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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.

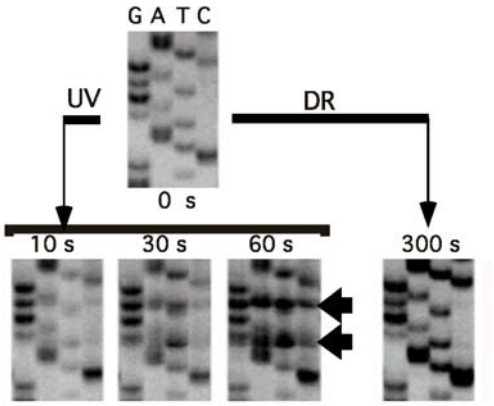
Exposure of the wheat germ DNA to 360 nm UV light for just 30 seconds had a large impact on the cloning efficiency. The plating efficiency of the UV-exposed DNA was reduced 170-fold compared to that of the Dark Reader-exposed DNA. This demonstrates that the UV-damaged DNA is significantly compromised 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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