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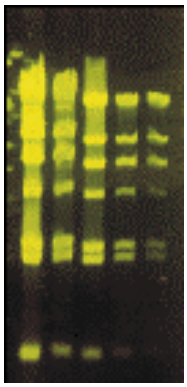
Revolutionary

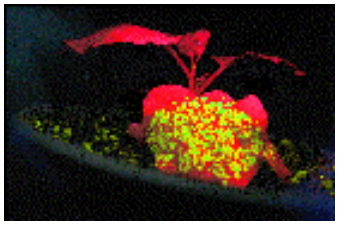
Fluorescence Detection

DARK READER

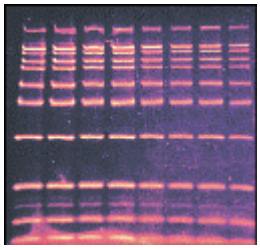


SYBR Gold

EGFP



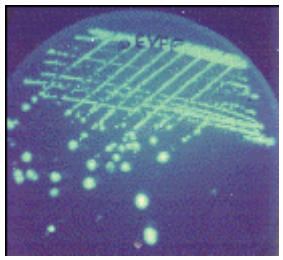
VISIBLE LIGHT

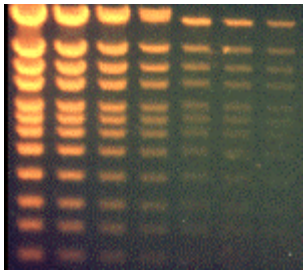


SYPRO
Orange

VERY
SENSITIVE

EYFP





Ethiduim bromide

VERY SAFE

SYBR
Green

VERY VERSATILE

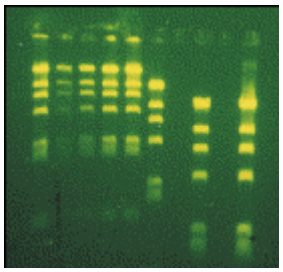


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TABLE DES MATIERES

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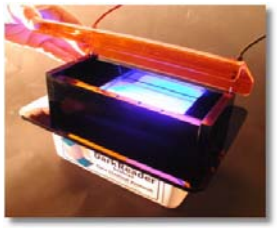
Produits Dark Reader™


Transilluminateurs

Les transilluminateurs Dark Reader sont désormais disponibles en 3 tailles le modèle DR 45, idéal pour les mini gels, le DR 88 pour les gels de taille classique et le DR 195 pour visualiser des gels jusqu'à 45 x 30 cm.

Lampes portables

La lampe et le spot Dark Reader sont des outils très polyvalents pour visualiser tous types de supports fluorescents dans le laboratoire. Les lampes sont notamment adaptées pour la visualisation des protéines GFP dans les animaux et plantes transgéniques.

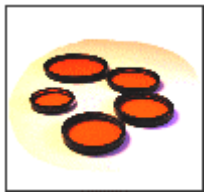


Electrophorèse

Le Dark Reader Gel-Head est une cuve d'électrophorèse unique intégrant un transilluminateur Dark Reader, permettant ainsi de visualiser les bandes fluorescentes d'ADN directement pendant leur migration dans le gel.

Accessoires Dark Reader

Chaque produit Dark Reader est livré avec une paire gratuite de lunettes de visualisation. Les filtres de caméra Dark Reader sont disponibles dans une grande variété de taille.




Référence	Désignation	Conditionnement
DR-45M	Transilluminateur Dark Reader 21x14 cm	1
DR-88M	Transilluminateur Dark Reader 25x22 cm	1
DR-190M	Transilluminateur Dark Reader 42x28 cm	1
DL-09B	Lampe Portable Dark Reader	1
SL-7S	Spot Dark Reader	1
DG-345i	Cuves d'Electrophorèse Dark Reader	1
AG-15	Lunettes Dark Reader format classique	1
AG-16	Lunettes Dark Reader	1

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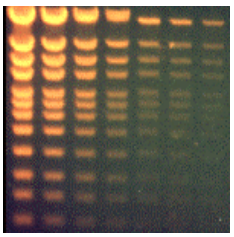
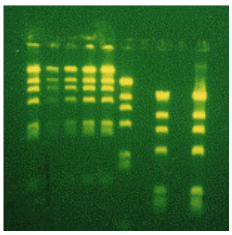
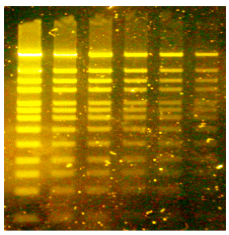
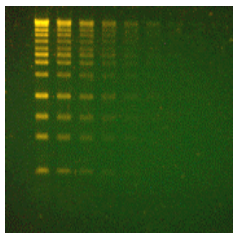
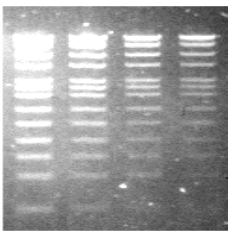
Applications des produits DARK READER™

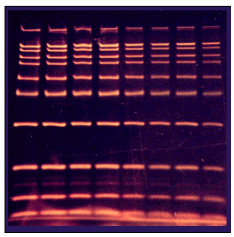

Bromure d'Ethidium

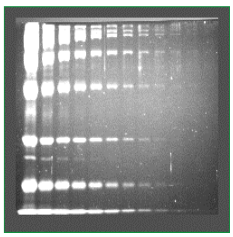

SYBR® Green

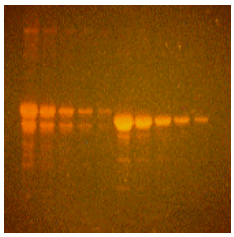

SYBR® Gold

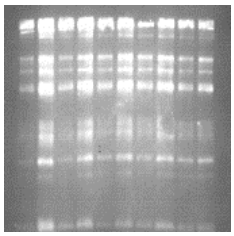

SYBR® Safe

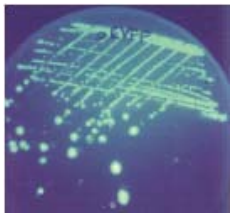

Gel Star

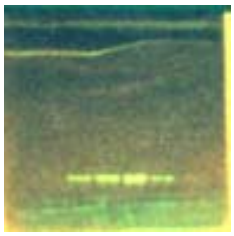

SYPRO® Orange


SYPRO® Ruby


Pro-Q™ Diamond


AttoPhos

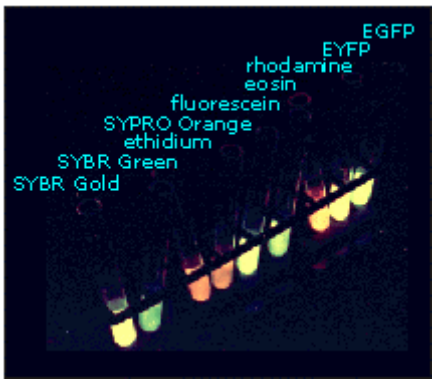

EYFP


EGFP in gel


EGFP in plant

More?

dye	ex/em	viewability
fluorescein	492/525	Good
lucifer yellow	428/533	Good
NBD	465/535	Good
ethidium bromide	518/605	Fair
eosin	524/544	Fair
acridine orange	500/526	Good
tetramethyl rhodamine	555/580	Fair
rhodamine X	580/605	Fair
SYBR Gold	495/537	Good
SYBR Green I	494/521	Good
SYPRO Orange	475/580	Good
SYPRO Red	545/635	Fair
Nano Orange	485/590	Good
PicoGreen	502/523	Good
OliGreen	498/518	Good
GelStar	493/527	Good
Vistra Green	497/520	Good
AttoPhos	440/560	Good
EGFP	488/507	Good
EYFP	513/527	Good
EBFP	380/440	Low
BODIPY FL	502/510	Fair
ATTOTAG	486/591	Good
Hoescht 33258	350/460	Low
fluorescamine	381/470	Low



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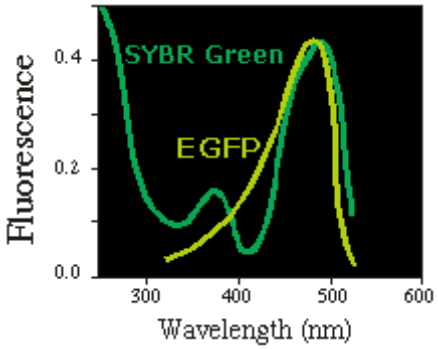
Comment marche la technologie Dark Reader™ ?

Many Fluorophors Absorb Visible Light



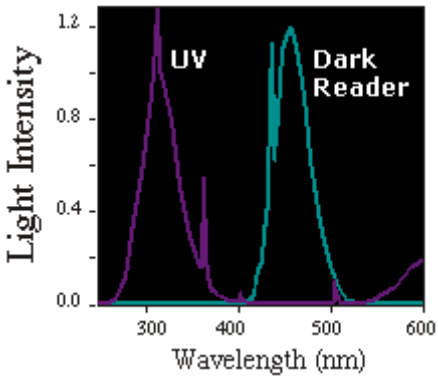
The excitation maxima for many popular dyes, including SYBR Green and red-shifted GFPs, are around 500 nm – **not** in the UV. This wavelength corresponds to blue-green light which is well within the visible light spectrum.

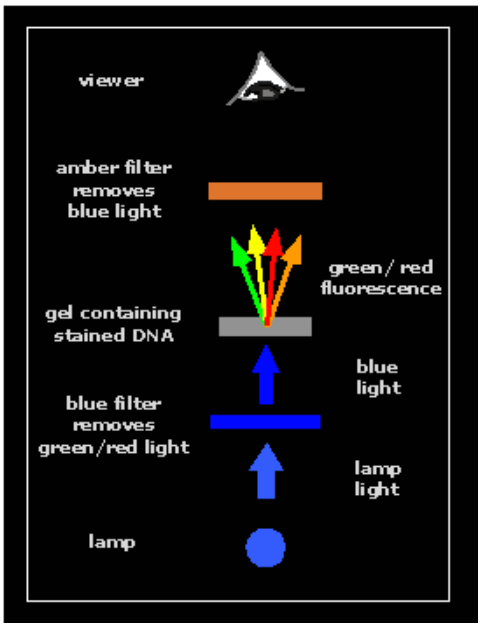
The excitation spectra of DNA stained with SYBR Green and of EGFP

A comparison of the output of the Dark Reader and a 312 nm UV transilluminator

The Dark Reader uses Visible Light

The lamp in the Dark Reader generates maximum light output between 400 and 500 nm - close to where dyes such as SYBR Green, SYPRO Orange, fluorescein and red-shifted GFPs are excited. UV transilluminators, on the other hand, typically output light around 300 nm, well removed from the absorption maxima of most common dyes.





If visible light is used for excitation of a fluorophor, any fluorescence from the sample is not directly detectable by the naked eye due to the large amount of incident light from the light source itself that reaches the observer.

The Dark Reader achieves the removal of incident light in 2 steps. The first filter is between the light source and the DNA. This removes any green and red components from the lamp and allows through to the DNA only blue excitation light.

A second filter is placed between the DNA and observer that removes the blue incident light but allows passage of the red and green fluorescent components.

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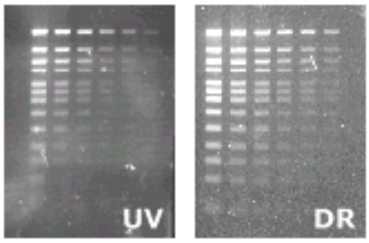
Détection de l'ADN

Ethidium bromide (EtBr) has long been the DNA stain of choice for many scientists. However, it is gradually being replaced by a new generation of stains which are more sensitive and reportedly less toxic. These include Vistrar® Green, GelStar® , PicoGreen® , OliGreen™, SYBR® Green I, SYBR Green II, and SYBR Gold stains.

The fluorescence enhancement of EtBr upon binding to nucleic acids is only on the order of 30-fold. Consequently, the background fluorescence from unbound ethidium dispersed throughout the gel is significant. The new generation of stains, on the other and, are almost completely non-fluorescent in the absence of nucleic acids but, upon binding to nucleic acids, the fluorescence intensities are enhanced approx. 1000-fold, resulting in very high signal-to-background ratios.

SYBR Stains

SYBR Green (CCD)

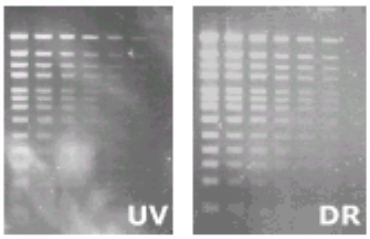


SYBR Green I was the first of the new generation of DNA stains introduced in 1994. Using a Dark Reader transilluminator it is possible to detect less than 100 pg of SYBR Green-stained DNA by eye (Table 1) and 10 pg using a CCD or Polaroid camera system.

Apart from its superior sensitivity, SYBR Green has a number of other advantages over EtBr :

- It is much less mutagenic, as shown by researchers at Molecular Probes, who compared the mutagenicity of SYBR Green I stain with that of EtBr in Ames tests.
- SYBR Green can be added directly to the DNA sample prior to electrophoresis and will remain bound during the separation run. This technique allows DNA fragments to be directly visualized as they migrate through the gel using the Dark Reader integrated electrophoresis-transilluminator unit (ETU).

Gelstar (CCD)

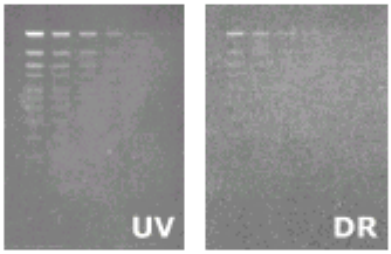


SYBR Gold stain is one of the most sensitive of the new generation of dyes. It is possible to see less than 50 pg of dsDNA by eye using a Dark Reader transilluminator (Table 1) and in combination with a CCD or Polaroid camera it is possible to detect as little as 10 pg of dsDNA.

GelStar Stain

GelStar (BMA, Inc.) can be used for the sensitive detection of dsDNA, ssDNA, oligonucleotides and RNA in gels. The detection limit of dsDNA stained with GelStar and viewed using a Dark Reader is comparable to that of SYBR Green and SYBR Gold stains. GelStar also effectively stains ssDNA and RNA.

EtBr (Polaroid)



Ethidium bromide

Ethidium bromide (EtBr) is intrinsically not as good a stain for the detection of DNA as the new generation of dyes. This is mainly due to the fact that the background fluorescence from unbound EtBr is relatively high.

The background problem is greatest when viewing EtBr-stained DNA gels with a DR transilluminator. As a result, the DR not as sensitive as 300 nm UV-based devices for the detection of EtBr-stained DNA.

Stain	amount of DNA detected (pg)					
	CCD		Polaroid		Eye	
	UV	DR	UV	DR	UV	DR
SYBR Green	15	9	44	19	119	60
SYBR Gold	15	9	34	15	73	35
GelStar	15	9	31	15	120	44
EtBr	89	623	125	500	500	2560

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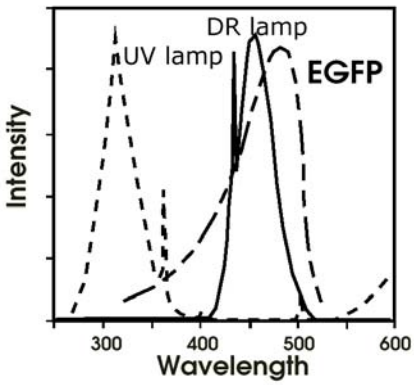
Détection des Protéines Fluorescentes

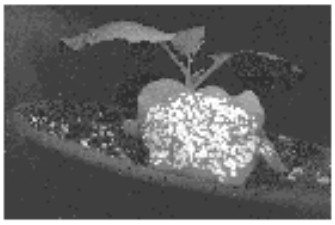
The optical performance of DR technology is perhaps most spectacular when viewing Green Fluorescent Proteins. The new generation of red-shifted GFPs have excitation and emission properties that are very well suited for viewing with the DR. For example, EGFP (ex/em = 488/507 nm) can be detected, by eye, down to concentrations of less than 100 pM. EYFP (ex/em = 513/ 527 nm) is also highly fluorescent under DR light.

The DR also works well with the red fluorescent protein DsRed even though the excitation maximum of this variant is around 550 nm. This is because DsRed is still significant excited by light of less than 500 nm.



GFP in vivo

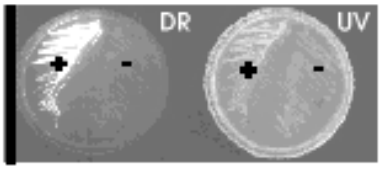
The Dark Reader Hand Lamp is proving very popular for the detection of GFP expression in a variety of plants and animals including tobacco, mice and shrimps.



Dr. Anton Callaway at North Carolina State University provided the accompanying photograph of *Nicotiana benthamiana* plants inoculated with turnip vein-clearing tobamovirus engineered to express an endoplasmic reticulum-localized form of EGFP. The photographs were taken 6 days post-inoculation using a Dark Reader Hand Lamp. The green fluorescent spots (against a background of red chlorophyll fluorescence) show the expanding foci of virus-infected cells.

Simple, Safe Selection

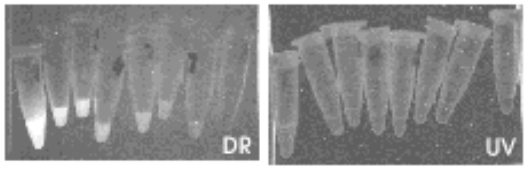
As illustrated below, distinguishing those bacterial colonies expressing GFP from other colonies is a simple exercise using the Dark Reader. In addition, the absence of harmful radiation from DR units can be crucial to the success of an experiment when viewing in vivo systems expressing GFP.



Two E. coli cultures (one expressing EYFP and the other not) were streaked on an agar plate and grown overnight before viewing on either a DR45 transilluminator or a 312 nm UV device. The GFP-expressing colonies (+) are very clearly distinguishable from the non-expressors (-) when using the DR.

X-Ray Vision !?

The benefit of using DR light rather than 300 nm UV light for viewing GFPs is particularly pronounced when viewing GFPs contained within a glass or plastic unit such as a gel apparatus, Petri dish or test-tube. In the example shown on the right, a serial dilution of EGFP (ClonTech) was viewed on either a DR45 transilluminator or a 312 nm UV device. Using the DR45, it was possible to detect, by eye, EGFP down to 50 pM. With UV illumination, the detection limit was over 2 orders of magnitude worse.



Using a DR Hand Lamp (DL-09), red-shifted GFPs can even be viewed as they migrate through polyacrylamide gels - a feat that is virtually impossible with UV illumination.

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Détection des Protéines par coloration

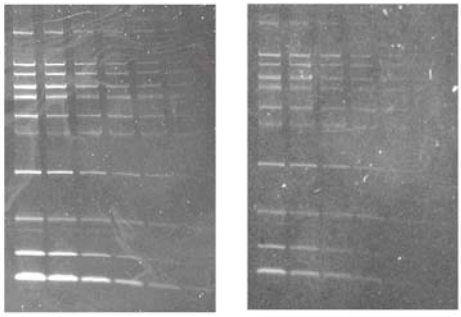
Following SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of protein mixtures the individual protein bands are typically visualized using either Coomassie brilliant blue R-250 or silver staining. Several new fluorescent protein stains have been recently developed by Molecular Probes, Inc. These SPYRO stains display excellent sensitivity similar to that of silver staining, less protein-to-protein variability than silver, a greater quantitation range, a simple one step staining procedure, and do not interfere with subsequent downstream characterization techniques. These stains are now becoming widely used in proteomics studies and can be very effectively detected using the Dark Reader.

"We find that SYPRO Ruby is highly compatible with the Dark Reader and particularly recommend your instrument for investigators who manually excise bands from gels for proteomic investigations. Spots may be excised from gels with no hazard to personnel from UV injury and no photobleaching of the stain."

Wayne F. Patton, Ph. D Group Leader, Molecular Probes

SYPRO Ruby

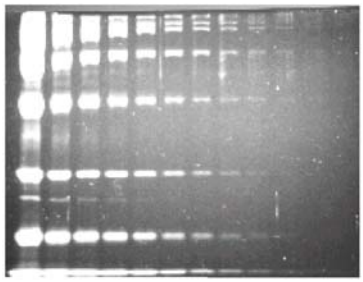
The family of SYPRO Ruby stains can be used to detect proteins in SDS-polyacrylamide gels, isoelectric focusing gels and on membranes. The dyes are maximally excited at 470 nm and the emission peak is about 610 nm - ideal spectroscopic properties for use with the Dark Reader. About 2 ng of SYPRO Ruby-stained protein can be detected directly by eye in an SDS-polyacrylamide gel using a Dark Reader transilluminator and about 8 ng after transfer to a PVDF membrane. The Ruby stains have become particularly popular for the detection of proteins following the 2-D electrophoretic separation of samples in proteomic studies.



An SDS gel of protein standards was stained with SYPRO Orange and photographed on either a DR or a UV transilluminator. The amount of protein per band ranged from 32 ng (lane 1) to 1 ng (lane 6)

SYPRO Orange

SYPRO Orange is a novel fluorescent stain for the detection of proteins separated by SDS polyacrylamide gel electrophoresis. The staining procedure is simple, rapid and sensitive. The detection limit for Orange-stained proteins using a DR transilluminator is around 2 - 4 ng both by eye and using either a CCD or Polaroid camera. This level of sensitivity, especially by eye, is significantly greater than that obtained using a UV transilluminator which about 20 ng by eye.

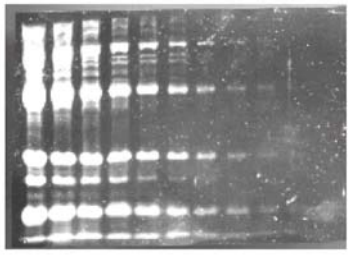


Polaroid photographs of an SDS gel (above) and a PVDF membrane (below), stained with SYPRO Ruby. Both contain serial dilutions of molecular weight standards ranging from 2000 – 1 ng per protein band. Both were viewed using a Dark Reader DR45M transilluminator.
(Thanks to Dr. K. Berggren.)

Photobleaching

The photobleaching of fluorophors upon exposure to light can become a significant problem, particularly when the experimental protocol takes a few minutes. This situation arises, for example, when proteins are isolated from 2-D electrophoresis gels for downstream analysis.

In a simple comparison, Orange-stained proteins samples were exposed on either a DR or a 312 nm UV transilluminator. UV exposure caused a ~40% decrease in the fluorescence intensity of the protein bands. The DR exposure, on the other hand, resulted in only a ~10% or less decrease in band intensity.



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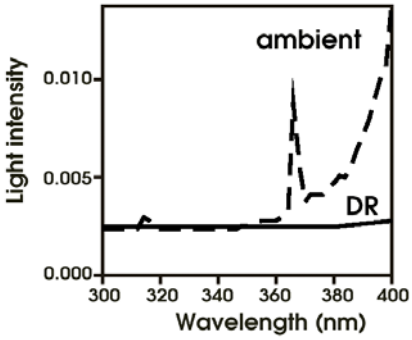
Les effets des radiations U.V.

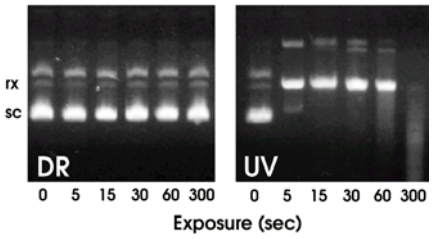
Many users of UV transilluminators have experienced, at one time or another, either a mild case of sun-burn or « spots before the eyes » as a result of spending too long either examining a gel or cutting out bands. The potentially harmful effects of shorter wavelength light are well documented and were even the subject of a report from the Council on Scientific Affairs of the American Medical Association[1]: high-intensity UV radiation can cause erythema, degenerative and neoplastic changes in the skin, retinal damage and cataracts, and modification of the immunologic system of the skin. Even the fluorescent lamps commonly used in homes and businesses emit sufficient UV light to cause mutagenesis in Salmonella[2] upon prolonged exposure. This latter effect is eliminated by the use of a filter that blocks light of less than 370 nm.



As shown in Figure 1, the emission spectrum of the lamp / blue filter system used in the Dark Reader optical system contains less UV light than the standard fluorescent lighting used in most offices and laboratories. Because the Dark Reader transilluminator emits almost immeasurably low levels of light below 400 nm, there is essentially zero risk of UV radiation causing eye or skin damage, making it much safer to use than a traditional UV transilluminator.

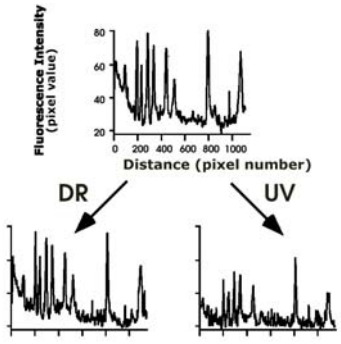
In vitro DNA damage

It is well known that DNA samples undergo a number of reactions when exposed to UV light including pyrimidine dimerization, breaks in the sugar-phosphate backbone and interstand cross-links. There have also been several reports regarding the deleterious effects of UV irradiation on the biological integrity of DNA samples and cloning protocols.



Because the Dark Reader does not emit any UV light, it can be predicted that the extent of damage to DNA when viewed on a DR device will be drastically reduced compared to the damage produced by the use of a UV table. This is borne out by the results of the simple experiment shown in Fig. 2 in which supercoiled plasmid was exposed to DR or UV light for various times and then incubated with T4 endonuclease V, which excises pyrimidine dimers, generating the relaxed form of the plasmid. This can then be resolved from the intact supercoiled form by gel electrophoresis. As little as a 5 sec exposure to UV light was sufficient to convert almost 100% of the supercoiled plasmid (sc) into the relaxed form (rx) by endonuclease V and after 300 sec of UV exposure the DNA was completely fragmented. In contrast, a 300sec exposure on the DR transilluminator resulted in no detectable DNA damage. This result suggests that the efficiency of downstream cloning protocols can be enormously improved by using a DR transilluminator, rather than a UV device, to visualize and excise DNA bands from gels.

Photobleaching



The photobleaching of fluorophors upon exposure to light can become a significant problem, particularly when the experimental protocol is prolonged. This situation arises, for example, when proteins are being isolated from 2-D electrophoresis gels for downstream analysis. Clearly, if photobleaching can be minimized then the usable life of a gel can be extended accordingly, without the need to re-stain the gel. To determine the extent of photobleaching that occurs upon exposure of Orange-stained proteins in an SDS gel to DR and UV light, samples were variously exposed for 8 minutes on either a DR or a 312 nm UV transilluminator. The results (left) show that UV exposure caused a 40% or more decrease in the fluorescence intensity of the protein bands. The DR exposure, on the other hand, resulted in only a ~10% or less decrease in band intensity.

[1] Council on Scientific Affairs, JAMA, 1989, 262, 380-384
[2] Hartman, Z., et al., Mut.res., 1991, 260,25-38

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Amélioration de l'efficacité de clonage

A number of recent studies make it clear that even brief exposure to UV light can seriously damage precious DNA samples and severely impact down-stream cloning protocols [1, 2, 3].

Epicentre Technologies was so impressed with the improved cloning efficiencies resulting from use of their in-house Dark Reader (DR) transilluminator that they decided to add the DR to their product line! Summarized below are the results from several experiments performed by Epicentre scientists that show the dramatic improvements that can be achieved when DNA samples are viewed on a DR rather than a UV transilluminator.

Maximize Cloning Efficiency

To evaluate DNA integrity after exposure to DR and UV light, cosmid libraries were constructed using Epicentre's pWEB ™ Cosmid Cloning Kit with wheat germ DNA as the nucleic acid source. In a second experiment, biological activity of exposed DNA was assayed by making cosmid constructs containing T7 DNA, and assaying for plaque formation.

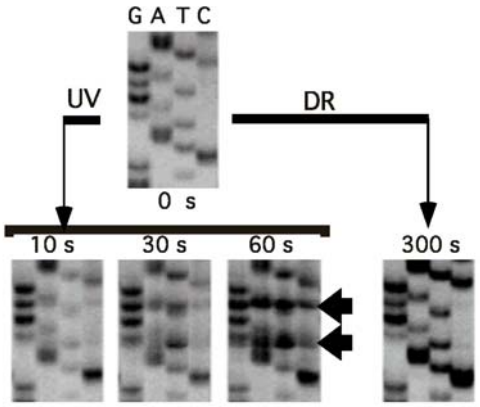
Exposure of the wheat germ DNA to 360 nm UV light for just 30 seconds had a large impact on the cloning efficiency. The plating efficiency of the UV-exposed DNA was reduced 170-fold compared to that of the Dark Reader-exposed DNA. This demonstrates that the UV-damaged DNA is significantly compromised in its ability to function well in such critical applications as the development of genomic libraries, particularly from larger genomes.

DNA Type	Cloning Efficiency (10^6 pfu/µg insert)	
	DR	UV
T7 phage	2.20	0.01
wheat germ	1.70	0.02

The successful development of a T7 library requires that the cloned T7 DNA in each cosmid produce the active proteins necessary for T7 replication. DNA recovered from a gel visualized on the Dark Reader Transilluminator produced a 220-fold greater number of plaques than DNA recovered from a gel exposed to 360 nm UV light.

Maximize DNA Sequencing

The exposure of DNA to a light source can potentially have adverse effects on the outcome of other down-stream molecular biology protocols besides transformation. This is illustrated in the qualities of the DNA sequencing data (left) obtained after exposure of DNA samples to either 360 nm UV or DR light.



DNA that was not exposed to any light yielded an excellent-looking sequencing gel. However, as the exposure time of the DNA on a 360 nm UV transilluminator was increased, several 'bands-across-all-four-lanes' (BAFLs) began to appear and within less than 60 sec of UV exposure it was impossible to read the sequence in multiple areas of the gel with the BAFLs obscuring two or more bases at that point in the 'read'. In contrast, the sequencing gel obtained even after prolonged exposure (300 sec) on a DR transilluminator was virtually identical to the gel obtained with DNA template unexposed to light of any kind, indicating no damage to the DNA.

(1) Hartman, P.S. Biotechniques, 1991, 11, 747-748. (2) Hoffman, L., Epicentre Forum, 1996, 3, 4-5 (3) Grundemann, D., Schomig, E., BioTechniques, 1996, 21, 898-903

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