



**SMOBIO**  
Small Bio, Smart Tool

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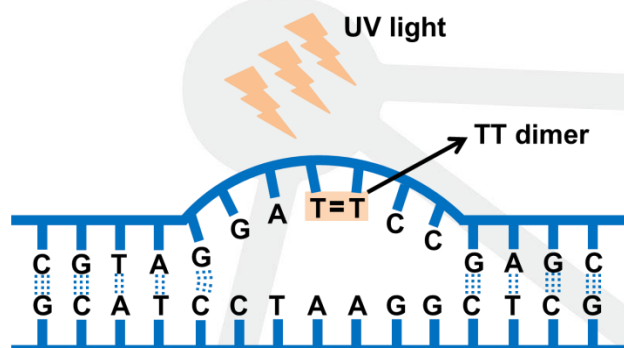
## 3 Things to Enlighten Your Cloning Life

By: *SMOBIO Technology*

### 1. Blue Light vs. UV Light

Safer substitutes for EtBr has been in the market for several years. However, EtBr as fluorescent dyes and ultraviolet (UV) light as excitation source have still been the staple method in most molecular biology labs. Hence, several safe dyes continue to use UV light to discount the need of purchasing another blue light device. The author may suggest the concurrent usage of both UV light and EtBr but to solely use blue light has the following advantages:

- High energy UV light causes the double thymine (T) of the DNA to be linked to each other and produce thymine-thymine dimer (TT-dimer) with a direct effect of changing the DNA structure as shown in Figure 1. Therefore, errors will occur during DNA replication that may lead to mutation.



**Fig. 1. UV light and its effects on DNA structure.**  
High energy UV produces TT-dimers in the DNA.

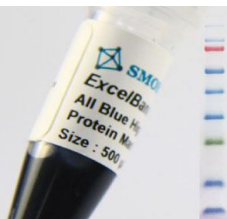
- UV light causes changes in the DNA structure and greatly affects the subsequent gene construction or even the success rate of cloning.
- Exposure to UV light entails unavoidable health hazards to the user, thus we would like to recommend the [B-BOX™ Blue Light LED epi-illuminator](#) as an alternative. The device can detect DNA as little as 0.1 ng used in conjunction with [FluoroStain™ DNA Fluorescent Staining Dye \(DS1000\)](#) or [FluoroDye™ DNA Fluorescent Loading Dye \(DL5000\)](#), significantly reducing the adverse effects of UV to both the sample and the user to produce a safe environment for your laboratory.



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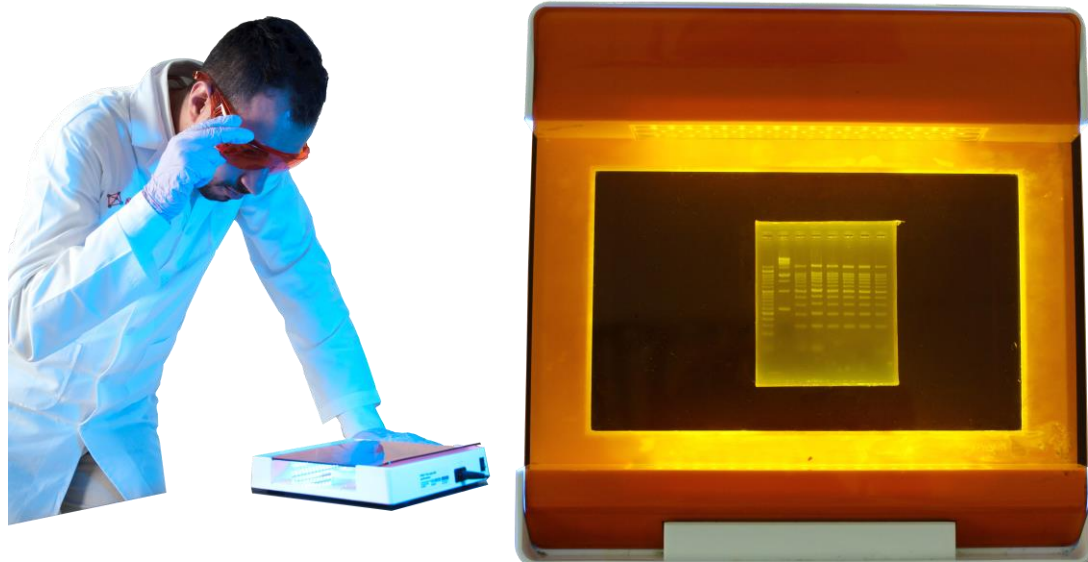


Fig. 2. B-BOX™ Blue Light LED epi-illuminator.

## 2. Safe Fluorescent Dyes Excited by Blue Light

SMOBIO produces several types of safe fluorescent dyes and here are the top 3 products for detecting nucleic acids in the agarose gel through different ways:

- **In-Gel Staining.** [NS1000 FluoroVue™ Nucleic Acid Gel Stain](#) is specially designed for *in-gel* use. It is a fluorescent stain which offers high sensitivity detection of double-stranded or single-stranded DNA and RNA in a convenient manner.
- **Post-Staining.** [DS1000 FluoroStain™ DNA Fluorescent Staining Dye](#) offers at least 10 times sensitivity in DNA detection levels, and is capable of detecting double stranded DNA (dsDNA) fragments up to 0.04 ng in electrophoresis analysis.
- **Pre-Staining.** [DL5000 FluoroDye™ DNA Fluorescent Loading Dye\(Green, 6X\)](#) contains sensitive fluorescent dye with high specific affinity towards double stranded DNA (dsDNA), being used by directly mixed DNA samples for direct loading into the gel. It shows negligible background and renders destaining process unnecessary.



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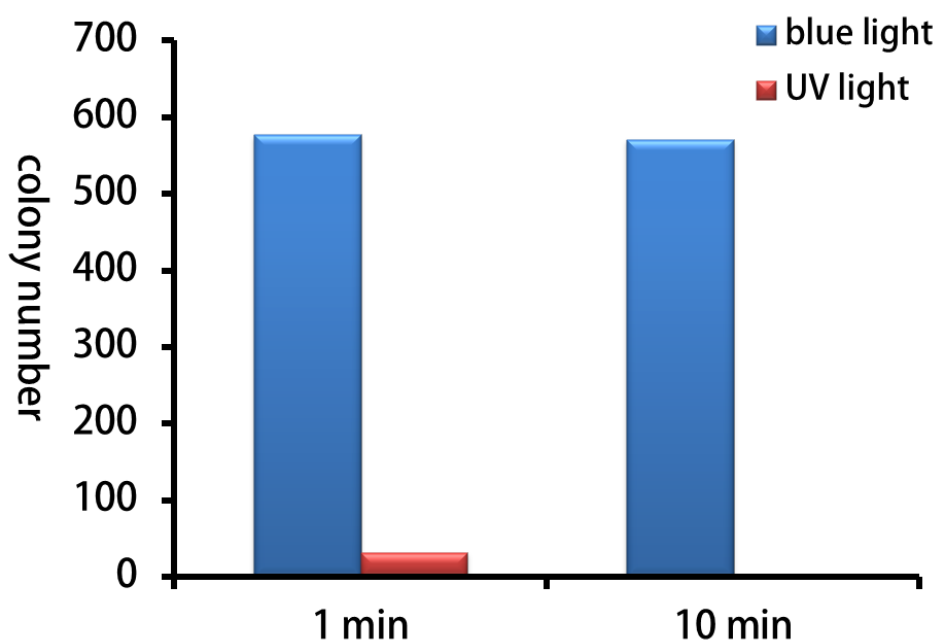


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The conventional method of using EtBr and UV light for detection causes negative interference in both DNA quality and transformation. To provide a more vivid comparison on the damaging effects of UV light on DNA, a simple experiment was conducted wherein a one-minute exposure of PCR products to UV light significantly decreased the colony number after ligation and transformation; no colony was even observed in a 10 min UV exposure as shown in Figure 3. Therefore, the use of blue light photodetector can help in significantly increasing the cloning success rate. According to our experience, ~100 folds increase of cloning efficiency can be achieved if the PCR product is isolated from the gel in blue-light environment instead of UV.



**Fig. 3. Comparison of cloning efficiency for PCR fragment in exposure to UV and blue light**  
The cloning efficiency after exposure to UV light and blue light presents the significant difference in colony numbers after 1 minute and 10 minutes of exposures to different light sources.



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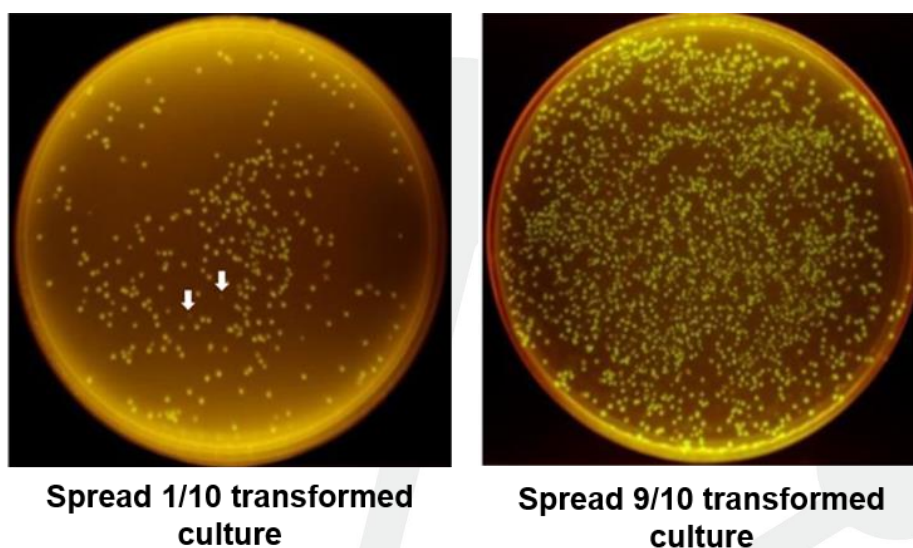
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### 3. Cloning Vectors with Low Background and High Efficiency

In recent years, there has been a steady increase on the use of PCR processes with proof reading enzyme (ex: [TF1000 ExcelTaq™ SMO-HiFi polymerase](#)) that produces blunt end PCR amplicons without the adenine overhangs at their 3' ends required for traditional cloning. [The GetClone™ PCR Cloning Vector \(CV1000, CV1100\)](#) is a low background cloning vector adopting a positive selection mechanism, designed to accept blunt end DNA and amplicons with high efficiency and convenience. It contains a lethal gene which can be disrupted after insertion of a blunt end DNA fragment at the cloning site. Therefore, only colonies with inserted vectors are able to propagate, eliminating the need for IPTG and X-Gal for blue/white screening. Figure 4 exhibits the cloning efficiency of CV1000 to be greater than 90%. Moreover, GetClone™ vector accepts a wide range of insert/vector ratios from 0.5:1 to 12:1. It also accepts insert sizes from 6 bp to 12 kb, which is vastly better than other cloning vectors on the market. The phosphorylation of insert fragments is not required.



**Fig. 4. High number of GFP transformants on the agar plate by using GetClone™ vector for cloning** GFP gene was PCR-cloned by use of GetClone™ vector. More than 90% of the colonies were illuminated under blue light, presenting a highly successful and low background cloning of GFP gene. Only a few colonies (white arrow) were in absence of green fluorescent signal under B-BOX blue light excitation.



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