NIPPON Genetics EUROPE

Influence of Ethidium Bromide and UV-light on the cloning efficiency - Improved cloning efficiency with Midori Green Direct and Blue LED light

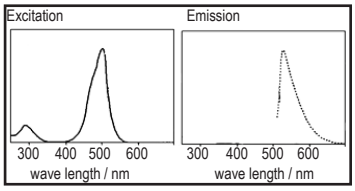
Cat. No. MG05 & MG06

Introduction

Ethidium Bromide is a carcinogenic and mutagenic agent. Nonetheless, it is still the standard nucleic acid staining dye used in many life science laboratories. Here, we present our investigation on the consequences of Ethidium Bromide on the cloning efficiency and demonstrate that cloning efficiency can be enhanced when a next generation dye, Midori Green Direct, is used.

Product Description

The Midori Green Direct is a non-intercalating nucleic acid stain which is directly applied to the sample, therefore considerably reducing background fluorescence. It can be used as a safe alternative to Ethidium Bromide, showing comparable sensitivity.



Midori Green Direct is compatible with a wide variety of gel reading instruments. It has a fluorescent excitation peaks in the UV-length spectrum (~290 nm) found on traditional UV tables as well as a strong excitation peak is found at ~490 nm, thus enabling an UV-independent and less destructive detection of nucleic acids. The emission peak is centered at ~530 nm.

Safety

Midori Green Direct DNA stain is non-carcinogenic. According to the Ames test, significantly fewer mutations were detected when using Midori Green Direct compared to Ethidium Bromide. Additionally, Midori Green Direct is unable to penetrate the cell wall of living cells as well as latex gloves. It is non-toxic to aqueous life and being classified as non-hazardous waste according to the CCR Title 22 Hazardous Waste Characterization. It can be disposed in a filtered sink or in a regular laboratory waste, saving special waste costs.

Quick Notes

- Increase in cloning efficiency
- Non-carcinogenic and significantly less mutagenic
- No DNA intercalation
- Comparable fluorescence to Ethidium Bromide

Methods

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, before being reinserted into the vector.

Influence of Ethidium Bromide vs. Midori Green Direct staining on cloning efficiency

The vector and insert were were separated on a 1% gel for the vector or on a 2 % gel for the insert, containing Ethidium Bromide or directly mixed with Midori Green Direct. The bands containing the vector or the insert were purified using a commercially available kit, ligated using a T4 ligase (Thermo Scientific, USA) and transformed into DH5α (BL21) competent cells using a heat shock procedure.

Exposing stained DNA to UV-light or visible Blue LED light

The vector and insert were exposed to UV and LED light for different period of time(15 to 120 seconds) prior to gel extraction to analyse the influence of the combination of DNA stain and the recommended light on the cloning efficiency. The DNA samples (vector and insert) were purified using a commercially available kit, ligated as described previously and transformed into chemically competent DH5α (BL21) cells.

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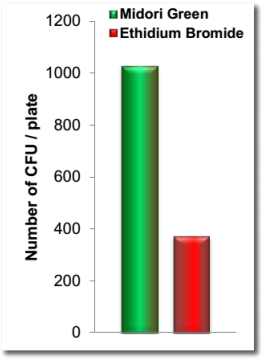
Results

The cloning efficiency was determined by counting the number of colony forming units (CFU) on a selective media plate. The number of CFU/plate of DH5α (BL21) transformed with DNA stained with Midori Green Direct is more than twice as high when compared to the number of CFU of DH5α (BL21) transformed with DNA stained with Ethidium Bromide (Figure 2.A).

The influence of the stained DNA detection light was further investigated. The detection of nucleic acid stained with Ethidium Bromide is normally performed using UV-Light. Previously, we showed that UV-light was a strong inhibitor of cloning efficiency. Again, the CFU/plate was considerably reduced when the DNA used in the transformation was stained with Ethidium Bromide and exposed to UV-light. Only few colonies were visible after 30 seconds of exposure. (Figure 2.B, 30 seconds) and no colonies were detected thereafter.



A

Using Midori Green Direct showed a successful transformation resulting in many colonies on the selective medium plates. No visible difference could be detected in the first 60 seconds of exposure. In contrast to the UV-light exposed DNA, even after 120 seconds of exposure several colonies are visible.

Conclusion

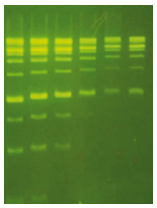
The standard nucleic acid stain Ethidium Bromide is a strong cloning inhibitor. The results suggest that ligation and insertion of DNA intercalated with Ethidium Bromide into a host, is difficult and resulting in a less effective transformation.

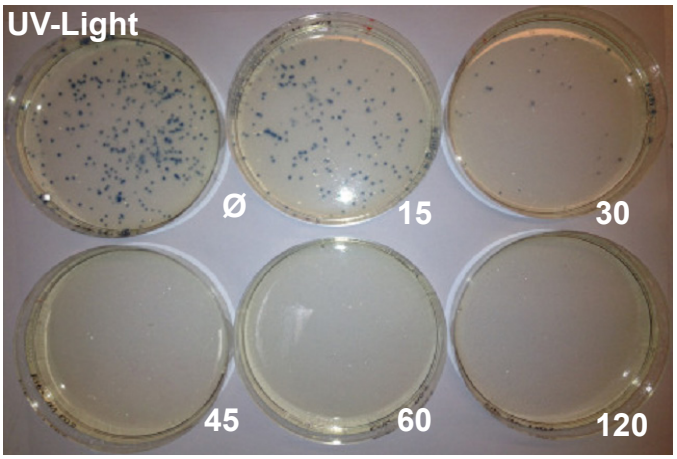
The new generation of nucleic acid stains is a safe alternative for the user and the cloning product. In combination with the blue LED detection technology it enables a less mutagenic and destructive detection of nucleic acids improving the cloning efficiency. Hence,

Midori Green Direct is the dye of choice for precise and efficient cloning.

Midori Green Direct (MG-05 & MG-06)

The next generation nucleic acid dye





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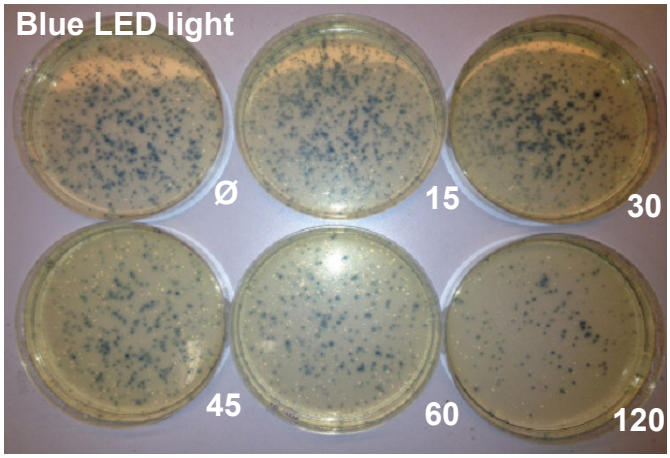

C

Figure 2 Cloning efficiency of DNA stained with Ethidium Bromide vs. Midori Green Direct. (A) Number of colonies forming unit (CFU) of succesful transformations counted on each plate for DNA stained with Midori Green Direct (green bar) or with Ethidium Bromide (red bar). **(B)** Selective LB-Agar plates showing results of the transformation of DNA stained with Ethidium Bromide and exposed to UV-light. The numbers next to the plates indicate the exposure period in seconds. Ø indicates no light exposure. **(C)** Selective LB-Agar plates showing results of the transformation of DNA stained with Midori Green Direct and exposed to blue LED-light. The numbers next to the plates indicate the exposure period in seconds. Ø indicates no light exposure.

Next generation nucleic acid dyes

Cat.No.	Product	Vol.
MG-05	Midori Green Direct[Sample]	50 µl
MG-06	Midori Green Direct	1 ml
MG-07	Midori Green Direct (w/o loading dye)[Sample]	50 µl
MG-08	Midori Green Direct (w/o loading dye)	1 ml
MG-03	Midori Green Advance[Sample]	50 µl
MG-04	Midori Green Advance	1 ml

Additional Questions and Information

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

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