SYBR Dyes Detection via Blue and White Light Transilluminator

INTRODUCTION

Bio-Safe dyes now a day becoming more popular in the molecular labs as well as the safe dye excitation devices. BW-20 is a blue/white light transilluminator capable to get excellent visualization result as well as UV-transilluminator. Below will state SYBR Green I, SYBR Gold, and SYBR Safe excited by BW-20 and detected by KETA G to get quality images. Wealtec also provide optional model, B-20 as a high intensity blue light transilluminator.

MATERIALS

- SYBR Green I / SYBR Safe /SYBR Gold (Invitrogen)
- TBE buffer (Uni-Region Bio-Tech)
- 100bp DNA Ladder(Genomics)
- SolGent™ 2x Taq PLUS PCR Smart mix (SolGent, Korea).
- KETA G imaging system / GES/ SEDI thermo cycler (Wealtec)
- BW-20 transilluminator (Wealtec)
- MD-25 UV transilluminator (Wealtec)

PROCEDURES

1. Prepare PCR reaction solution with series diluted Template DNA solution from Dr. Hu's lab as below formula.

Solution	Conc.	Volume
ddH_2O	-	17.5μL
2X Master Mix		25μL
Template DNA	0.075, 0.15, 0.3, 0.6, 1.2, 2.4 ng/μL	2.5μL
5'-Primer		2.5μL
3'-Primer		2.5μL
	Total Volume	50μL

2. Run with following PCR program to prepare the DNA samples:

Step	Temp.	Time	GoTo	Cycles
Step 0	95°C	OFF		
Step 1	95°C	2 mins		
Step 2	95°C	30 sec		
Step 3	56°C	45 sec		
Step 4	72°C	1 mins	Step 2	25 cycles
Step 5	72°C	5 mins		
Storage	6°C	ON		

3. According to different instructions of SYBR dyes, proceed the experiment as below:

(a) **SYBR Green I**:

(1) Run the DNA samples with 0.5x 2% TBE Agarose gel with the DNA ladder.

(2) Apply with constant voltage 100V and run for 50 minutes.

(3) While running the electrophoresis, prepare staining solution. Prepare 500 mL 0.5x TBE and adjust the pH to 8.0. Add with 50 µL SYBR Green I dye and mix well.

(4) Immerse the agarose gel into staining solution for over than 2 hours.

(5) Detect the result with BW-20 under the KETA G image system with WK101 filter.

(b) **SYBR Gold**:

(1) Prepare 1x 1.5% TBE agarose gel 100mL. As the gel cooling down to around 60°C, add with 20µL SYBR Gold stock solution before pool into the casting module. Make sure to cover with foil paper during the gel solidification.

(2) Run the DNA samples with pre-stained agarose gel with 100 V and 80 minutes.

(3) Detect the result with BW-20 under the KETA G image system with WK101 filter.

(4) Adjust the brightness and contrast to have better result during capturing.

(c) **SYBR Safe**:

(1) Prepare 1x 1.5% TBE agarose gel 100mL. As the gel cooling down to around 60°C, add with 10µL SYBR Safe stock solution before pool into the casting module. Make sure to cover with foil paper during the gel solidification.

(2) Run the DNA samples with pre-stained agarose gel with 100 V and 80 minutes.

(3) Detect the result with BW-20 under the KETA G image system with WK101 filter.

(4) Adjust the brightness and contrast to have better result during capturing.

RESULT

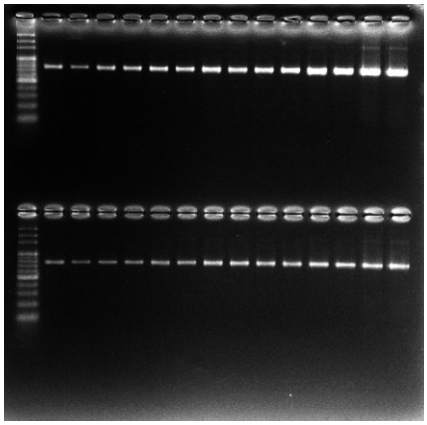
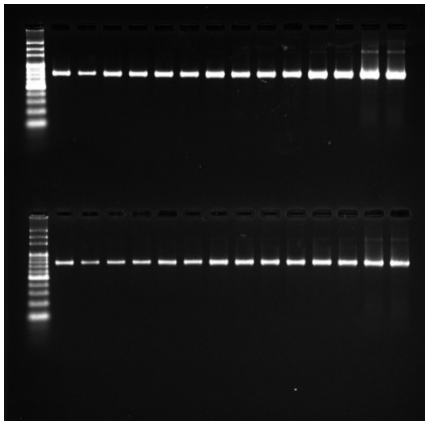
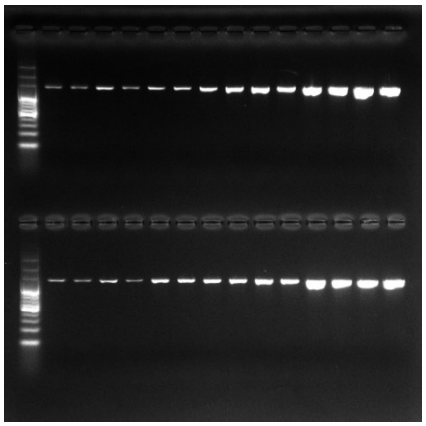
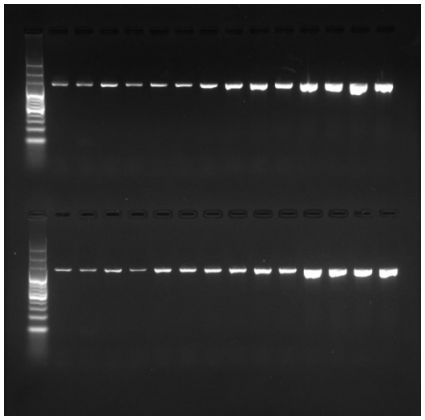
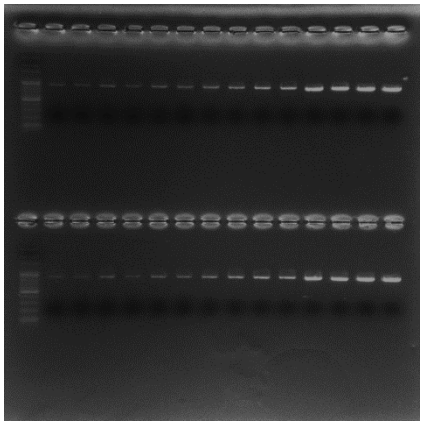
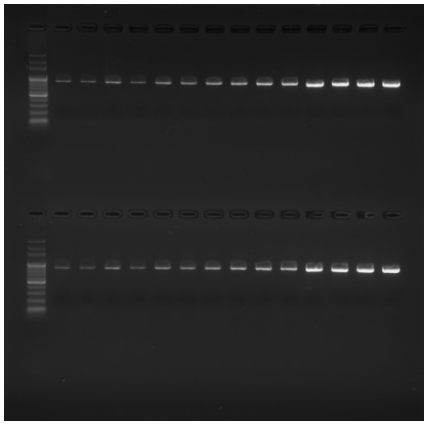
	BW-20	MD-25 UV transilluminator
SYBR Green I		
SYBR Gold		
SYBR Safe		

Figure 1. *BW-20 Transilluminator compare to UV-transilluminator*

DISCUSSION

BW-20 is an illumination device with combination of white light and blue light with its emission peak centered at 470nm. SYBR Green I has excitation wavelength at approximately 497 nm and SYBR Gold is about 495nm. In the fig. 1, both SYBR Green I and SYBR Gold stained gel had darker background where SYBR Safe was brighter. By extending the exposure time in both SYBR Green I and SYBR Gold stained gels can get good image quality with blue light excitation of BW-20, which is similar with result in a UV-transilluminator.

However, SYBR Safe has excitation wavelength at 507 nm, is a bit far from the blue light emission peak centered at 470 nm, the visualization result by BW-20 in SYBR safe stained gel is about one-fourth intensity weaker than UV transilluminator. Accordingly, it would be highly recommended to adjust the brightness, contrast, and gamma value to get a better visualization result for SYBR Safe stained gel by using blue light excitation.

Moreover, due to the SYBR dyes' characteristics are different, so does the staining protocols, observation method and result. Users should follow the instruction manual from the safe dyes' supplier to perform the experiment.

The application by Bio-Safe dyes are more important and popular to get rid of risk of toxic chemicals and hurt to human beings. BW-20 with its high and even intensity of blue light source for SYBR Green I, SYBR Gold, and SYBR Safe dyes excitation is considered a safer solution to replace the UV light excitation to decrease the risk in exposure of UV hazard.

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