Prevent DNA Damage by Visible Light Excitation

INTRODUCTION

Experiments that need to manipulate the nucleic acid which all use of electrophoresis to separate the sample mixture. Specific length or molecular weight of sample is usually selected as a target for the next experiment starting point. Agarose gels were commonly not only used to separate the sample mixture but also to accumulate the target sample. The most common way to detect the sample on the agarose gel is staining with the fluorescent dyes and detecting through the UV light source. However, energy from the UV light was too strong that might cause the nucleic acid damage and lost. Substitution light source with lower energy and which would lower down the damage of DNA samples. Specifically, the blue light source was evaluated by comparing to the traditional trans-UV light source.

MATERIALS

- KETA ML imaging system (Wealtec)
- Ethidium Bromide (Sigma); SYBR Gold (Invitrogen)
- UV/Blue light converter plate

PROCEDURES

- 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.
- Prepare the 1.2% TBE and 1.0 % TAE agarose gel with pH = 8.0.
- Run the DNA samples with 100 V in agarose gel for 1 hour.
- Stain the agarose gel with different staining buffer for 1 hour:
  EtBr: 10 μL (10 mg/mL) EtBr in 100 ml TBE buffer
  SYBR Gold: 10 μL stock (1:10000) in 100 mL TAE Buffer (pH 8.0)
- Observe the result with KETA ML imaging system through WK101/WK102 filter.

RESULT

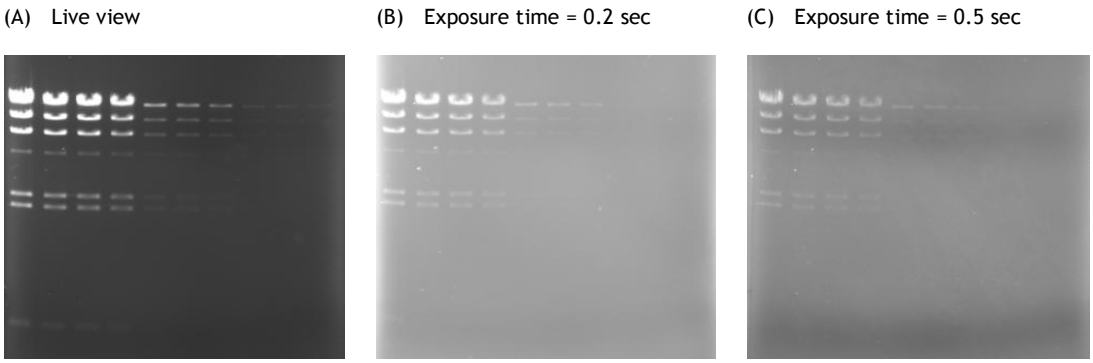

Figure 1. *Observation of EtBr stained agarose gel in KETA ML with WK101.*
Serial diluted DNA samples in the agarose gel were excited by (A) High intensity Trans-UV light (312nm), (B) Epi-Blue LED light (460 nm), and (C) UV/Blue converter plate.

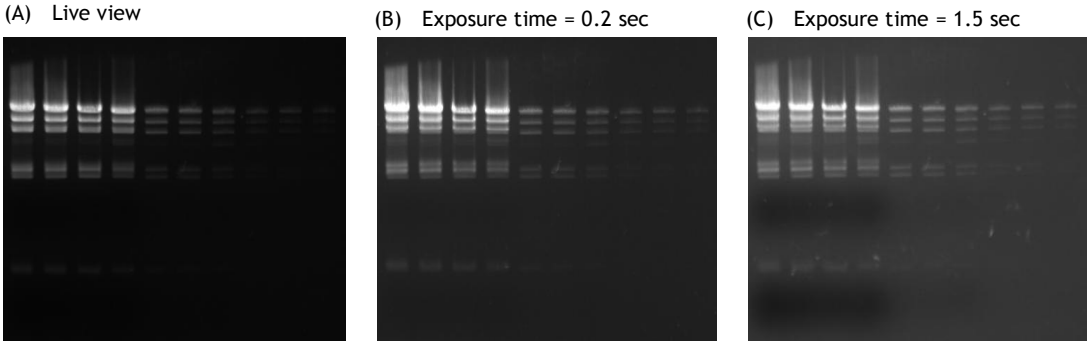

Figure 2. *Observation of SYBR Gold stained agarose gel in KETA ML with WK102.*
Serial diluted DNA samples in the agarose gel were excited by (A) High intensity Trans-UV light (312nm), (B) Epi-Blue LED light (460 nm), and (C) UV/Blue converter plate.

DISCUSSION

Refer to the spectrum characteristic of both EtBr and SYBR Gold, they both can be excited under the blue light observation and have the emission wavelength around 600 and 537 nm. Respectively, they can be observed through WK101 and WK102 filter. As in

the *fig. 1* and *fig. 2*, comparing to using of trans-UV light source result, DNA samples can be observed with the same detection limit with extending the exposure time when excited with blue light sources. Although as the exposure time was extended, the background signal would also been enhanced, samples can still be distinguished from the background. Comparing two different blue light source observations, samples can be detected better in the epi-blue LED light excitation with stronger signal than with UV/Blue converter plate. However, the reflection on the gel from the epi-blue LED light source sometimes interfere the observation result.

In conclusion, as it had been proved that using of blue light as excitation light source can be a substitution way to detect EtBr or SYBR Gold stained gels. Users concern about the damage that caused by the UV-light source can choose blue light instead as the excitation light source. In case to lose the detection of tracing nucleic acid samples, users should extend the exposure time to have better observation result while using the blue light source. Even if users intend to perform the gel excision, blue light is a better observation light source with less damage toward human body.

Yi-Ta, Chen
Product Manager
Email: info@wealtec.com

Wealtec Bioscience Co. Ltd.
27Fl. No. 29-1 Sec.2, Jungjeng E. Rd., Danshuei Jen, Taipei, Taiwan 25170
TEL: +886-2-8809-8587 FAX: +886-2-8809-8589; http://www.wealtec.com