dna revealed

Ethidium bromide, a potent mutagen

In research laboratories, ethidium bromide and similar fluorescent compounds are normally used to visualise DNA on a gel. Unfortunately, ethidium bromide and its breakdown products are potent mutagens and carcinogens and therefore *they should not be used in schools*. Such dyes are often flat molecules with similar dimensions to DNA base pairs. When ethidium bromide binds to DNA, it slips between adjacent base pairs and stretches the double helix. This explains the dye's mutagenic effect — the 'extra bases' cause errors when the DNA replicates. In addition, short-wavelength UV light (which itself is harmful) is required for ethidium bromide to fluoresce and reveal the DNA. For reasons of safety and because UV light of this wavelength causes unwanted mutations in the DNA being studied, several researchers have sought alternative methods of revealing DNA.

Safer alternatives

Crystal violet binds to DNA in a similar way to ethidium bromide and although it is a mutagen, it is not thought to be as harmful as ethidium bromide. Because it can be viewed in normal daylight (avoiding the need for damaging UV light), some researchers have advocated its use where functional DNA is to be recovered from a gel.

Thiazin dyes

The most widely used alternatives to ethidium bromide are methylene blue and its oxidation products, such as Azure A, Azure B, Toluidine blue O and Brilliant cresyl blue. These dyes are used individually or as mixtures (often in proprietary formulations). Although their exact mode of action is unknown, they are thought to bind ionically to the outside of nucleic acids (to the negatively-charged phosphate groups) and can therefore be used to detect both DNA and RNA.

Such dyes are not as sensitive as ethidium bromide, and some of them colour the gel heavily. Consequently, prolonged 'destaining' may be necessary before the DNA bands can easily be seen. Several dyes also fade rapidly after use — methylene blue falls into both categories and is therefore not ideal for staining DNA on a gel.

All of the thiazin dyes may be used in aqueous solution at a concentration of about 0.02–0.04% and applied to the gel after it has been run. They may also be dissolved in mild alkaline solutions (*e.g., running buffer; not over about pH 8*). Destaining with dilute acetic acid may be necessary for alkaline solutions.

The age of the dye may have a considerable effect upon the results achieved. For example, old samples of methylene blue will almost certainly contain a proportion of other dyes (such as Azure A and B) and these breakdown products may be responsible for much of the staining. Dye solutions are best stored in glass bottles (some dyes will stain plastic containers), either wrapped in foil or kept in the dark.

Staining DNA on the move

Recently, several commercial products have emerged that enable the DNA to be seen as it moves across the gel. Suppliers seldom reveal their composition, but several of these stains contain Nile blue sulphate (also known as Nile blue A), a dye which had not previously been noted for its ability to stain DNA. Adkins and Burmeister (1996) give useful guidance as to its use as well as hints for identifying other dyes which may be useful for visualising DNA.

All of the dyes used for staining 'mobile' DNA are cationic — that is, they are positively charged in the gel buffer, at pH 8. They move through the gel in the opposite direction to the DNA, latching onto the DNA molecules as they meet them. So that sufficient dye remains in the gel, it is added to both the gel and the buffer above it. However, a far lower concentration (1–3 μg per cm^3) of dye is necessary for this method than for post-electrophoresis staining. This is because too much dye will neutralise the negatively-charged DNA fragments, slowing their movement and reducing the resolution or even preventing the DNA from moving at all. Consequently, there is a compromise to be struck between visibility and resolution. Better results are usually achieved by staining the DNA *after* the gel has been run, rather than staining during the run.

Drying gels

It is also possible to dry a gel after the dye has been applied, and thereby to concentrate the dye in bands which would otherwise be difficult to see. So that the gel dries evenly, it is advisable to place the wet gel on a sheet of good-quality writing paper, and to place this on several sheets of filter paper. Moisture from the gel soaks into the filter paper, while the writing paper layer stops too much of the dye from soaking out of the gel. Gels should be dried at room temperature.

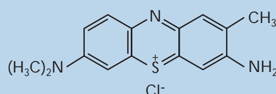
Light boxes

White light boxes (such as those sold for viewing photographic negatives or transparencies) are ideal for observing stained gels; the larger models tend to be more robust and reliable. A yellow-coloured filter may help when photographing gels that have been stained with blue dyes.

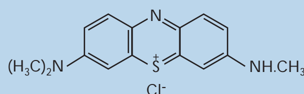
Safety

Although several dyes that can be viewed in normal daylight are thought to be relatively safe, they have not been as intensively studied as the fluorescent dyes for long-term toxic effects. Some of these visible dyes, apparently, intercalate DNA like ethidium bromide so they too have a potential for mutagenesis and, depending on absorption and metabolism, a potential for carcinogenesis. As with all laboratory chemicals, suitable safety precautions should be exercised when handling any dyes, particularly when they are in dry, powdered form.

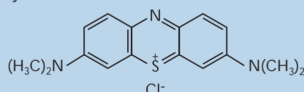
Toluidine blue O



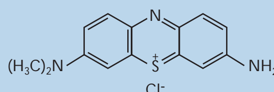
Azure A



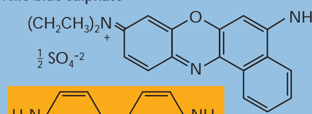
Methylene blue

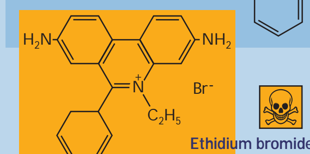


Azure B



Nile blue sulphate





Ethidium bromide

resources

Methylene blue
Yung-Sharp, D. and Kumar, R. (1989) Protocols for the visualisation of DNA in electrophoretic gels by a safe and inexpensive alternative to ethidium bromide. *Technique* **1** (3) 183–187.

Brilliant cresyl blue
Santillán Torres, J. and Ponce-Noyoia, P. (1993) A novel stain for DNA in agarose gels *Trends in Genetics* **9** (2) 40.

Nile blue
Adkins, S. and Burmeister, M. (1996) Visualization of DNA in agarose gels as migrating colored bands: Applications for preparative gels and educational demonstrations *Analytical Biochemistry* **240** (1) 17–23.
www-personal.umich.edu/~steviema/blueDNA.html

Crystal violet
Rand, N. (1996) Crystal violet can be used to visualise DNA bands during gel electrophoresis and to improve cloning efficiency *Technical Tips Online* www.biomednet.com/db/tto