Compare the SYBR Gold Signal by Epi-blue Light, UV-transilluminator, and UV/Blue Converter Plate

INTRODUCTION

As blue light was used to excite the fluorescence dyes in the daily experiments as a harmless light source toward nucleic acid, Wealtec introduced epi-blue LED lights in Dolphin View II stand alone imaging system. Since the high intensity epi-blue LED is strong enough to penetrate the gel, Dolphin View II provides more ways of excitation to not merely been used for normal gel documentation but also for fluorescence image capturing. While using epi-blue LED light source to excite the stains, materials that put under the gel may have reflection and interferes the image by enhancing or decreasing the signal. Take SYBR Gold as an example of blue light source excitation target to distinguish the differences of using different light sources.

MATERIALS

- Dolphin View II with epi-blue LED (470 ± 50 nm) (Wealtec)
- UV/Blue converter plate (Wealtec)
- High transparent amber filter (550 ~ 675 nm) (Wealtec)
- SYBR Gold (Invitrogen) Ex/Em: 300, 495/537 nm
- λDNA/*Hind*III Markers (Wealtec)

PROCEDURES

1. Prepare the serial diluted Lambda DNA/*Hind*III markers with the following amount: Lane 1 to 10: 1.6, 1.0, 0.8, 0.5, 0.1, 0.08, 0.05, 0.01, 0.008, 0.005 µg DNA.
2. Prepare the 1.0 % TAE agarose gel with pH = 8.0.
3. Run the DNA samples with 100 V in agarose gel for 1 hour.
4. Prepare SYBR Gold TAE solution with 10 µL stock (1:10,000) in 100 mL TAE Buffer
5. Stain the agarose gel with SYBR Gold TAE staining buffer for 1 hour
6. Observe the result with imaging system through high transparent amber filter.
7. The gel was placed on top of different surface and excited by different light source.

RESULT

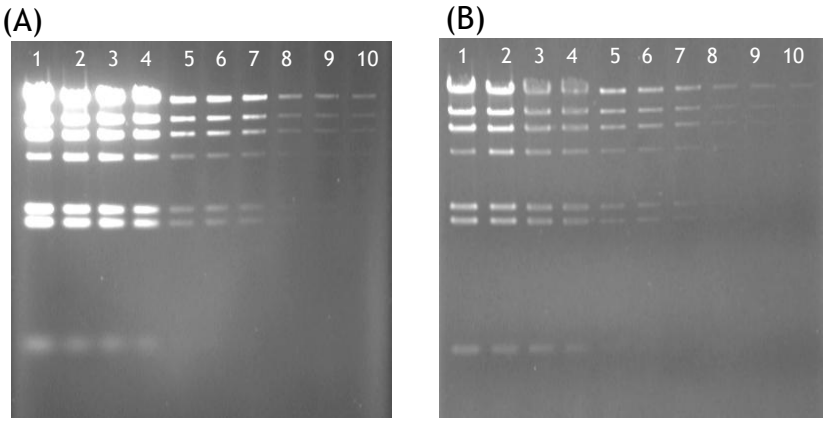

Figure 1. *SYBR Gold stained agarose gel was excited by UV transilluminator in Dolphin View II. (A) Trans-UV light without integration times; (B) UV-Blue convert plate with 20 integration times.*

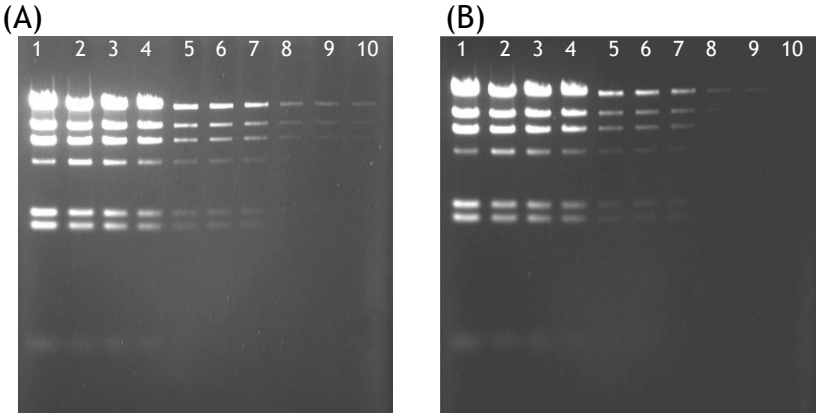

Figure 2. *SYBR Gold stained agarose gel was excited with Epi-Blue LED in Dolphin View II wihtout setting of integration times. Gel was placed on top of (A) UV/Blue converter plate and (B) UV transilluminator.*

DISCUSSION

SYBR Gold was claimed having better detection limit than EtBr. Taken as typical nucleic acid stain, when observing the SYBR Gold stained gel, users can excite the stain with 300 and 495 nm light source and observe at 537 nm. Compare the signal intensity in *fig*. 1 and 2, SYBR Gold that was excited by 312 nm UV transilluminator had the strongest signal intensity through amber filter observation. However, the damage toward nucleic acid was also the worst. Using of high intensity epi-blue LED light can prevent damage and

have the same detection limit while comparing with the UV light excitation. Besides, under the epi-blue light excitation condition, the signal intensity was increased when the gel placed on top of the UV-blue converter plate, because of been enhanced by the reflection light from the converter plate. Considering about the detection limit, when placing gels on top of the converter plate, it can have better detection ability than placing on top of UV transilluminator.

In conclusion, while using the epi-blue LED light as excitation light source to observe SYBR Gold stained samples in Dolphin View II, users can get almost the same quality as using trans-UV light, especially when they put the sample on top of the white surface, such as on the UV/Blue converter plate. For those users that need further processing of DNA/RNA samples, in order to prevent DNA from been damaged, epi-blue light is the best choice for exciting proper stains.

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