

Comparing the influence of Ultra Violet to visible Blue LED light on the cloning efficiency

Cat. No. FG-05, FG-06, FG-08

Introduction

It is well established that UV-light is a major mutagenic agent. The energy rich radiation spectrum has a wavelength between 100 nm and 400 nm and acts on the chromophores present in the DNA. Nonetheless, it is still the standard light source for nucleic acid detection in life science laboratories.

The development of dyes that are compatible with the visible light spectrum enabled an UV-light independent detection of nucleic acids. Midori Green Advance (MG-03 or MG-04) and Midori Green Direct (MG-05 or MG-06) are examples of these next generation nucleic acid dyes which are excitable by the blue LED light.

Here, we present the consequences on the cloning efficiency, when DNA is exposed to UV-light and compare it to the cloning efficiency achieved using blue LED light exposed DNA. In order to prevent interaction with a dye, the DNA is separated without any staining. The in-sample dye, Midori Green Direct (MG05 & MG06) was used to locate the position of the unstained DNA samples, being added to identical samples on parallel wells.

Product Description

The Blue LED illuminators are equipped with LEDs that emit light at a wavelength of 470 nm. This wavelength excites both Midori Green dyes, bound to DNA, causing a fluorescent emission at a wavelength of ~530nm. The FastGene® Illuminators are available as direct illuminator (Figure 1.A, FG-05) or as transilluminator (Figure 1.B, FG-06).

The FastGene® Blue/Green Transilluminator (FG-08) is the latest generation of nucleic acid transilluminator and uses blue LEDs, enhanced by the addition of green LEDs. These create an additional light source with the wavelength of 530 nm, enabling the transilluminator to be used with the standard nucleic acid dye Ethidium Bromide.

The FastGene® FAS Digi Imaging System (GP-05LED) enables the recording of images using the standard FastGene® blue LED transilluminator as a light source as well as the new FastGene® Blue/Green Transilluminator, therefore being a complete solution for the detection and documentation of nucleic acids.

Quick Notes

- Blue LED light does not influence cloning efficiency
- Non-hazardous light source
- Available as illuminator and transilluminator
- Next generation transilluminator uses a blue/green LED combination

Methods

1.Preparing the plasmid

The vector, pUC19 (2686bp), was double digested with NdeI and HindIII to remove a 264bp region of the LacZ gene. A short double stranded oligonucleotide sequence with the same sticky ends (NdeI and HindIII) was ligated to the remaining pUC19 vector to re-circularize the plasmid. A white colony was selected for further vector amplification to ensure the absence of the LacZa gene in the vector backbone. The modified pUC19 vector was then re-cut with NdeI and HindIII (vector backbone). The 264bp LacZ region was PCR amplified and re-cut with NdeI and HindIII (insert).

2.Exposing pUC19 to UV-light or blue LED light

The vector and insert were run on a 1% and 2% gel, respectively, without any DNA stains. A small aliquot of the vector and insert was stained using Midori Green Direct, run next to the unstained vector and insert, and used to determine the cutting position of the bands on the gel. The vector and insert were exposed to UV and LED light (FG-06, Nippon Genetics Europe) for the specified time periods (0 to 120 seconds) prior to gel extraction, using a commercially available kit, ligated using a T4 ligase (Thermo scientific) and transformed into chemically competent DH5α (BL21) cells.

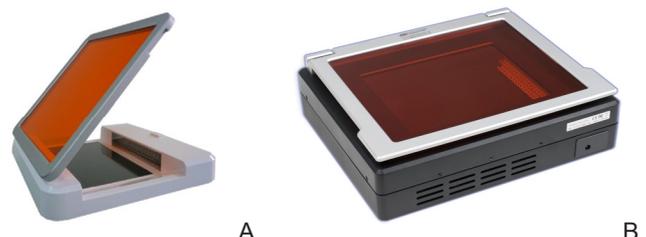


Figure 1 Fastgene® Blue Light LED: (A) Illuminator (FG-05) or (B) Transilluminator (FG-06)

Results

Overall, a clear difference between the cloning efficiency of DNA exposed to UV-light and to blue LED-light is visible after 30 seconds.

Exposing the insert or the vector to blue LED light for up to 120 seconds did not cause any detectable decrease in cloning efficiency (Figure 2.A, blue bar Figure 2.B, top row).

The number of colony forming units (CFU) on the selective growth medium stayed constant at $\sim 1.9 \times 10^3$ per plate.

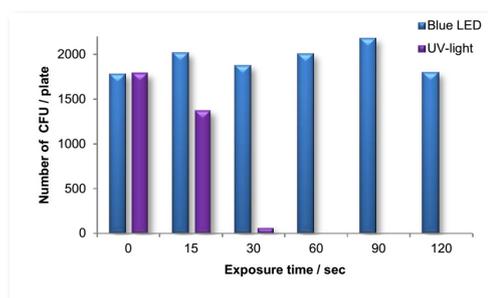
In contrast, exposing the DNA to UV-light for 15 seconds, started to inhibit cloning efficiency (Figure 2.A, violet bar). The number of colony forming unit (CFU) was considerably reduced after 30 seconds of UV-light exposure (Figure 2.A and B, bottom row, third plate). Impressively, no transformation could be detected after exposing the DNA to UV-light for 60 seconds or longer (Figure 2.B, bottom row, plates 4 to 6), suggesting severe DNA damages.

Conclusion

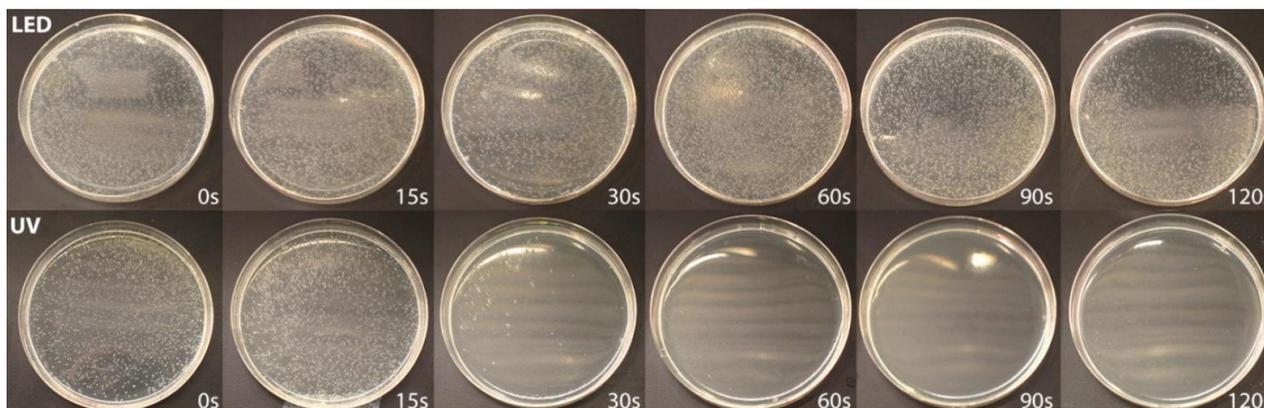
UV-light is a major DNA-damage agent. Here we demonstrate that this DNA damaging is considerably reducing the cloning efficiency. The transformation was completely abrogated after 1 minute exposure time.

Blue LED light is a safe alternative, which does not cause any efficiency reduction. The efficiency is maintained for up to 2 minutes without any decrease.

Blue LED is therefore the light source of choice for cloning and general nucleic acid detection.



A



B

Figure 2 Cloning efficiency of DNA treated with UV-light vs Blue LED. (A) Number of colonies forming unit (CFU) of successful transformations counted on each plate and each time point. (B) Selective LB-Agar plates showing results of the transformation of pUC19 vector exposed to the FastGene® Blue LED Transillumination (top row) or to UV-light (bottom row). The exposure time varied from unexposed (0s) to 120 seconds of exposure.

Blue LED nucleic acid detection



- Mupid™ LED Illuminator (MU4)
- FastGene® Blue LED Illuminator (FG-05)
- FastGene® Blue LED Transilluminator (FG-06)
- FastGene® Blue/Green Transilluminator (FG-08)
- FastGene® Gel Pic Imaging System (GP01-LED)
- FastGene® Gel Pic Imaging Box (GP03-LED)
- FastGene® FAS Digi (GP05-LED)

Additional Questions and Information

- Please contact us for additional information info@nippongenetics.eu
- Please contact us for support at support@nippongenetics.eu