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Drug Discovery

Tutorial: Dark Reader Transilluminator

Maintains Biological Activity and Cloning Efficiency when Viewing DNA in Gels

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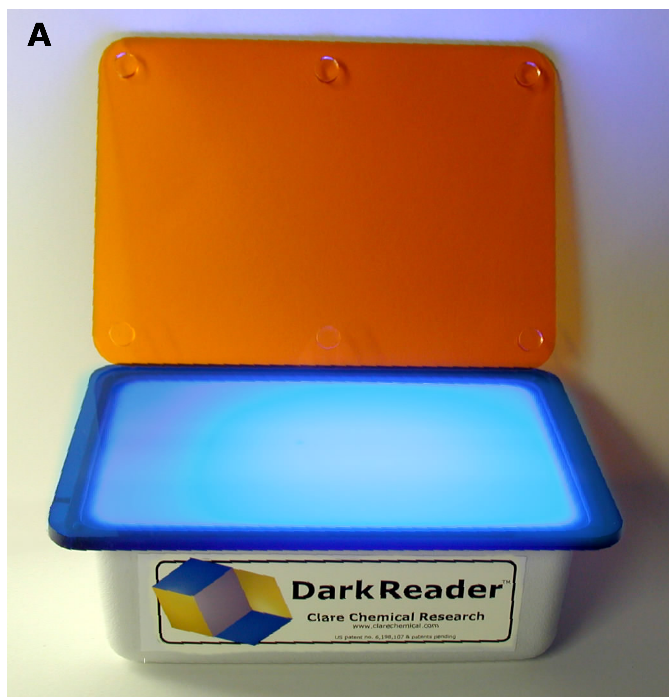
Detection of nucleic acid fragments after separation by gel electrophoresis typically involves staining the DNA with either ethidium bromide (1) or one of the new generation of dyes, such as SYBR® Gold stain[2] or GelStar® stain[3], and then using an ultraviolet (UV) transilluminator to visualize the fluorescent DNA bands.



It is often assumed that UV-induced damage is of minor consequence to subsequent DNA manipulation when visualizing the DNA on a transilluminator. However, a number of reports[4-10] show that exposure of DNA samples to UV radiation can, in fact, have a significant detrimental impact on downstream protocols involving cloning or biological activity.

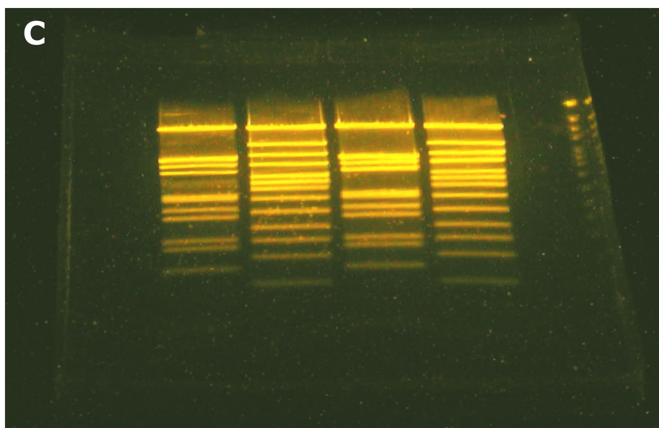
Transformation, transcription and polymerase chain reaction (PCR) efficiencies are all reduced by 2 - 3 orders of magnitude when DNA samples are exposed to UV light - even for the brief period of time that it takes to excise a band from a gel[8]. Also, the use of UV-exposed DNA templates in PCR reactions can promote polymerase jumping that results in reduced fidelity and incorrect DNA replication[9].

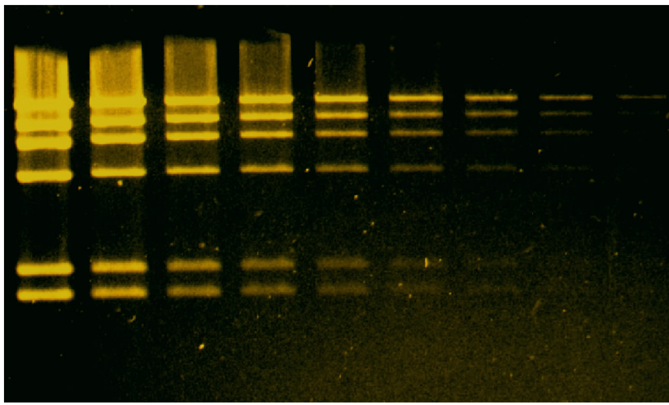
Not only the efficiencies and fidelities of many DNA-dependent reactions can be adversely affected by UV-exposure, but also the biological

The Dark Reader Transilluminator. Scientists at Clare Chemical Research have developed an innovative optical system that uses 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 first blue filter removes the traces of green and red wavelengths from the lamp resulting in a 'true blue' excitation (A). The second amber filter is placed between the sample and the viewer and blocks the blue excitation light. The only wavelengths that passed through the amber filter to the viewer are the green and red fluorescent emissions of the sample (B).

Using the Dark Reader, bright sample fluorescence is viewed in an almost black background environment as shown in the enlarged image (C) of the SYBR Gold-stained DNA agarose gel on the transilluminator surface.





The DR optical system is remarkably efficient and sensitive. Using a DR transilluminator to view DNA bands separated by gel electrophoresis it is possible to see, by eye, around 100 pg of DNA stained with SYBR Gold. In a Polaroid photograph the detection level is around 70 pg and less than 20 pg of stained DNA can be imaged using a CCD camera.

The (colorized) Polaroid photograph (courtesy of Jill Hendrickson, Ph.D., and Matt Beaudet) shows a 2-fold dilution series of lambda DNA samples (Lane 1 contains a total DNA load of 125 ng) cut with HindIII and fractionated by agarose gel electrophoresis. The gel was stained with SYBR Gold stain and then photographed using Polaroid 667 film.

The Dark Reader works well with many other fluorophors excited in the visible region including DNA stains such as SYBR Green, GelStar and ethidium bromide, the fluorescent protein stains such as SYPRO Ruby and SYPRO Ruby , red-shifted GFP variants, such as EGFP, EYFP and DsRed and common fluorophors such as fluorescein and rhodamine derivatives.

integrity of proteins encoded by the exposed DNA[10].

Until recently there has been no simple and practical alternative to the use of UV light to locate DNA fragments in gels. The Dark Reader™ (DR) series of transilluminators utilize a novel optical system that does not emit any UV light (11). Instead, the Dark Reader optical system uses a visible blue light source in combination with 2 optical filters to view the fluorescence emission that results from excitation of the visible region of the excitation spectrum of many common fluorophors[11]. The system is at least as sensitive as a UV transilluminator for the detection of many fluorophors and can be used to detect about 50 pg of SYBR Gold-stained dsDNA by eye. Given the absence of any UV radiation from Dark Reader devices, it can be predicted that the extent of damage to DNA samples will be significantly reduced compared to the damage produced by the use of a UV device, resulting in enhanced cloning efficiencies and biological activities. To test this prediction we performed the following experiments.

DNA Integrity

We determined the extent to which DNA integrity is compromised by exposure to long wavelength (302 nm) UV light as compared to exposure to the Dark Reader transilluminator's light source in 2 independent assays. First, wheat germ DNA was exposed to each excitation source, and then the cloning efficiency of the DNA was measured by using it to construct cosmid libraries using the pWEB™ Cosmid Cloning Kit (EPICENTRE Technologies).

In a second experiment, biological activity of bacteriophage T7 DNA exposed to UV or Dark Reader light was assayed by cloning the DNA in a cosmid, and assaying for the ability of the cloned DNA to make T7 plaques.

T7 DNA (40 kbp) or wheat germ chromosomal DNA (sheared to an average fragment length of 40 kbp by passing it through a 22 Gauge syringe needle) was prepared by standard methods[12]. The DNA (20-40 µg) was then incubated according to the pWEB Cosmid Cloning Kit protocol (45 minutes at room temperature) with End-Repair Enzyme Mix (EPICENTRE Technologies), in the presence of dNTPs and ATP, to ensure that all DNA fragments were both blunt-ended and phosphorylated.

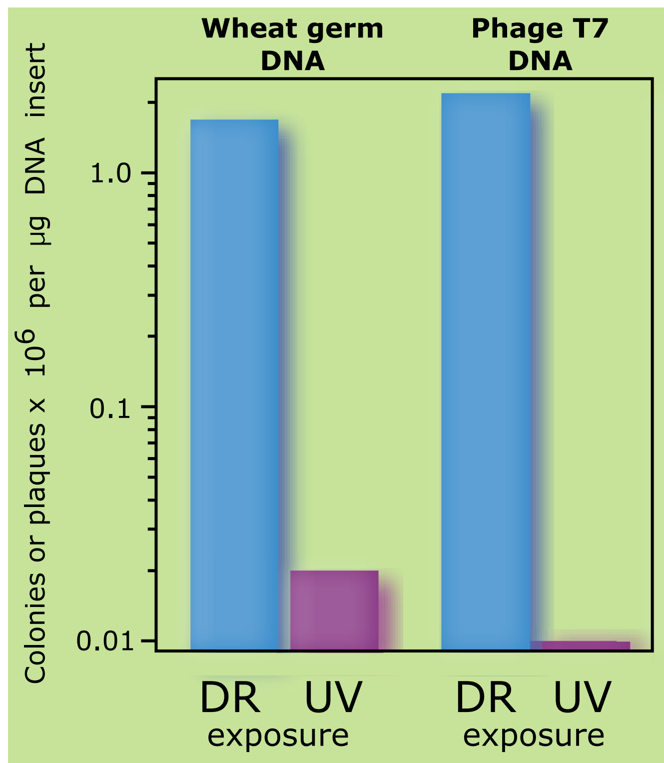
After end-repair, the blunt-ended, phosphorylated 40 kbp DNA fragments were resolved by electrophoresis through a low melting temperature agarose gel (1%, SeaPlaque GTG agarose [BioWhitaker, Rockland, ME, USA], 20 cm long gel, 30 Volts for 16 hours) that was subsequently stained with a 1:10,000 dilution of SYBR Gold Gel Stain (Molecular Probes, Eugene, OR, USA) in 40 mM Tris-Acetate – 1 mM EDTA (TAE) for 30 min.

In order to locate and excise the DNA fragments, the stained gel was then placed either on a 302 nm UV transilluminator (T1202, Sigma Chemical Co., St. Louis, MO, USA) or on a Dark Reader™ transilluminator (Clare Chemical Research, Denver, CO, USA). It required 30 seconds to excise the DNA fragments from the gel.

The excised UV- and DR-exposed T7 and wheat germ 40 kb DNA fragments were then purified using GELase™ Agarose Gel Digesting Preparation (EPICENTRE Technologies) following the manufacturer's recommended procedure.

The DNA recovery was quantified using a fluorometer and Hoechst 33258 Dye. The 40 kb DNA (600 ng; ~0.02 pmol) was ligated to the linearized and dephosphorylated pWEB Cosmid Vector (1 µg, ~0.2 pmol) using Fast-Link™ DNA Ligase (EPICENTRE Technologies) following the protocol provided in the pWEB Cosmid Cloning Kit.

The ligated DNA was packaged into lambda particles using the MaxPlax™ Lambda Packaging Extracts (EPICENTRE Technologies) following the manufacturer's recommended procedure. EPI305™ plating cells (EPICENTRE Technologies) were then transfected, plated, and incubated overnight, according to the protocols in the pWEB™ Cosmid Cloning Kit. Either plaques (for T7 DNA), or colonies (for wheat germ DNA) were counted.



Summary of the number of transformants obtained after exposure of DNA to either Dark Reader or ultraviolet light. Wheat germ and T7 DNA were fractionated by agarose gel electrophoresis, stained with SYBR Gold stain and then visualized on either a Dark Reader (DR) or ultraviolet (UV) transilluminator and the 40 kb fragments excised from the gel. The purified DNA was then used to transform cells and the number of transformants scored. DR-exposure of the wheat germ and T7 DNA yielded 1.7 x 10^6 cfu/µg DNA and 2.2 x 10^6 pfu/µg DNA respectively. UV-exposure of the wheat germ and T7 DNA yielded 2 x 10^4 cfu/µg DNA and 1 x 10^4 pfu/µg DNA, respectively.

Exposure of the wheat germ DNA to 360 nm UV light for 30 seconds had a large impact on the cloning efficiency. The plating efficiency of the UV-exposed DNA was 2 x 10^4 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 1) demonstrate that UV-exposed DNA is significantly compromised in its ability to function well in such applications as the development of genomic libraries *(Graph)*.

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 Dark Reader 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[13, 14].

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 Dark Reader 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.

Ultraviolet Transilluminators

Ultraviolet transilluminators have become important tools for preparation and analysis of nucleic acids for DNA cloning and library construction projects. UV light is a well-known mutagenic agent, introducing cyclobutane pyrimidine dimers into DNA[15]. Previous investigations[5-10] into the effects of UV exposure on DNA integrity have shown that mid-range UV radiation extensively damages DNA fragments ~2 kb in length, contrasting with earlier reports that indicate UV damage to DNA is relatively minor[4].

In this report we demonstrated that even brief 30 second exposure to long-wave (302 nm) UV light causes DNA damage of such magnitude that results of DNA cloning and library construction are severely affected. In contrast, the non-UV Dark Reader transilluminator did not adversely affect the integrity of DNA. Results obtained in construction of a pWEB Cosmid Library were as expected from high quality, biologically active, intact DNA.

The results presented here have important implications for the success of experiments and procedures that involve exposure of DNA samples to UV light, including cloning for gene expression, DNA sequencing, optimal and accurate PCR, *in vitro* transcription, and BAC or cosmid library construction.

The hugely improved transformation efficiencies obtained using DNA samples viewed using the Dark Reader transilluminator indicates that this approach should be employed in order to maintain the maximum integrity of the DNA.

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