Illumatool™ Tunable Light System: A Non-Destructive Light Source For Molecular And Cellular Biology Applications. John Fox, Lightools Research.

Fluorescent dyes and proteins are basic analytical tools used in many molecular and cellular biology applications. Fluorescent dyes such as ethidium bromide, SYBR Green I stain, SYPRO Orange I stain and Nile Red stain are used to detect nanogram amounts of DNA, RNA and protein separated by gel electrophoresis techniques. Auto-fluorescent proteins such as green fluorescent protein (GFP) and its blue and yellow variants have been used as reporter molecules for protein localization, gene expression, insertional cloning, and other applications. UV transilluminators commonly used to excite these molecules emit ultraviolet light in a broad band (approximately 150 nm wide) centered around 300 nm. The fluorescent yield of these dyes, in complex with their respective binding partners, is considerable when exposed to ultraviolet radiation. However, most of the fluorescent dyes and proteins in common use today have a bimodal excitation spectrum, with one excitation peak in the UV and another in the visible range. For example, wild-type GFP has excitation peaks at 395 and 475 nm (1), whereas SYBR Green I stain has excitation peaks at 296 and 494 nm (2).

The Illumatool Tunable Light System is a new illumination device for the optimal excitation of many of the fluorescent dyes and proteins used in molecular and cellular biology. The Illumatool TLS used in this study was equipped with an excitation bandpass filter at 480 nm (20 nm bandwidth), which coincides with the second excitation peak of many fluorescent dyes and proteins. Thus, the Illumatool Tunable Light System can be substituted for many of the same purposes as UV, with several important experimental advantages. First, ultraviolet radiation is a mutagen, carcinogen and an eye damaging agent requiring special safety precautions for the operator. Second, UV radiation rapidly damages molecular and cellular reagents. Indeed, UV lamps are commonly utilized as germicidal devices and to damage contaminating nucleic acid on plastic ware, reagents and pipetters used for PCR amplification. Purification of DNA from ethidium bromide stained gels exposed to UV for minimal lengths of time can result in a 2-3 order of magnitude reduction in transformation, transcription, and amplification efficiency (3).

The following results highlight some of the possible cell and molecular biology applications facilitated by Illumatool TLS. Fig. 1 shows a flask containing an overnight culture of ***E. coli*** expressing GFP. The culture has been illuminated with an Illumatool Tunable Light System and the green fluorescence is detected with the CCD camera based Speedlight Platinum GDS (Lightools Research) electronic documentation system equipped with a 600 nm bandpass filter. Plant and animal research using GFP to localize and quantify expression results can benefit from the macro-imaging capabilities of this system. For example, baculovirus containing green fluorescent fusion proteins can be used to assess gene expression noninvasively in infected caterpillar larvae, simply by monitoring the fluorescent intensity of the resulting green glow (4). Green fluorescent mice containing GFP integrated into their genome are used to study development (5). Biotechnology process chemistry, such as

GFP fusion protein purification can also benefit from this “real-time”, non-invasive, non-destructive detection capability (6).

Many cloning systems utilize insertional inactivation of a reporter gene, typically the ***lacZ'*** gene, to distinguish colonies containing recombinant inserts from parental, or empty vector. The GFP gene can also be used as a reporter system for cloning, as seen in Fig. 2. A GFP containing plasmid was linearized at a unique restriction site within the gene, ligated to similarly treated DNA fragments, transformed into ***E. coli*** and plated on antibiotic selection media. If a foreign DNA fragment disrupts the GFP gene the resulting colonies should not fluoresce. Empty parental plasmid results in a fluorescent colony that glows bright green when illuminated by the Illumatool Tunable Light System. A variety of cloning strategies can be implemented using GFP as the reporter molecule, such as promoter and terminator traps (7). Similar results are seen using eukaryotic cells transfected with a GFP reporter gene (8).

GFP expressing cells can also be used in a microtiter plate format for high-throughput screening of compounds against various organisms, such as ***Mycobacterium tuberculosis*** (9). Fig. 3 illustrates such an application, where ***E. coli*** containing a GFP fusion protein was grown in various media supplements. Aliquots from the treatments were dispensed into a black-walled microplate for analysis of gene expression. This assay was designed to show which incubation medium was optimal for production of the fusion protein.

Illumatool Tunable Light System can also be used to detect fluorescent dyes, such as SYBR Green I stain and SYPRO Orange I stain. Fig. 4 is a comparison of the same SYBR Green I stain (left side) or ethidium bromide (right side) stained DNA agarose gels illuminated with UV (Fig.4A) or Illumatool TLS (Fig.4B). The detection sensitivity is similar between the two light sources. However, a primary advantage is that DNA purified using Illumatool Tunable Light System illumination shows none of the ultraviolet radiation damage which impairs transformation, amplification and transcription by 2-3 orders of magnitude. Fig. 5 compares the cloning efficiency of pUC19 exposed to UV radiation or the Illumatool Tunable Light System for the indicated time periods. Exposure of pUC19 for as long as 8 minutes to 480 nm light has no apparent effect on the transformation efficiency. In contrast, exposure to 30, 60, and 120 seconds of 302 nm UV reduces the transformation efficiency 33 fold, 500 fold, and 41,000 fold respectively. These results show that a significant improvement in cloning efficiency can be realized using the Illumatool Tunable Light System, instead of a conventional UV transilluminator, for the gel purification of DNA fragments

The Illumatool Tunable Light System, in conjunction with the Speedlight Platinum GDS documentation and analysis system, can extend the range of lab applications to include the non-destructive detection and purification of DNA and the non-invasive assay of gene expression. The Illumatool Tunable Light System is a tunable system which can deliver a large range of light at specific wavelengths. This tunability will permit a much larger range of fluorescent applications, compared to the UV transilluminator based systems.

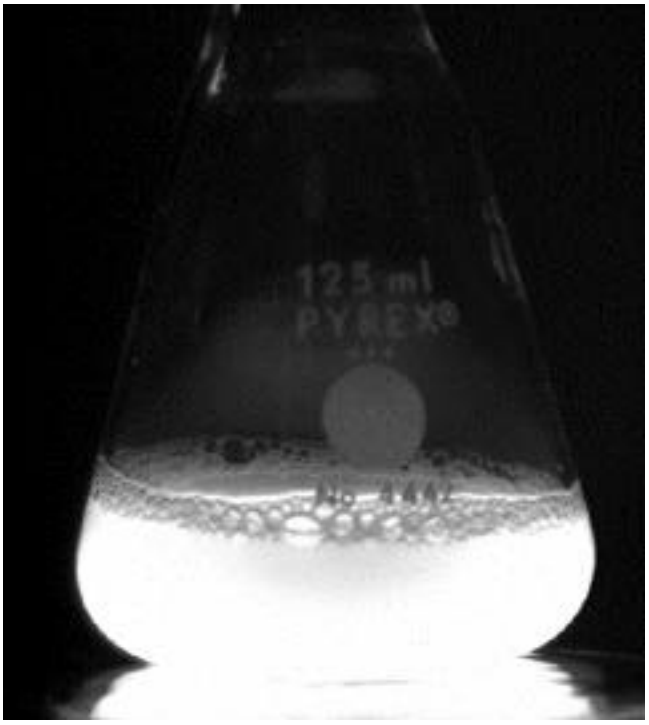

Figure1. Illumatool Tunable Light System illumination of an overnight culture of ***E. coli*** containing green fluorescent protein.

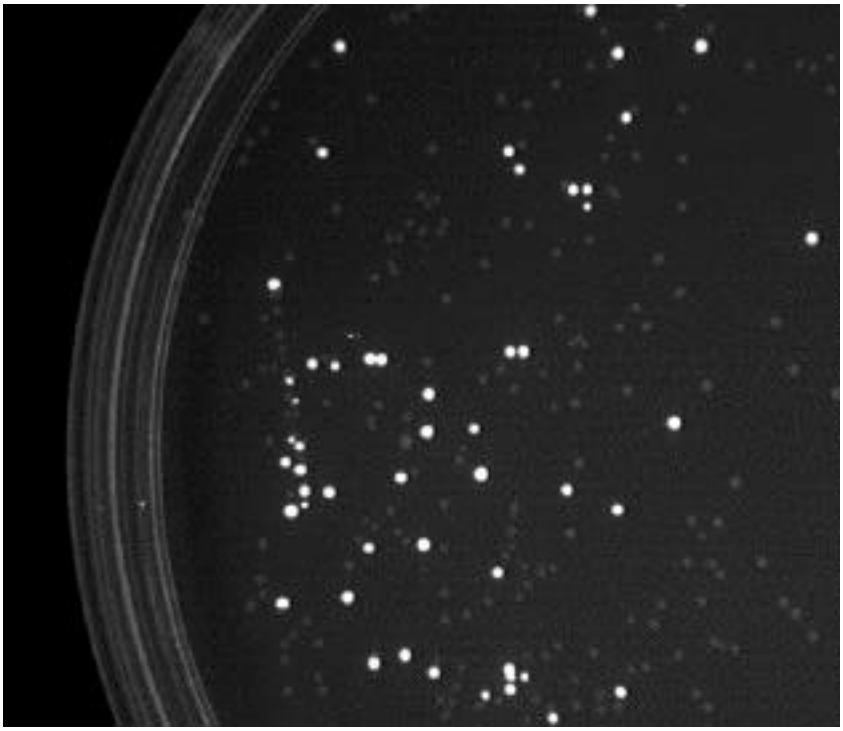

Figure 2. Insertional inactivation cloning assay using a GFP plasmid and Illumatool TLS detection. ***E. coli*** colonies containing recombinant inserts are dark, the parental GFP vector results in a green glowing colony phenotype.

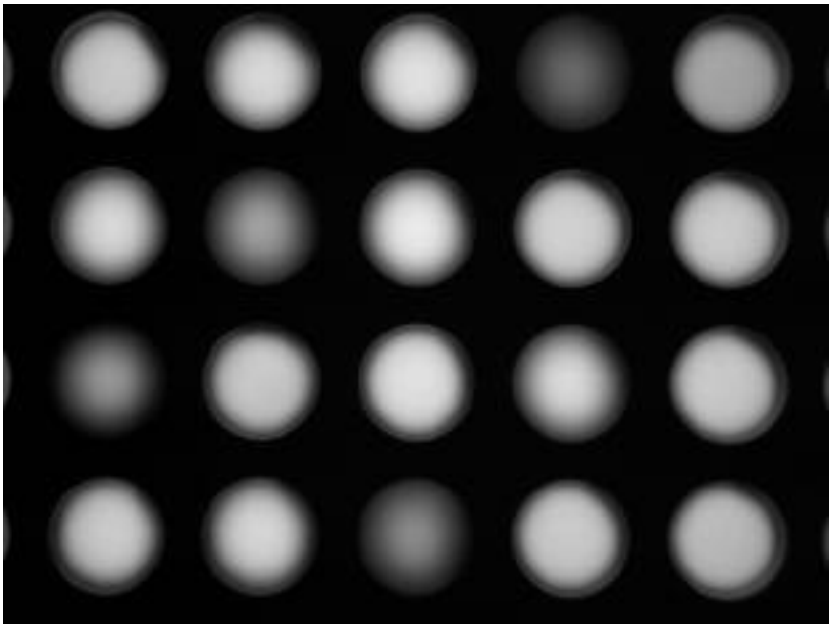

Figure 3. Microtiter plate assay of various growth media to optimize production of ***E. coli*** using a GFP fusion protein as the biosensor.

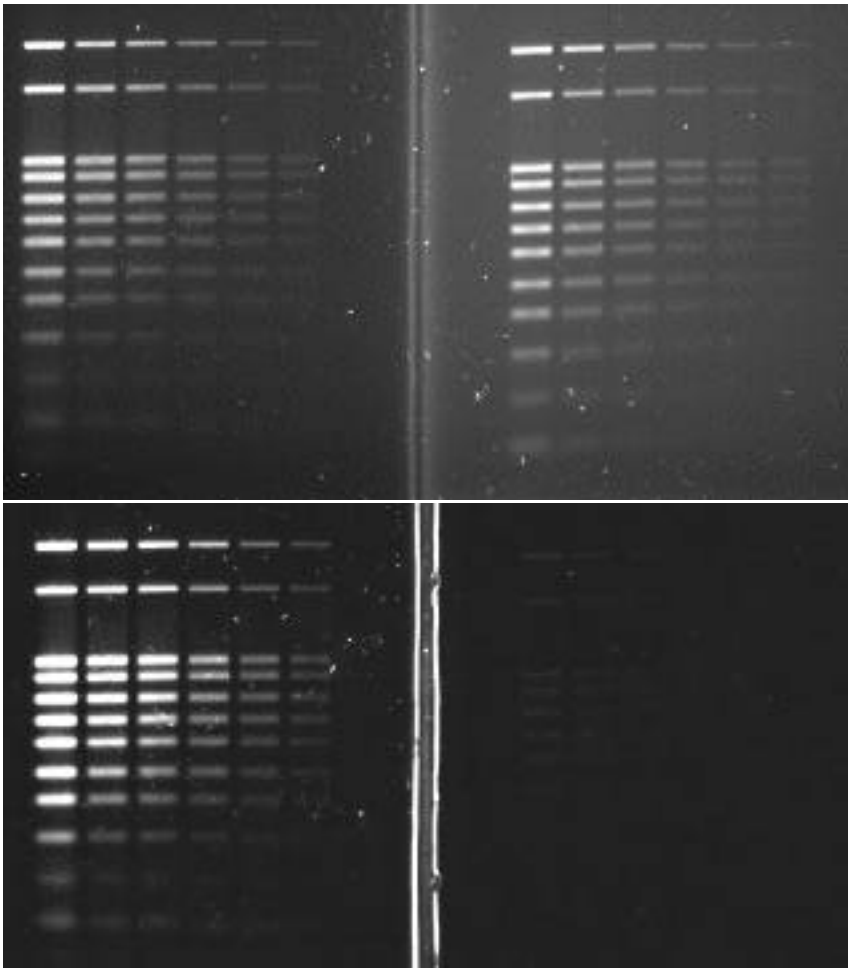

Figure 4. SYBR Green I stain (left side) or ethidium bromide (right side) stained DNA agarose gels illuminated using UV (upper) or Illumatool Tunable Light System (lower) light sources.

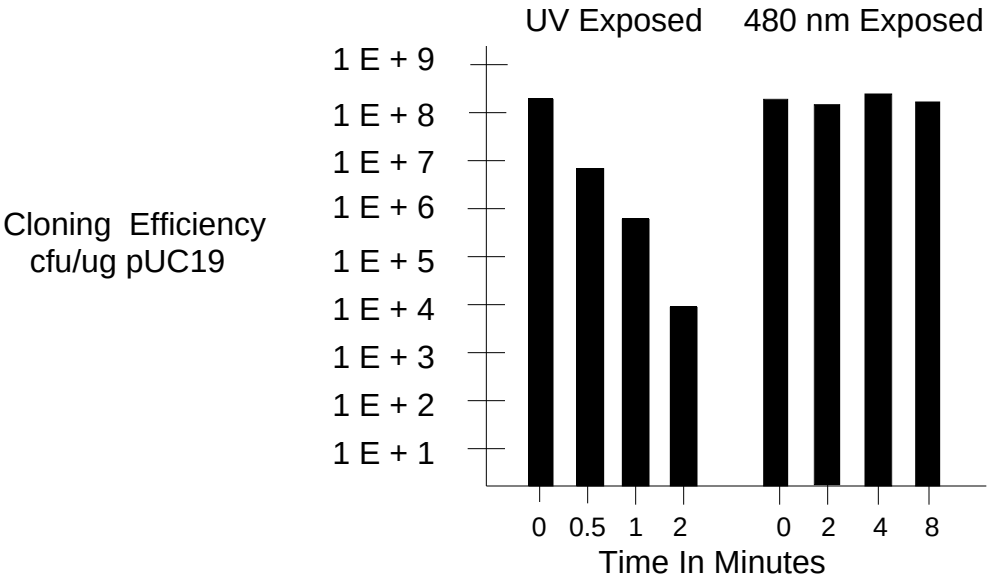

Figure 5. Cloning efficiency of pUC19 plasmid DNA purified from agarose gels illuminated with UV (hatched bars) or 480 nm light from the Illumatool TLS (solid bars) for the indicated times.

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