# Cutting back on UV damage

*Using a novel transilluminator, researchers maintain the cloning efficiency of DNA.*

BY HENRY A. DAUM, III AND MARK SEVILLE

Genomic libraries are key resources that, among other things, can be screened for genes of interest as potential drug targets. A high quality library should contain the greatest possible number of unique DNA fragments, providing a complete and unbiased representation of the entire genome of an organic being. An important factor in achieving this goal is maintaining the integrity of the DNA fragments during the several steps involved in library construction.

## Lightly toasted

One such step is the purification of the DNA fragments by gel electrophoresis. Detection of the DNA fragments in the gel typically involves staining with either ethidium bromide or one of the new generation of dyes, such as SYBR Gold stain or GelStar stain, and then using an ultraviolet (UV) transilluminator to visualize the fluorescent DNA bands.

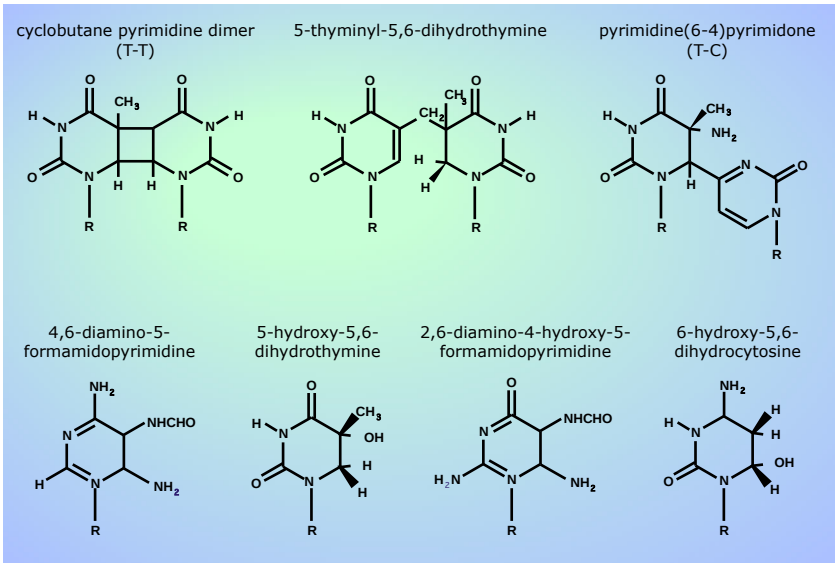


Figure 1. Products formed in UV-damaged DNA

Unfortunately, numerous lesions are induced in DNA by exposure to relatively low doses of ultraviolet light. These include pyrimidine cyclobutane dimers and non-cyclic dimers such as pyrimidine (6-4) pyrimidone adducts and 5-thyminyl-5,6-dihydrothymine (Figure 1). Additional reaction products include 6-hydroxy pyrimidine hydrates that are generated by addition of a water molecule across the C5-C6 double bond, and other monomeric derivatives such as 4,6-diamino-5-formamidopyrimidine, 2,6-diamino-4-hydroxy-5-formamidopyrimidine and 5-hydroxy-5,6-dihydothymine. Photochemical reactions of purine bases are less common and less well characterized though 8,8-adenine dehydrodimers have been identified in poly(dA) exposed to UV light. Yet other types of lesions induced by UV-irradiation include breaks in the sugar-phosphate backbone and cross-links between DNA strands.

Though many of the DNA lesions that result from exposure to UV light have been shown to be mutagenic, it has often been assumed that any damage caused by visualizing DNA on a UV transilluminator is of minor consequence to subsequent DNA manipulations. However, a number of reports now make it clear that exposure of DNA samples to UV radiation can, in fact, have a significant detrimental impact on downstream protocols involving cloning and biological activity. Grundemann & Schomig showed that plasmid DNA and cDNA samples separated by agarose gel electrophoresis, stained with ethidium bromide and subsequently cut from the gel using 300 nm UV illumination, are 100-1000 times less effective as substrates for transcription, transformation and PCR reactions compared with DNA samples prepared 'blind' (1). UV exposure of DNA templates also results in reduced fidelity and incorrect replication during PCR amplification (2). The extent of UV-induced damage is dependent on the size of the DNA (3), but even even small dsDNA fragments are damaged by brief exposure to 300 nm light (4). Also, the integrity of proteins encoded by DNA that has been exposed to UV light can be severely compromised (5).

## Blueprint for success

Until recently there has been no simple and practical alternative to the use of UV light to locate DNA fragments in gels. To get around this problem, scientists at Clare Chemical Research (Denver, CO) decided to go back to first

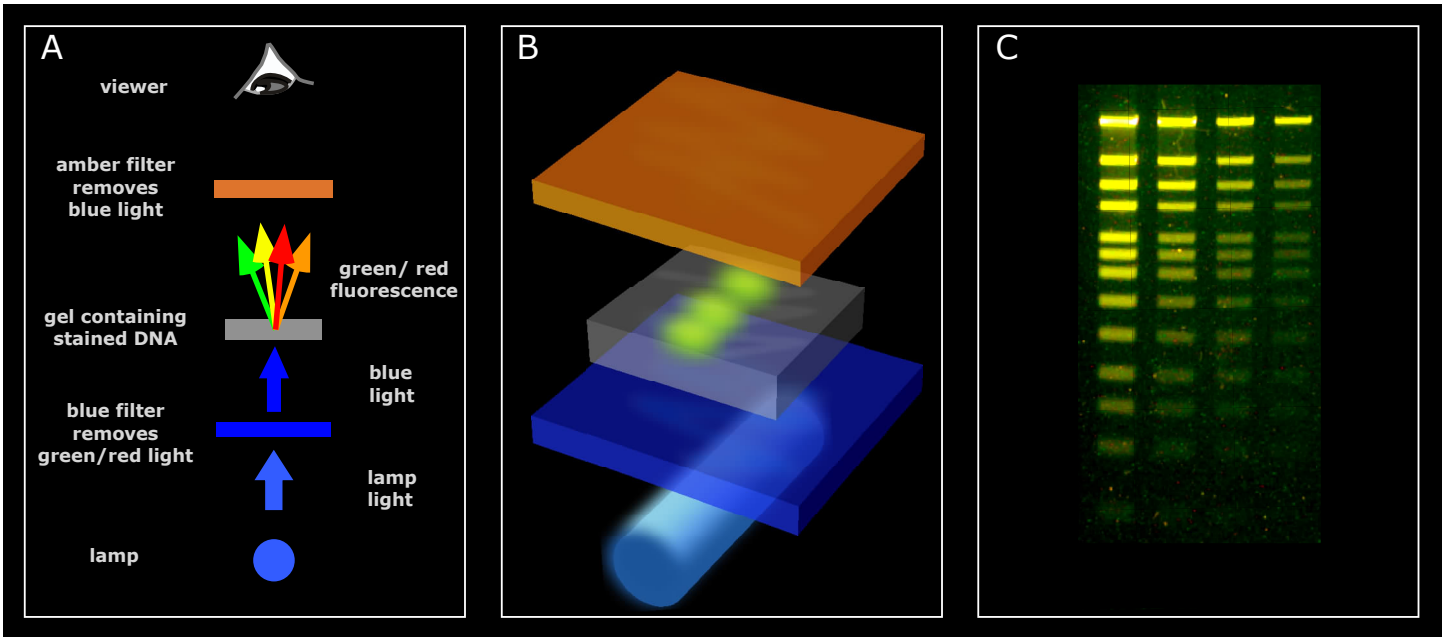


**Figure 2. The Dark Reader optical system.** (A, B) Optical components and relevant light paths of the Dark Reader design. (C) DNA samples separated by agarose gel electrophoresis, stained with SYBR Green and viewed on a Dark Reader transilluminator. The image was recorded with a CCD camera.

principles. If UV light was responsible for the damage but was necessary to see the stained DNA in gels, was there a way to see the DNA, directly by eye, using other wavelengths of light? What they developed was an innovative optical system that used visible blue light as an excitation source in combination with 2 optical filters that allowed the direct viewing of the fluorescent patterns of stained DNA bands, as well as many other widely used fluorophors.

The whole idea of using visible light for excitation seemed impractical: any faint fluorescence emission would be almost completely swamped by the much brighter excitation light. Scientists at Clare overcame this problem by using a combination of blue and amber filters. The first blue filter removed the traces of green and red wavelengths from the lamp resulting in a 'true blue' excitation. The second amber filter was placed between the sample and the viewer and blocked the blue excitation light. The only wavelengths that passed through the amber filter to the viewer were the green and red fluorescent emissions of the sample (Figure 2).

To be useful for viewing fluorescence from large sample areas such as electrophoresis gels, the filters needed to be correspondingly large. Typically, optical filters are not only tiny but also very expensive. Clare Chemical scientists overcame this limitation by using acrylic sheets charged with various pigments chosen to absorb the appropriate wavelengths of light.

The initial tests were striking: bright sample fluorescence viewed in an almost black background environment. Every researcher who has used a UV table is well aware that powerful UV light floods the entire room, making for some spectacularly fluorescent white shirts, and even teeth! Because of the almost complete absence of extraneous light from the Clare Chemical device, the researchers dubbed their system Dark Reader (DR).

Further optimization and testing revealed the DR system to be both remarkably efficient and sensitive. Using a DR transilluminator to view DNA bands separated by gel electrophoresis it was possible to see, by eye, less than 100 pg of DNA stained with, for example, SYBR Gold and around 10 pg of stained DNA could be imaged using a CCD camera (6).

The remarkable sensitivity of fluorescence detection achieved with the Dark Reader system is partly a consequence of the cut-off/cut-on characteristics of the blue and amber filters. This 'wide-open' optical design is highly efficient and maximizes the available excitation and emission energies across a broad wavelength range. This design has the added advantage that it allows a broad range of fluorophors to be viewed, either alone or simultaneously.

Given the absence of UV radiation from the Dark Reader transilluminator, the extent of photodamage to DNA samples should be significantly reduced compared to that produced by the use of a UV device, resulting in enhanced cloning efficiencies and biological activities.

## Proof of concept

A typical method of genomic library construction involves the use of cosmids which are genetic entities that can contain a relatively large piece of foreign DNA (about 40 kb in length), flanked by

sequences that allow the DNA to be packaged into the heads of the lambda bacterial virus.

We determined the extent to which DNA integrity is compromised by exposure to either UV light or the Dark Reader's visible light source using wheat germ chromosomal and T7 viral DNA as test samples for cosmid library construction. To construct the libraries, the DNA samples were purified by agarose gel electrophoresis and stained with SYBR Gold (Molecular Probes). The fluorescent DNA bands were cut from the gel on either a UV or a DR transilluminator. The purified DNA was then ligated to the pWEB Cosmid Vector (EPICENTRE Technologies) and the ligated DNA was packaged into lambda particles using the MaxPlax Lambda Packaging Extracts (EPICENTRE Technologies). *E. coli* plating cells were then transfected, plated, and incubated overnight. Either plaques (for T7 DNA), or colonies (for wheat germ DNA) were counted.

Exposure of the wheat germ DNA to UV light had a large impact on the cloning efficiency. The plating efficiency of the UV-exposed DNA was 2 x $10^4$ colony forming units per microgram (cfu/µg) DNA. In contrast, the plating efficiency of the DR-exposed DNA was 85-fold higher, with 1.7 x $10^6$ cfu/µg DNA. These results (Figure 3) demonstrate that UV-exposed DNA is significantly compromised in its ability to function well in such applications as the development of genomic libraries. For library construction an 85-fold reduction in cloning efficiency can be very significant, particularly for larger genomes. If biologically intact, non-UV irradiated DNA is used for ligation, one is more likely to obtain a sufficient number of clones in the primary library, which normally is sufficient to find a single copy gene. The use of the DR transilluminator to view DNA bands and excise them from gels obviates the need to amplify the library in order to allow screening of more clones, a process which often leads to the loss and under-representation of some genomic segments from the library.

Viral infection with T7 phage requires that the cloned T7 DNA in each cosmid express the gene products, in active form, necessary for T7 phage production. DNA recovered from a gel visualized on a DR transilluminator produced a 220-fold greater number of plaques (2.2 x $10^6$ pfu/µg DNA) than DNA recovered from a gel exposed to light from a 302 nm UV transilluminator (1 x $10^4$ pfu/µg DNA). These results are even more dramatic than those obtained with the wheat germ DNA. This clearly indicates that, in addition to affecting ligation and transformation efficiencies, the 30-second UV exposure compromised the activity and function of one or more of the gene products necessary for T7 phage production.

The hugely improved transformation efficiencies achieved using DNA samples viewed using the Dark Reader transilluminator rather than the UV unit suggest that this approach should also be employed in other protocols where maintaining DNA is a major factor in the overall success of the experiments including (a) maintaining the function of genes for use in expression vectors, (b) increasing confidence in the accuracy of DNA sequencing, (c) ensuring the integrity of a DNA template for PCR or in vitro transcription, and (d) optimal cosmid and bacterial artificial chromosome (BAC) library construction.

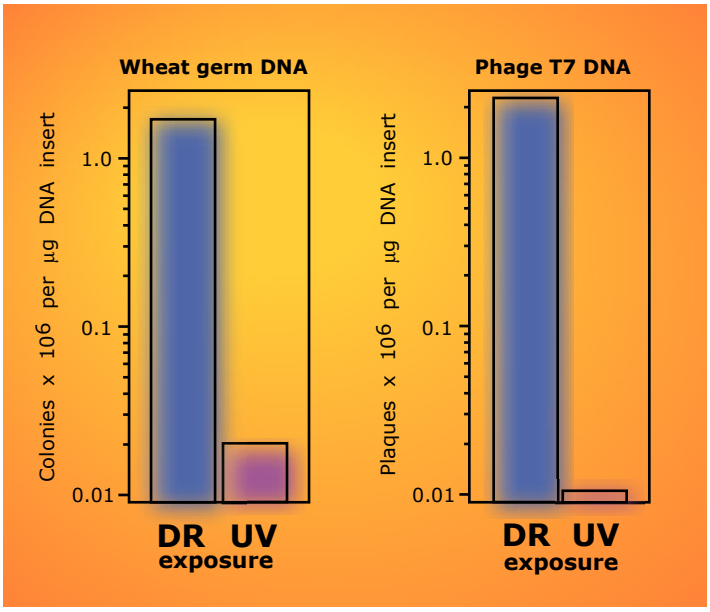


**Figure 3. Cloning results obtained using a DR or UV light source.** DNA was purified by agarose gel electrophoresis, stained with SYBR Gold and then viewed on either a Dark Reader (DR) or ultra-violet (UV) transilluminator to allow excision of the 40 kb fragments from the gel. The DNA fragments were then used to transform cells.


## Acknowledgments

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**Henry A. Daum III** is a technical consultant at EPICENTRE Technologies Corporation (Madison, WI). **Mark Seville** is a senior scientist at Clare Chemical Research (Denver).