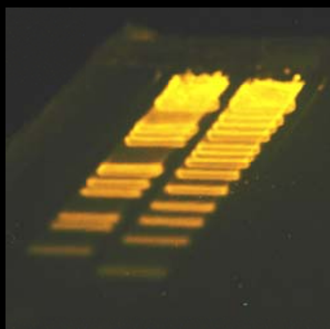
Revolutionary Fluorescence

The Dark Reader® HandBook

SYBR Gold



EGFP

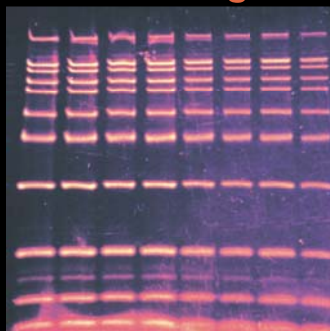
Visible Light

Dark Reader Transilluminators and Hand Lamps reveal fluorescent DNA, RNA, and proteins using a revolutionary combination of a VISIBLE blue light source and two color filters.

Very Sensitive

Dark Reader apparatus is at least as sensitive as UV for the detection of many fluorophors and can be used to see, directly by eye, less than 100 pg of stained DNA.

SYPRO Orange

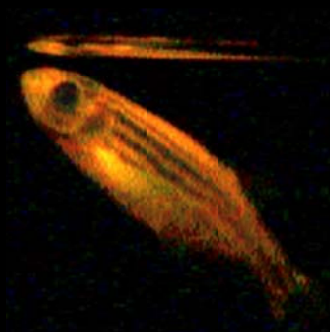


EYFP

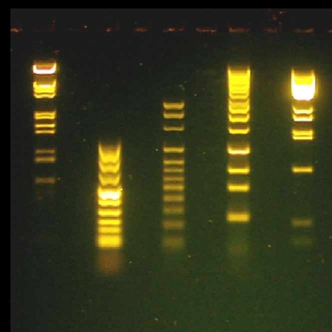
Very Safe

Because Dark Readers emit no UV radiation, there is essentially zero risk of eye or skin damage, the extent of DNA sample damage is drastically reduced, and cloning efficiency is increased over 100-fold.

RFP



GelStar



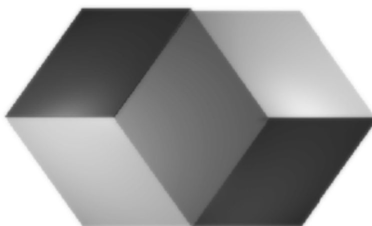
Very Versatile

The Dark Reader visible light source is particularly effective for viewing fluorophors through glass and plastic, and even in transgenic plants and animals.

Dark Reader® devices including transilluminators, hand lamps and electrophoresis units as well as associated accessories such as viewing glasses, screens and camera filters are for use under one or more of US patents 6198107, 6512236, 6914250 and international patent pending.

The Dark Reader HandBook

Clare Chemical Research



Products
- Transilluminators, Hand Lamps and Electrophoresis

Performance
- As sensitive as UV - detect 100 pg of DNA by eye
- As sensitive as laser scanning - detect 1 nM fluorescein
- Great for the detection of GFPs

Fluorophors
- SYBR Green, SYBR Gold, Ethidium, GFPs, AttoPhos,
- SYPRO Orange, SYPRO Ruby, PBXLs, fluorescein
- rhodamine & more

No UV
- Very safe to use - No sunburn. No sample damage

Theory
- How Dark Reader Technology Works

Ordering
- Available world-wide

FAQ
- Useful tips

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Dark Reader Transilluminators

Clare Chemical Research currently offers 3 transilluminators:

DR45M: (14 x 21 cm viewing surface) suitable for mini gels and smaller samples.

DR88M: (22 X 25 cm surface) suitable for most standard gels and larger samples.

DR195M: (30 x 46 cm surface) suitable for very large gels.

A DR45M transilluminator is shown above 'at rest' and viewing a SYBR Gold-stained DNA gel.

Included with each Dark Reader transilluminator are an amber viewing screen (which also acts as a camera filter) and a pair of DR glasses. The glasses are particularly useful for cutting bands out of gels.

The Dark Reader series is compatible with all imaging systems. Separate camera filters are available if required.

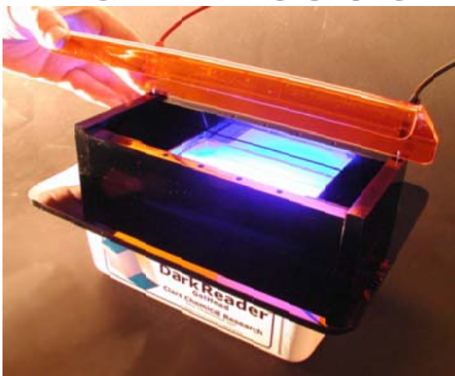
Dark Reader Lamps

The DR Hand Lamp (HL-32T) provides for the incredibly versatile viewing of fluorophors such as GFPs, SYBR Green, SYPRO Orange and fluorescein.

Like all DR devices, the Hand Lamp uses visible light and so (unlike ultra-violet devices) the DR Hand Lamp can "see" through just about any kind of transparent construction whether it be glass, polystyrene, polyethylene or acrylic.

Clare has recently introduced the DR Spot Lamp (SL7S). This mighty mite (shown on the right) generates a concentrated light beam with nearly twice the intensity of the Hand Lamp AND runs off a standard alkaline battery!

Dark Reader GelHead Electrophoresis



The Dark Reader DG-345 is a unique electrophoresis unit that contains an integral DR transilluminator allowing DNA fragments to be directly viewed as they migrate. With a footprint of 25 x 10 cm, the GelHead is the same size as an ordinary mini-gel apparatus

Using the DG-345, the researcher can continuously monitor the progress of a DNA fractionation, and so, a gel only needs to be run until the DNA band(s) of interest are separated.

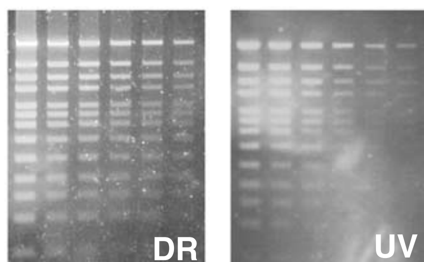
Performance

Revolutionary

The **DARK READER (DR)** is a non-UV transilluminator that uses a novel patented technology to visualize fluorescently stained DNA, proteins and other biological samples. DR devices use a **visible** light source that excites fluorophors between about 420 and 500 nm. This range includes many popular dyes such as fluorescein, SYBR Green, SYBR Gold, AttoPhos, GelStar, Vistra Green, SYPRO Orange and red-shifted GFP variants. The DR also works with dyes maximally excited above 500 nm such as rhodamine and ethidium bromide,

Detect 10 pg of DNA

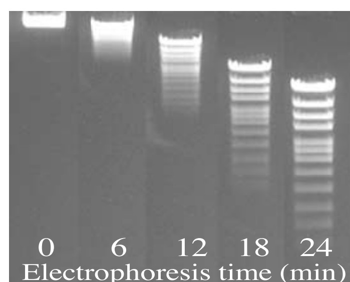
Using a unique combination of filters, the Dark Reader is at least as sensitive as a UV transilluminator for the detection of many dyes. For example, using SYBR Gold or SYBR Green stain it is possible to see, by eye, around 75 pg of DNA and 10 pg using a CCD camera.



In the gel on the left, (stained with SYBR Gold) Band 10 in Lane 6 contains 9 pg of dsDNA.

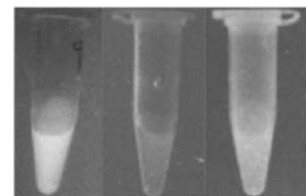
Monitor DNA Migration in Real Time

The Dark Reader GelHead allows DNA bands to be visualized in real time as they migrate through a gel. An example of the GelHead in action is shown to the right. The DNA used in this experiment was pre-stained with SYBR Green before loading. The gel was then photographed as the DNA was actually fractionating through the agarose.



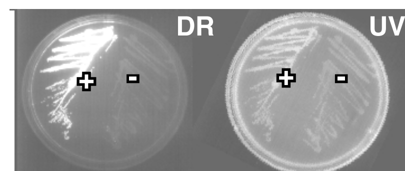
Much More Sensitive than UV

All **Dark Reader** products, including the Hand Lamp, are particularly effective for viewing fluorophors in places where UV light cannot reach - through glass and plastic.



Identical fluorescein solutions, in 1.5 mL tubes, were photographed on a DR45M ,or a UV device. The superior ability of the Dark Reader to "see" through plastic is obvious. Note how the glow from the tube itself, especially on the 312 nm unit, masks the fluorescence from the solution.

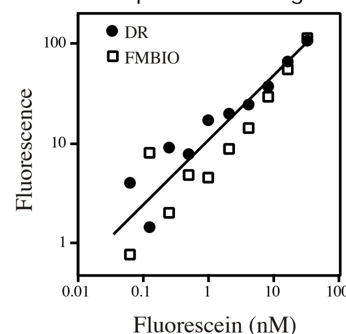
Ideal for GFPs



The optical performance of DR technology is perhaps most spectacular with the new generation of red-shifted GFPs that have ideal excitation and emission properties for viewing with the DR. Shown above, the EYFP-expressing E. coli (+) colonies are clearly distinguishable from the non-expressors (-) when using a Dark Reader Hand Lamp.

Head to Head: DR vs Laser Scanner

The graph below shows a comparison of a DR45M transilluminator (coupled with a CCD camera) and a FMBIO II laser scanner. 100 microlitre samples of a serial dilution of fluorescein were placed in a 96-well plate and imaged using either the DR45 or the FMBIO.



The DR performs as well as the FMBIO and can be used to reliably detect fluorescein down to less than 1 nM or 0.1 pmol.

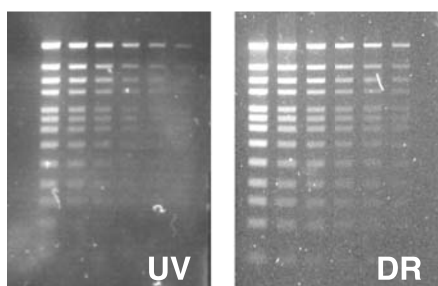
DNA Detection

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 Vistra® 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

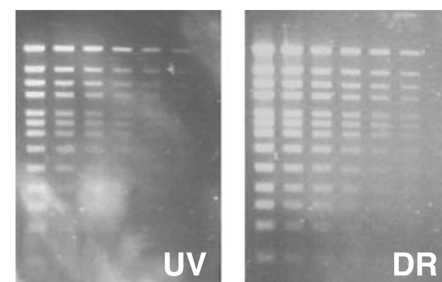
SYBR Green (CCD)



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 GelHead.

GelStar (CCD)

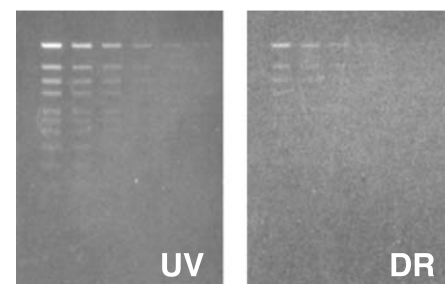


SYBR Gold stain is one of the most sensitive of the new generation of dyes It is possible to see less than 50 pg of dsDNA by eye using a Dark Reader transilluminator (Table 1) and in combination with a CCD or Polaroid camera it is possible to detect as little as 10 pg of dsDNA.

GelStar Stain

GelStar 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 the SYBR stains. GelStar can be added to the agarose before electrophoresis, allowing DNA bands to be visualized as they migrate.

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 is 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

Protein Detection

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 SYPRO® 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.

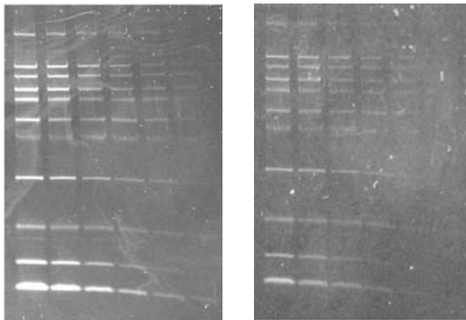
" We find that SYPRO Ruby is highly compatible with the Dark Reader and particularly recommend your instrument for investigators who manually excise bands from gels for proteomic investigations. Spots may be excised from gels with no hazard to personnel from UV injury and no photobleaching of the stain."

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

SYPRO Ruby

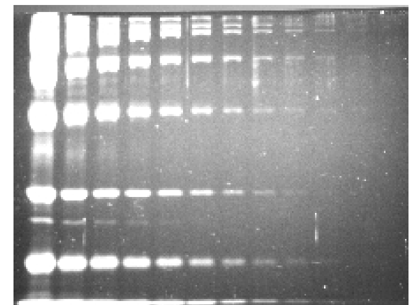
The family of SYPRO Ruby stains can be used to detect proteins in SDS-polyacrylamide gels, isoelectric focusing gels and on membranes. The dyes are maximally excited at 470 nm and the emission peak is about 610 nm - ideal spectroscopic properties for use with Dark Reader equipment. About 2 ng of SYPRO 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.

SYPRO Orange

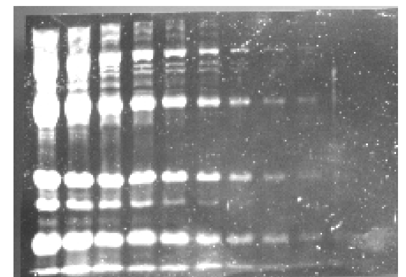


An SDS gel of protein standards was stained with SYPRO Orange and photographed on either a DR or a UV transilluminator. The amount of protein per band ranged from 32 ng (lane 1) to 1 ng (lane 6)

SYPRO Orange 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.)



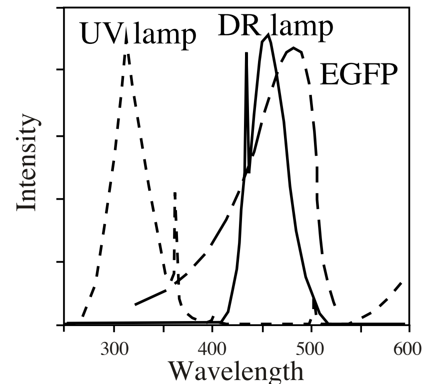
Photobleaching

The photo-bleaching 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.

Green Fluorescent Proteins

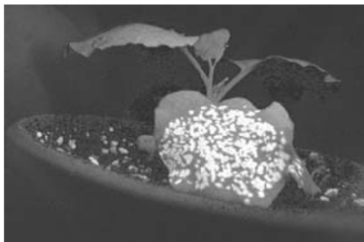
The optical performance of DR technology is perhaps most spectacular when viewing Green Fluorescent Proteins. The new generation of red-shifted GFPs have excitation and emission properties that are very well suited for viewing with the DR. For example, EGFP (ex/em = 488/507 nm) can be detected, by eye, 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

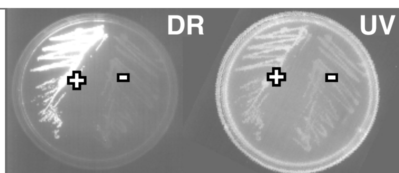
The Dark Reader Hand Lamp is proving very popular for the detection of GFP expression in a variety of plants and animals including tobacco, mice and shrimps.



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.

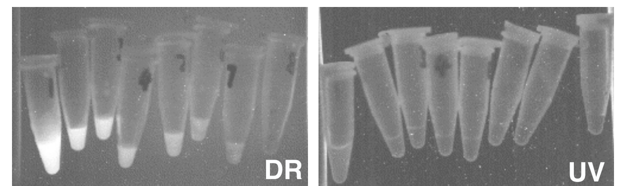
Simple, Safe Selection

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, red-shifted GFPs can even be viewed as they migrate through polyacrylamide gels - a feat that is virtually impossible with UV illumination.

More Fluorophors

The Dark Reader works with almost all your Favorite Dyes.........

Dark Reader Technology can be used to detect many different fluorophors. In general, the ideal spectral characteristics for a 'DR-dye' are an excitation maximum between 420 - 500 nm and an emission maximum above 520 nm.

It should be emphasized that the Dark Reader can also be effectively used to detect dyes that have maxima outside the above ranges. The only criterion is that at least a portion of the fluorescence excitation and emission fall within these ranges. Stated another way, the DR can be used to detect almost any dye excited in the visible range that does emit exclusively in the blue.

The table on the right lists a few commonly used dyes and their viewability using the Dark Reader. It should be noted that the listed qualifications are relative to an OPTIMAL excitation source and emission detector, such as a tunable fluorometer - not a transilluminator that can only provide excitation in the UV.

Dye	Ex/Em Maxima (nm)	Viewability
acridine orange	500/526	+ + +
aminoacridone	425/531	+ + +
AttoPhos	440/560	+ + +
ATTO-TAG	486/591	+ + +
BODIPY FL	502/510	+ +
Cy3	552/568	+ +
DDAO-phosphate	478/628	+ + +
DsRed	558/583	+ + +
EBFP	380/440	+
ECFP	434/477	+ +
EGFP	488/507	+ + +
eosin	524/544	+ +
ethidium bromide	518/605	+ +
EYFP	513/527	+ + +
fluorescamine	381/470	-
fluorescein	492/525	+ + +
GelStar	493/527	+ + +
Hoescht 33258	350/460	-
lucifer yellow	428/533	+ + +
NanoOrange	485/590	+ + +
NBD	465/535	+ + +
OliGreen	498/518	+ + +
PicoGreen	502/523	+ + +
PyMPO	415/570	+ + +
SYBR Gold	495/537	+ + +
SYBR Green I	494/521	+ + +
SYPRO Orange	470/570	+ + +
SYPRO Ruby	450/610	+ + +
SYPRO Tangerine	490/640	+ + +
tetramethyl rhodamine	555/580	+ +
Vistra Green	497/520	+ + +

......all around the Laboratory

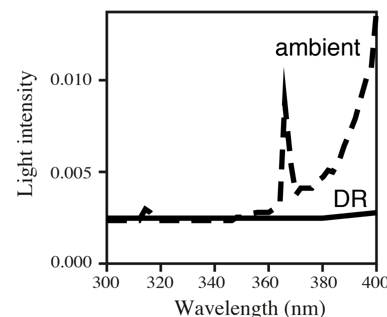
The handling and viewing of fluorescent dyes in the laboratory typically involves numerous types of media and containers including electrophoresis apparatuses, blotting membranes, test-tubes, etc. Consequently, the practical limits of fluorescence detection depend on a variety of factors besides the intrinsic optical properties of the dye and the viewing device. These factors include attenuation of the fluorescence excitation and emission as well as a possible background fluorescence contribution by the medium or container.

The data summarized below compare the sensitivity of DR and UV devices for the direct visual detection of fluorescein (FL, ex / em maxima = 492/525 nm) and tetramethylrhodamine (TMR, ex / em maxima = 555/580 nm) in a variety of typical laboratory settings. The data show that the performance of the DR optical system with both FL and TMR is, in many instances, significantly better than that of a UV device. This is directly attributable to the blue excitation band employed by the DR optical system which efficiently excites the fluorophors - even TMR which has a red-shifted excitation peak. In addition,

Dye environment	ratio of minimum detectable amount of dye (DR / UV)	
	FL	TMR
plastic wrap (trans)	2	0.5
nitrocellulose membrane (epi)	2	2
electrophoresis glass plate (trans)	4	4
96-well plate (trans)	8	4
1.5 mL polypropylene tubes (trans)	8	2
nitrocellulose membrane (trans)	16	8
electrophoresis acrylic (trans)	16	16

2-fold dilution series of fluorescein (FL) and tetramethylrhodamine (TMR) were variously aliquoted onto several media or laboratory-ware. The dilution series were then viewed by eye in either a 'trans' configuration on a DR transilluminator or a 312 nm UV transilluminator or in an 'epi' configuration using either a DR hand lamp or a 312 nm UV handlamp to illuminate from above, and the lowest detectable amounts of FL and TMR were recorded. For the sake of clarity, the results in the table are presented as the ratio of the lowest detectable amount of fluorophor using a DR device versus that using a UV device.

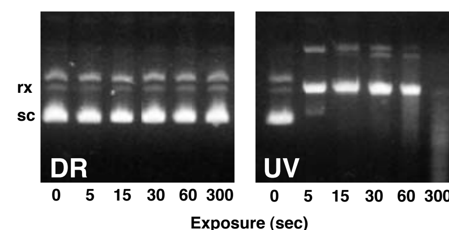
The Effects of UV Radiation



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.

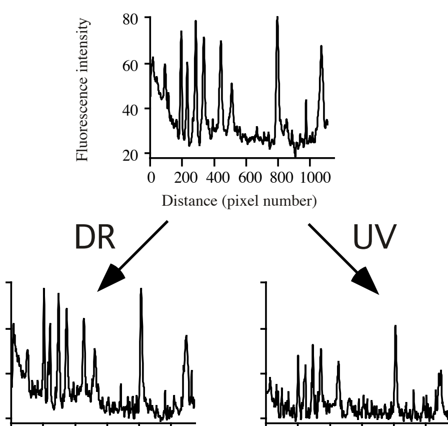
In vitro DNA damage



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 300 sec 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 photo-bleaching 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..

Maximize Your DNA using a **Dark Reader**

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.

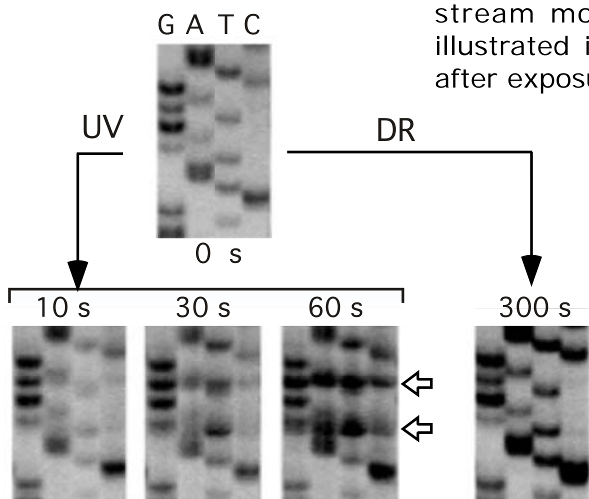
DNA Type	Cloning Efficiency (10^6 pfu/μg insert)	
	DR	UV
T7 phage	2.20	0.01
wheat germ	1.70	0.02

Exposure of the wheat germ DNA to 302 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.

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 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 302 nm UV or DR light.



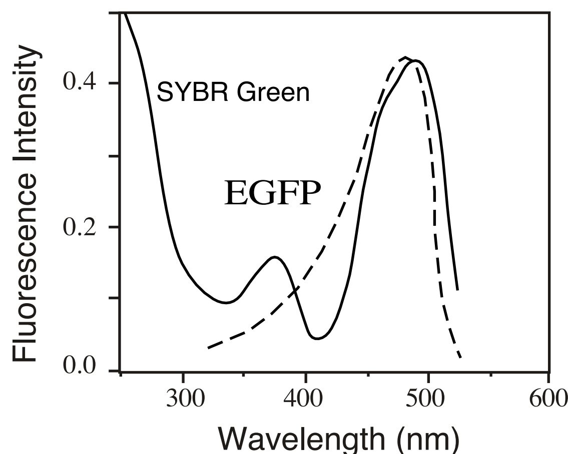
DNA that was not exposed to any light yielded an excellent-looking sequencing gel. However, as the exposure time of the DNA on a UV transilluminator was increased, several ‘bands-across-all-four-lanes’ (BAFLs) began to appear and within less than 60 sec of UV exposure it was impossible to read the sequence 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.

Theory

How Dark Reader Technology Works

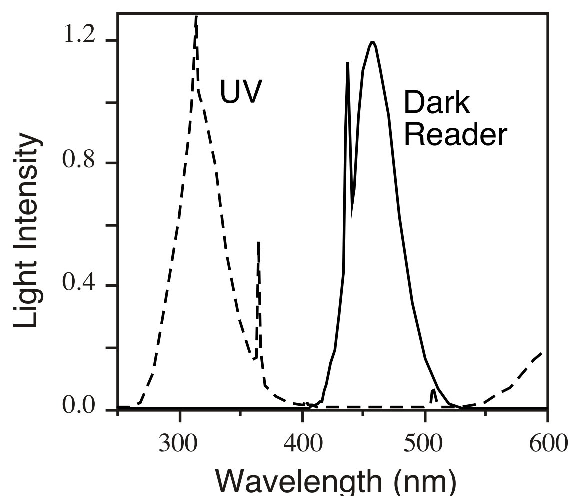
Many Fluorophors Absorb Visible Light



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.

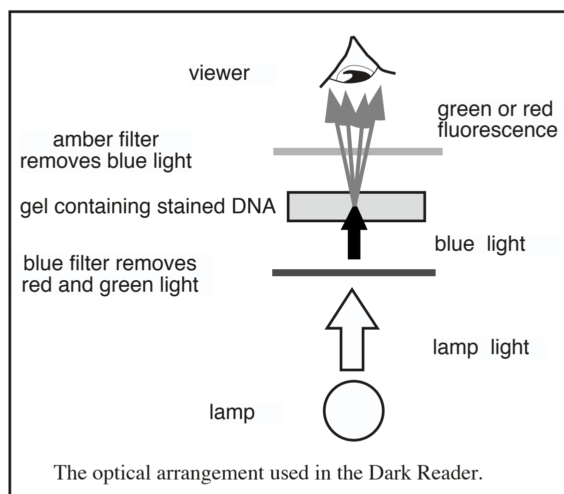
A comparison of the output of the Dark Reader and a 312 nm UV transilluminator.



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.

The Dark Reader uses 2 Filters to Reveal Fluorescence



The optical arrangement used in the Dark Reader.

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.

Ordering Information

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For current pricing, information on new products and a list of our distributors, both in the USA and world-wide, please visit out web page:
www.clarechemical.com/order.htm

Unit*	Model #
21 x 14 cm DR Transilluminator	DR-45M
22 x 25 cm DR Transilluminator	DR-88M
30 x 46 cm DR Transilluminator	DR-195M
DR Hand Lamp	HL-32T
DR Spot Lamp	SL-07S
DR GelHead Electrophoresis unit	DG-345

*All units come with a pair of DR glasses and an amber screen, except the DR Lamps which come with glasses only.

Accessories and Parts	Part #
DR glasses	AG16
DR 37 mm filter*	AF370
DR 40.5 mm filter	AF405
DR 46 mm filter	AF460
DR 49 mm filter	AF490
DR 49 mm filter (unmounted)	AF491
DR 52 mm filter	AF520
DR 75 x 75 mm filter	AF07
red-enhancing filter	AF09
extra comb set for DG345	AC201

* Note: the DR45, DR88, DR195 and DG345 are sold as complete packages and there is no requirement for a camera filter. See our web page for more details on camera filters:
www.clarechemical.com/filters.htm

Frequently Asked Questions

Do we have to use the orange glasses for eye protection or other reasons?
The glasses are not for eye protection. The glasses are especially useful for when you are cutting bands out of a gel. They save you trying to work around the amber screen.

How resistant is the blue screen to scratching?
The blue screen is made of plastic and will scratch more easily than a UV screen. However, this does not appear to be an important factor in performance of the Dark Reader - we have had a Dark Reader in use for several years in a cloning lab and it has been used (and abused) on a daily basis.

How long do the lamps last?
The lamps used in the DR-45 and DR88 transilluminators are rated for 10,000 hours (about 13 months of 24 hours-a-day operation) and the DR-195 lamps for 6,000 hours. Replacement lamps cost about $15 and $30 respectively. In contrast, typical UV bulbs are rated at 5000 hours and cost $40 each.

I would like to know if the DR45 transilluminator would work with our XYZ imager.
I am not familiar with the XYZ system but the answer is: Yes, in theory.
In practise there are a couple of issue:
- the physical size of the cabinet - is it big enough for the DR45.
- the electrical power supply into the cabinet. The XYZ may use a different style plug so you may need an adapter (a Radioshack part).
- the CCD camera may not have a built-in IR filter, so you will need to get a separate one.

We have a camera set up for EtBr gel photography using a UV transilluminator. Do you think we would need a new filter to take images using a Dark Reader?
You should always use a Dark Reader filter - either the amber screen or a separate DR camera filter. This filter is optimized to work with the Dark Reader. An amber screen is included in the basic transilluminator package.

Do you recommed to use a filter onto the camera of a geldoc or to use the amber screen?
The optical properties of the amber screen and the DR camera filters are identical, but having a separate filter is usually going to be a more flexible approach.

When I image a gel using my CCD camera, I get a very high background that almost looks like a flare. How can I fix this?
This sounds like IR radiation. All lamps emit IR radiation. Unfortunately, this is the region of the spectrum to which CCD chips are most sensitive. (Oddly enough, the more expensive the CCD camera, the less likely it seems it will have a built-in IR-blocking filter, but all the basic cameras from the likes of Olympus, Kodak and Nikon have a built-in IR filter) The lack of an IR filter will result in excessive background that effectively obscures any fluorescence signal.

The solution is to attach an IR-blocking filter to the camera. These are available from a variety of sources. Tiffen sell several different sizes for around $50.

How sensitive is the Dark Reader Transilluminator for DNA detection?
With SYBR Green, SYBR Gold and GelStar the detection limit, by eye, is 50 -100 pg of dsDNA. For ethidium bromide (EtBr) it is about 5 ng.
On Polaroid film, for SYBR Gold, SYBR Green and GelStar, the detection is 25 - 50 pg. The ethidium detection limit on Polaroid film is around 650 pg using our AF-09 camera filter - about twice as good as without the filter.
With a CCD camera system it is possible to detect 10 - 20 pg of SYBR- or GelStar-stained dsDNA. Ethidium detectability is about the same as with Polaroid film.
The new SYBR dye - SYBR Safe - is less sensitive than Green and Gold. It is possible to detect about 1 ng by eye and 500 pg using a CCD camera.

What is the cost of the new DNA dyes?
The purchase price of SYBR Gold and SYBR Green is significantly higher than that of EtBr. However, it is important to remember that, for example, SYBR Green is 5 -10 times more sensitive than EtBr and less mutagenic. Many people will consider these advantages sufficient to outweigh the additional cost.

Because the new generation of DNA stains are generally 5 - 10 times more sensitive than EtBr, it is possible to load correspondingly less DNA molecular weight standards and PCR reactions. This can result in significant savings. For example, a typical mini-gel with 2 lanes of DNA standards costs a total of about $1.86 if stained with EtBr and $1.43 if stained with SYBR Gold. If the samples loaded onto the gel are PCR reaction products, the use of SYBR Gold can result in savings of over $10 compared with EtBr.

My gel is very thin & fragile I would like to know if I can leave it placed on top of a glass plate whilst on top of the dark reader permanently to both view & capture an image via a digital camera?
Yes you can. No problem. Because the Dark Reader light is all visible blue, it passes easily through glass and plastic and there is virtually no loss of sensitivity. The Dark Reader also works very well for observing fluorophors in plastic tubes, Petri dishes, 96-well plates, etc.

Using EtBr on a UV transilluminator gave more concise DNA bands with less smearing. On the otherhand, the SYBR Green + Dark Reader results had very high levels of background smearing extending from the loading margin all the way down to the product.
Too much DNA! The SYBR + Dark Reader combo is at least 5 times as sensitive as the UV + EtBr method. So, if you are loading your 'usual amount' of DNA, you are going to be seeing all sorts of schmutz! Reduce the DNA loaded on the gel by a factor of 5 - 10.

Why `Dark Reader`?
Very simple: switch on a UV box in a darkened room and the walls (not to mention your teeth!) glow purple. Switch on the Dark Reader and the room remains just that - dark.

I would like to know if the fluorophor XYZ is compatible with the Dark Reader.
The ex/em maxima of XYZ are around 534/570. The excitation maximum would, at first glance, seem to be a bit on the long side. However, from looking at the spectrum there is a fair amount of excitation below 500 nm and this is what the Dark Reader would 'go after'. Tetramethylrhodamine (546/576) is the dye with the closest spectral characteristics we have experience with, and this works surprisingly well with the Dark Reader (though not quite as well as fluorescein, for example). Ultimately, the only way to find out if the Dark Reader is good enough for your particular XYZ application, is give it a try.

Does the DR work with Green Fluorescent Protein?
GFP has 2 excitation peaks at 395 and 470 nm. The 470 nm peak is maximally excited by the Dark Reader and the red-shifted variants (e. g., EGFP and EYFP from ClonTech) fluoresce superbly when illuminated with DR light. In addition, the DR does not cause the rapid photobleaching of GFP that occurs with exposure to UV. Visualization of wild-type GFP is usually not practical using a Dark Reader.

How well does the Dark Reader perform with 96-well plates?
Great. Because the excitation light used by the Dark Reader passes through most plastics and glass, the Dark Reader is up to 8 times more sensitive than a 312 nm transilluminator for the detection of, for example, fluorescein in 96-well plates, centrifuge tubes, etc.

Why should I worry about UV Exposure? I use a UV box inside a hood with a CCD camera.
You are well protected, but what about your DNA samples? By the time you have adjusted the focus, fixed the zoom, and set the exposure time your DNA is well cooked. If you intend to use the DNA for further reactions you will have to contend with a significant amount of DNA damage.