USING NEW CHEMISTRY AND PHYSICS FOR BETTER RESUTLS

WHEN SAFETY MET SIGNAL

DNA IMAGING SYSTEMS ARE MAINLY BASED ON UV-LIGHT, WHICH CAUSES DNA DAMAGES AND IS THEREFORE HARMFUL FOR THE USER. BASED ON THE NEGATIVE INFLUENCE OF UV-LIGHT ON DNA, DOWNSTREAM APPLICATIONS ARE VERY OFTEN UNSUCCESSFUL. THE BLUE/GREEN LED IMAGING SYSTEMS ARE A SOLUTION FOR THE PROBLEM BY USING HARMLESS VISIBLE INSTEAD OF UV-LIGHT APPLYING A NEW TECHNICAL APPROACH WITH A VERY HIGH SENSITIVITY FOR ALL AVAILABLE DNA STAINS.

Applications

- Detection of nucleic acids
- Very intense signal
- Gel documentation
- No DNA degradation
- Detection of GFP, etc.

FastGene® FAS-NANO: Transform a small transilluminator into a gel documentation system by taking images with any smartphone.

The detection of DNA is still standardly performed using ethidium bromide. This dye intercalates the DNA and has an excitation peak in the UV-light spectrum of about 300 nm. Hence, majority of the detection instruments are based on UV-light gel documentation systems. The high sensitivity, the low background signal and the affordable price are reasons for ethidium bromide still being used in the laboratories, although many alternatives are already available. However, not only ethidium bromide raises serious safety concerns, the UV-light itself can cause severe damages.

Consequences of UV-Light for Human

According to the World Health Organization (WHO), prolonged exposure to energy rich light with wavelengths in the UV-spectrum (up to 400 nm) has implications in many diseases1. It is widely known, that skin cancer and melanoma are caused by UV-light1. Recent studies also show that UV-light may increase susceptibility to infection and activate latent viral infections, e. g. Herpes labialis and Human Papilloma virus infections1.

The exposure of UV-light in a laboratory environment is or should not be prolonged. However, UV-transilluminators produce a highly concentrated light beam, not comparable with the sunlight filtered and reflected by the earth outer atmosphere2. Hence, the findings summarized in the WHO report must be taken very seriously.

Consequences of UV-light for the cloning efficiency

DNA is able to absorb light in the UV-spectrum. Consequences are the formation of pyrimidine dimers; ssDNA and dsDNA breaks, DNA-DNA interstrand crosslinks and DNA-to-protein crosslinks3.

Organisms are able to repair these DNA damages or suffer the consequences discussed above. DNA in agarose gels lack these repair mechanisms.

Additionally, the amount of DNA in a gel is much less when compared to a whole organism. Therefore DNA degradation caused by short-term UV light exposure was investigated.

pUC19 was enzymatically digested using NdeI and HindIII resulting in a 264 bp

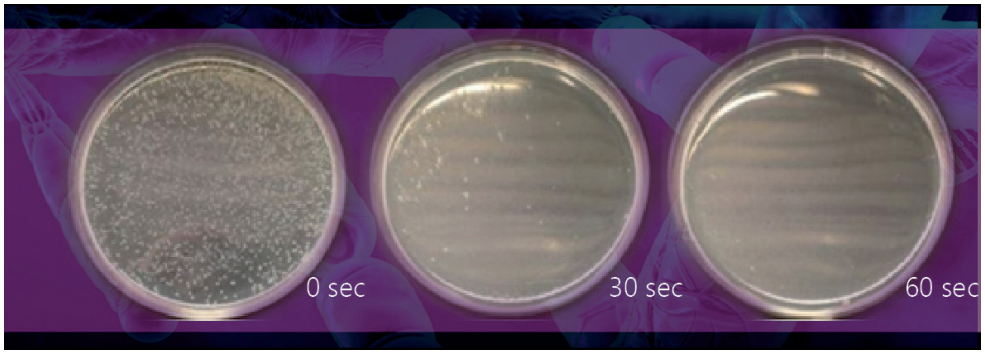


Figure 1 – Cloning efficiency of DNA exposed to UV-light.

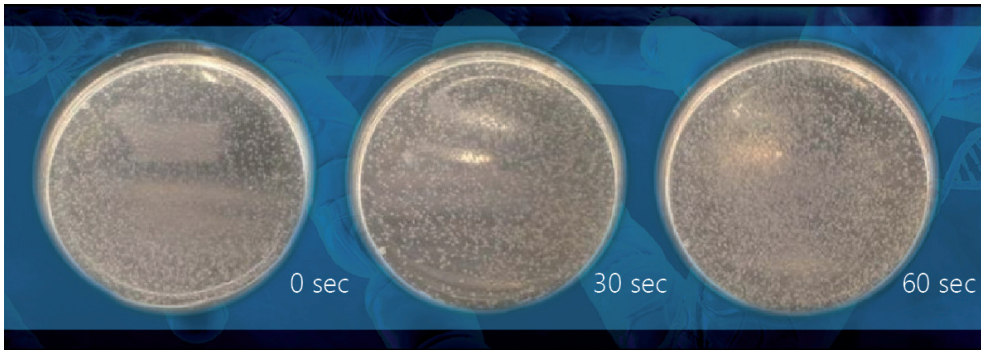
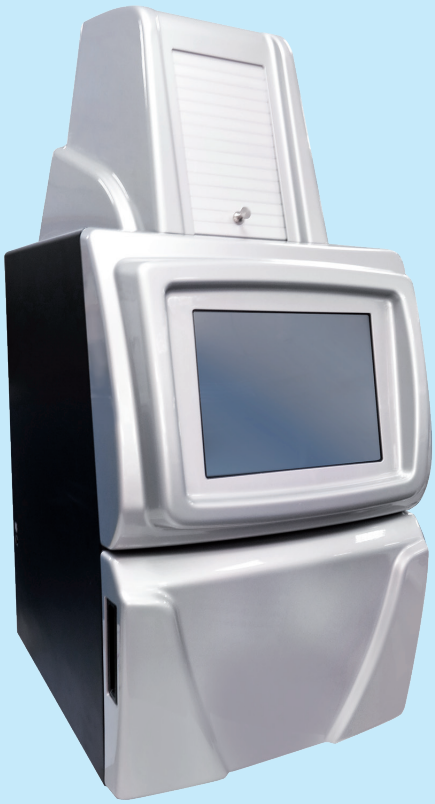


Figure 2 – Cloning efficiency of DNA exposed to light in the visible range (470 nm).



FastGene® FAS-V: High-end gel documentation system with very light-sensitive 2 MPixel CCD camera, XXL-transilluminator, built-in computer & 10,4" touch screen control.

product. This restriction digest product was amplified using PCR and run on a 2% TAE agarose gel without using DNA stains (one product was stained with MidoriGreen Direct as a reference).

After the electrophoretic run, the gel was exposed to UV-light for up to 120 seconds. The PCR product was subsequently isolated and cloned into a control vector, previously digested with NdeI and HindIII, before being transformed into Escherichia coli DH5α (BL21) cells. The results are shown in Figure 1.

The number of colony forming units (CFU) per plate decreases rapidly with the exposure of the PCR product to UV-light. After 30 seconds, the CFU number is dramatically reduced. Impressively, no transformation was observed at 60, 90 or 120 seconds UV-light exposure, suggesting sever DNA damage.

Early solution – Blue LED light

The light in the visible range is much harmless to the DNA. For example, the previously mentioned pyrimidine dimers are not detected from 400 nm onwards (Han et al.,1984 cited in Peak et al.3). Hence, in 2009 blue light emitting diodes (LED) transilluminators were introduced.

LED are semi-conductors able to transform electricity into light. The advantages are defined wavelengths, superb efficiency and a huge average life expectancy of 50 000 hours. In 2014, three Japanese scientists received the Nobel prize in Physics for the development of the blue LEDs. They emit light with a wavelength of 470 nm.

The experiment described above was repeated and the DNA was exposed to the blue LED light for the same amount of time. The result is shown in Figure 2:.As expected, the amount of CFU per plate did not have any significant change. The prevention of the DNA damage therefore vastly increased the cloning efficiency.

Problems with blue LED light

Although having the assets of safe and long living light source, using blue LEDS also had several drawbacks. Ethidium bromide, the most common DNA stain did only deliver poor results. The excitation peak of ethidium bromide bound to DNA is not at 470 nm between 480 and 525 nm. New dyes had to be developed.

New DNA dyes

Ehtidium bromide has been suspected for a long time to cause mutation in the genome. Additionally, according to the EU-regulation No 1272/2008 (CLP), ethidium bromide must be labelled as very toxic (T+). Hence, companies developed new dyes as an alternative to the mutagenic and toxic ethidium bromide.

The first dyes were emitting light in the red spectrum and had chemical and physical characteristics similar to ethidium bromide.

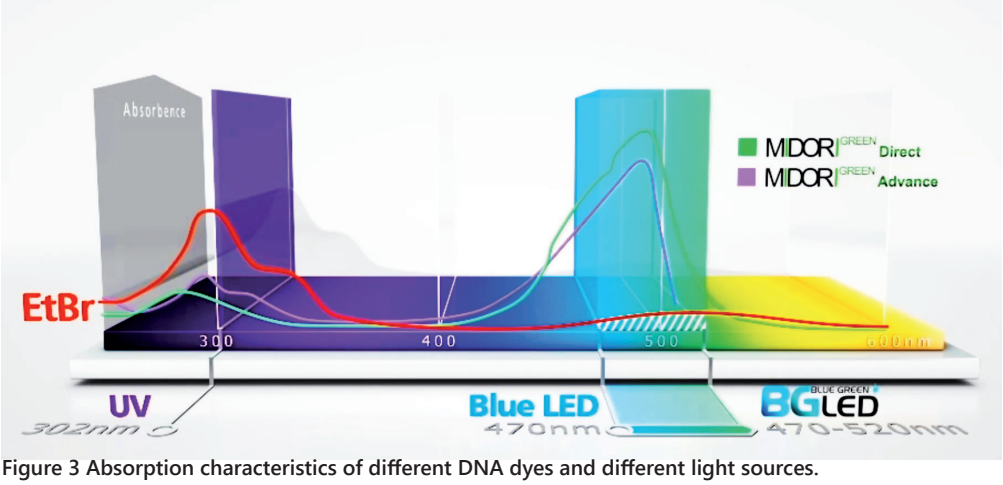


Figure 3 Absorption characteristics of different DNA dyes and different light sources.

Nippon Genetics developed a complete new chemical formulation with dyes emitting light in the green spectrum (>530 nm). The MIDORIGreen* Dye family: MidoriGreen Advance and MidoriGreen Direct. These new dyes are extremely safe and sensitive. Both dyes have successfully passed several toxicity and mutagenicity tests, e.g. Ames test and are therefore considered as non-toxic and non-mutagenic[5-6].

The BG LED Revolution

In the past, the physical properties of the different DNA dyes, i.e. their absorption maxima, resulted in the selection of the detection light type according to the dye. Blue LEDs were able to induce strong signals from green dyes. Red dyes, e.g. ethidium bromide, produced only a very week signal from blue light excitation.

NIPPON Genetics found a solution for this problem in 2014: Instead of using a single wavelength, a combination of wavelengths was created resulting in a spectrum of light with blue/green light of 470 nm to 520 nm. The Blue/green LEDs are able to excite dyes which were not excitable with a single wavelength of 470 nm, such as ethidium bromide. This can be achieved by the accumulated energy absoprtion of the dyes, as can be seen in figure 3 using ethidium bromide as an example of a red dye.

The following experiment shows the difference in signal intensity of ethidium bromide when excited with blue or with blue/green LEDs. DNA standards were separated on a 1 % 1 x TAE agarose gel by electrophoresis of 20 min at 100 V. The gel was then documentated using the FastGene® FAS Digi in combination of a blue LED transilluminator or a Blue/green LED transilluminator:

This idea led to the development of the FastGene® Blue/Green LED Transilluminator (FG-08): Signal intensities as high as from strong UV-transilluminators using safe wavelength were finally possible for all commercially available DNA dyes. Ethidium bromide and the safe alternatives deliver a much better signal with this unique technology (Fig. 4), with much better results in downstream applications as well as creating a safer laboratory environment.

FastGene® FAS-Digi: Gel documentation system with an high-end 16 MPixel camera, BG LED transilluminator.

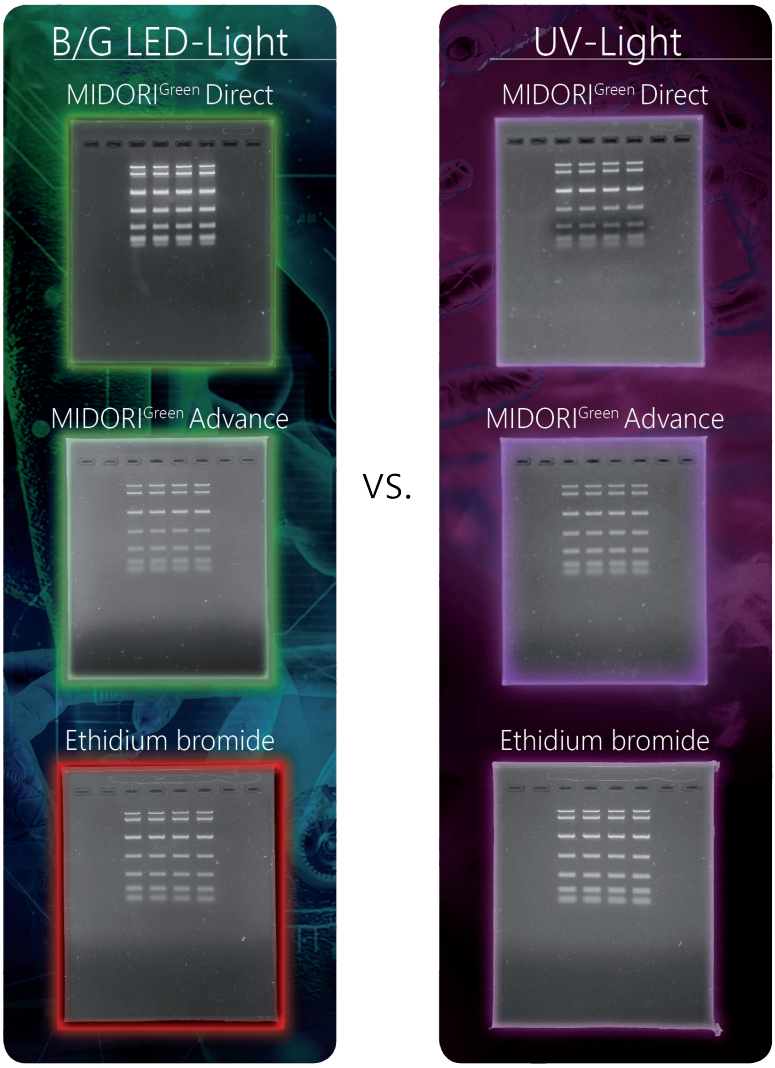
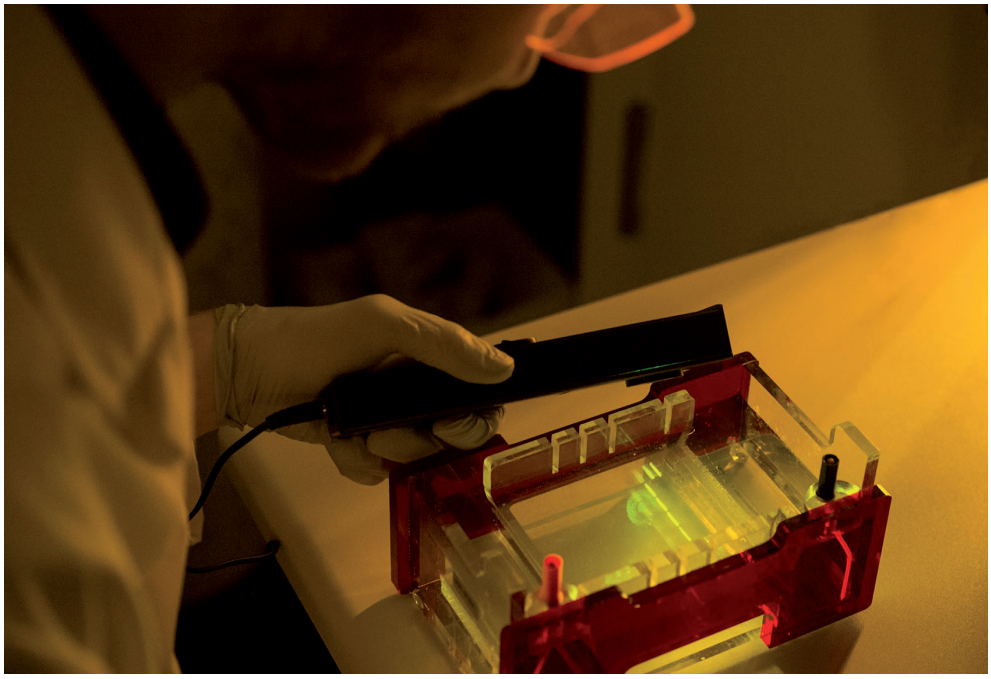


Figure 4 - Comparison of signal intensity of DNA dyes excited with blue/green LED or UV-light.



Blue/Green LED light: The unique LEDs technology combined with very good filters produces an uncomparable signal intensity using light in the visible range (demonstrated here with the B/G LED Flashlight - #FG-11).

FastGene® BG LED GelPicBox: Small gel documentation box containing three different light sources, including the Blue/Green LED technology.

References

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Bulldog Bio, Inc.
603-570-4248
info@bulldog-bio.com
www.bulldog-bio.com

Summary

UV-light is harmful

UV-light damages the DNA in agarose gels in a short amount of time. This degradation can be inhibited by using light in the visible range (>400 nm).

Blue/Green LED produces the best signal

Spectrum of light in the visible range able to excite any dye with a signal intensity which is comparable to UV-light.

Safe alternative: MIDORI^{GREEN}

Ethidium bromide is considered mutagenic and highly toxic. NIPPON Genetics developed a DNA dye family which is proven to be safe.

Ordering Information

Cat.No.	Description
MG04	**MIDORI^{GREEN} Advance**
MG06	**MIDORI^{GREEN} Direct**
FG-08	**FastGene® B/G LED Transilluminator**
FG-11	**FastGene® B/G LED Flashlight**
GP04LED	**B/G LED GelPicBox**
GP05LED	**FAS Digi**
GP06LED	**FAS Nano**
GP-FAS-V	**FAS-V**