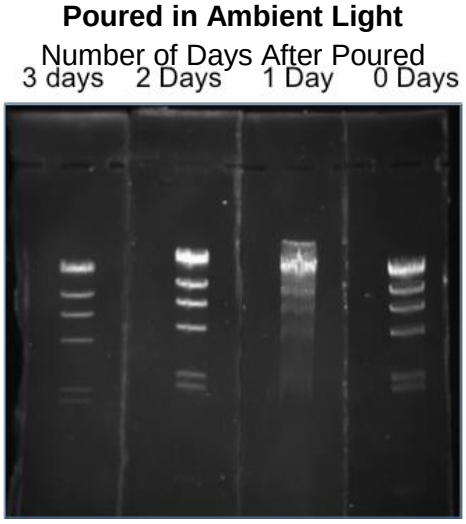

Figure 1. Titles indicate the number of days visualized after poured for agarose gel poured in ambient light

When poured under ambient light, all DNA bands were visible for least three days as long as gels were stored in the dark immediately after being prepared.

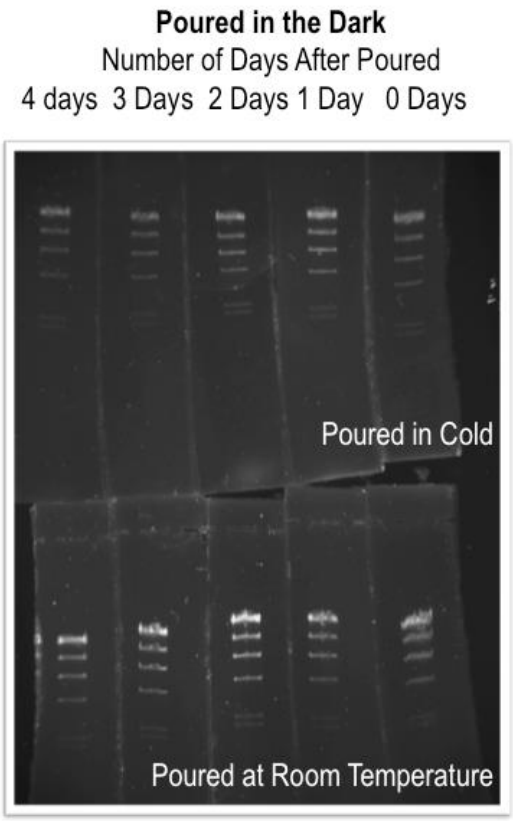

Figure 2. Clarity of DNA bands visualized on gels poured in the dark under cold and room temperature conditions

When poured in the dark in either cold (4º C) or at room temperature (approximately 24º C), all DNA bands were visible for at least four days as long as gels were stored in the dark immediately after being prepared.

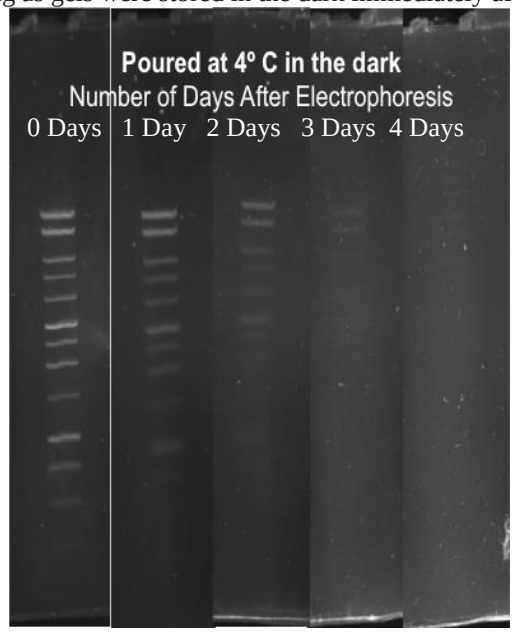

Figure 3. Clarity of DNA bands after passage of time, visualized on gels poured in the dark at 4º C

Figure 3 shows a SYBR® Safe containing DNA gel that was poured at 4º C in the dark and electrophoresed immediately in the dark. The entire amount of DNA loaded onto the gel was detected under UV light on day 0. Daily photo documentation of the gel indicated that after 24 hours (+/- 1 hour) of storage at 4º C, some DNA bands were undetectable and degradation increased for the duration of the experiment with all bands being undetectable by day 4.

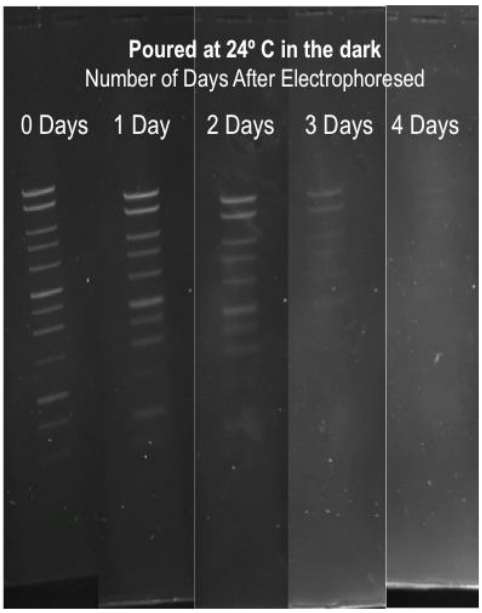

Figure 4. Clarity of DNA bands after passage of time, visualized on gels poured in the dark at 24º C

Figure 4 shows a SYBR® Safe containing DNA gel that was poured at 24º C in the dark and electrophoresed immediately in the dark. The entire amount of DNA loaded onto the gel was detected under UV light on day 0. After 24 hours (+/- 1 hour) of storage at 4º C, some DNA bands were undetectable and degradation increased daily. This supports the data shown in Figure 3 that SYBR® Safe signal degrades over time and that by day 4, regardless of the temperature that a gel is poured at, all lanes will be undetectable.

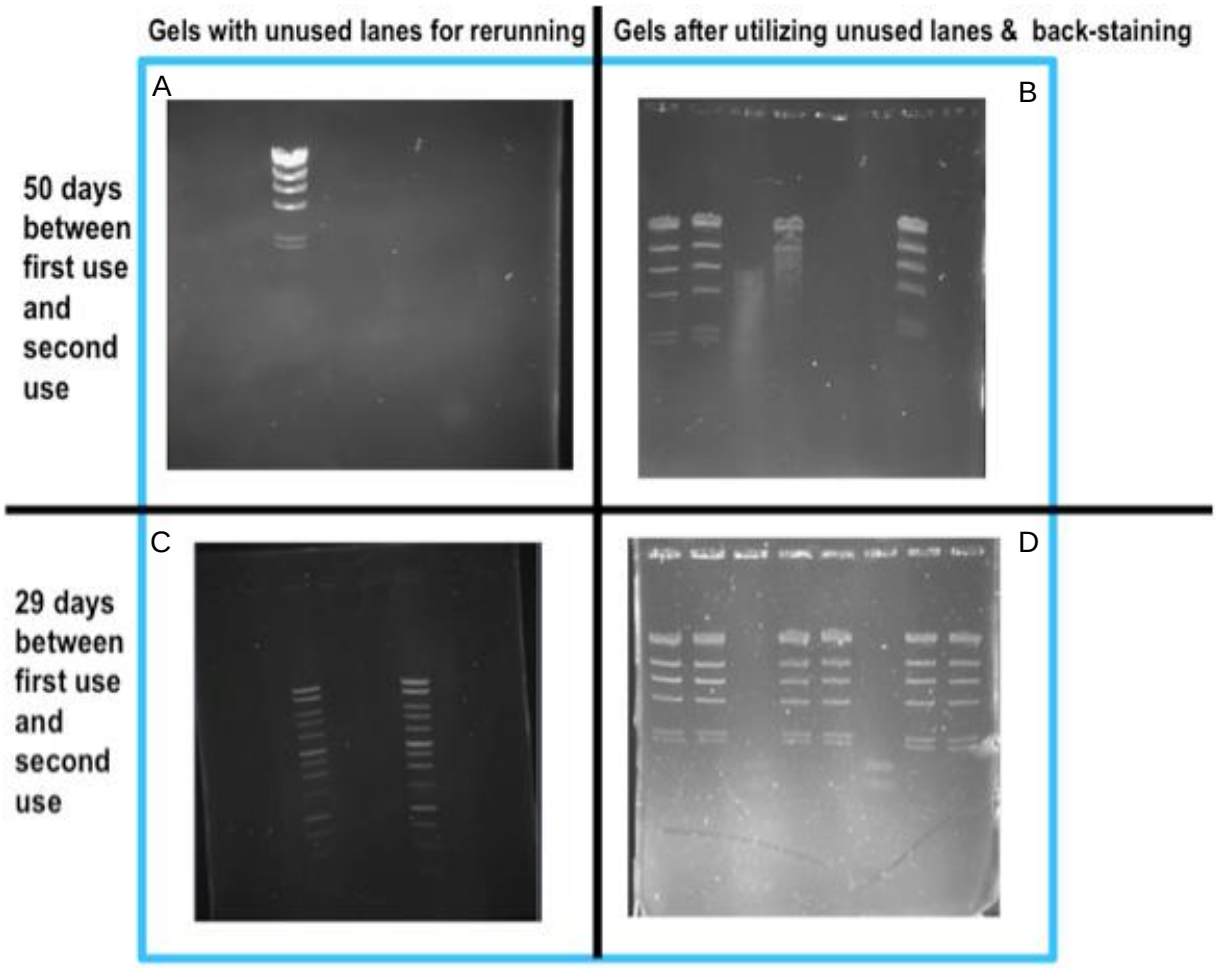

Figure 5. Previously unused lanes were loaded with new DNA ladders, electrophoresed, and back-stained

Figure 5 shows the use of stored gels. Gels poured and electrophoresed at least one month prior (A and C) were electrophoresed again after loading DNA into a previously un-used lanes (B and D). After back-staining the gels with SYBR® Safe, the expected DNA bands in the new lane were detectable under UV light.

3. Conclusion

This series of experiments sought to mimic several typical conditions, which might occur in research and teaching laboratories where interruptions in protocol might be the norm. Overall, it should be noted that gels poured in the cold became fragile and broke more easily than those poured at room temperature. Also, gels stored for a long period of time have the potential to grow mold.

Data in Figure 1 shows that a gel can be poured in ambient light up to at least 72 hours (+/- 1 hours), which was the duration of this experiment, before electrophoresing and provide reliable data. Figure 2 shows that a gel can be poured in the dark at least up to 96 hours (+/- 1 hours), which was the duration of this experiment, before being electrophoresed under the two indicated temperature conditions and all of the DNA bands will be detectable under UV light. The gels poured at 24º C had diffuse banding with higher intensity under UV light than the gels poured at 4º C. These figures show that regardless of the light and temperature preparation conditions, gels can be poured up to at least 72 hours (+/- 1 hour) in advance of electrophoresing the gel and still provide reliable data.

Another common interruption in protocol in a teaching or research laboratory might be an inability to capture data on an electrophoresed gel immediately. Gels poured, electrophoresed, and assessed for band brightness showed

progressive signal degradation from 24 to 96 hours (+/- 1 hour). Thus, to ensure that all DNA bands are visible under UV light, it is prudent to capture a photograph of data immediately. Lower base pairs may fail to appear if more time lapses.

Utilizing unused agarose gel electrophoresis lanes and back-staining gels is also a common practice in research and teaching laboratories. Back-staining can be performed on gels using SYBR® Safe, which allows for gels to be reused. Unused lanes can be loaded and most new DNA will be detectable under UV light. After back-staining the gels with SYBR® Safe, the expected DNA bands in the new lane were detectable under UV light; however, the utilization of this technique should take into account time and reagent costs. Since gels can be poured up to five days in advance of electrophoresing, it might be more cost effective (at least in regards to SYBR® Safe) to pour a new gel, rather than to reuse a gel, since in these experiments back-staining used two times more SYBR® Safe than pouring a new gel. In the interest of time, back-staining takes about a half an hour less time than preparing a new gel.

Through our experiments, we strove to elucidate user information for SYBR® Safe. In teaching settings, it is not uncommon for gels to be stored for periods of time before use or documentation. In conclusion, while it would appear that one could have a delay in use of the gels, it is imperative that photographic capture of data occurs within 24 hours, as long as the gel is stored in the dark. Further experimentation could lead to more precise recommendations regarding the duration of time between electrophoresis and documentation of a gel that would allow for accurate data capture.

4. Acknowledgements

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5. References

1. Invitrogen™, "SYBR® Safe DNA Gel Stain," LifeTechnologies.com, https://tools.lifetechnologies.com/content/sfs/manuals/mp33100.pdf.

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