Crystal violet can be used to visualize DNA bands during gel electrophoresis and to improve cloning efficiency

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I have found that it is possible to visualize DNA bands as they separate during agarose gel electrophoresis by including crystal violet in the gel and running buffer. This is particularly useful for isolating DNA fragments for cloning work. Bands can be cut out as soon as they are sufficiently resolved, and the DNA is not exposed to the damaging effects of ultraviolet (UV) light, as occurs when ethidium bromide is used as a marker.

For preparation of fragments, I routinely use Agarose NA (Pharmacia) dissolved in TAE (40 mM Tris-acetate, 1 mM EDTA, pH 7.2). Just before pouring, crystal violet (Sigma) (C 3886) is added to a final concentration of 10 µg/ml. The same concentration of dye is used in the TAE running buffer. DNA samples are loaded in 2% Ficoll 400 with 0.002% xylene cyanol; Bromophenol blue should not be used as it interacts with the crystal violet and interferes with the visualization of some bands. The gel apparatus is placed on a light box to allow illumination from below during electrophoresis. For maximum sensitivity, the running buffer should only just cover the gel.

The sensitivity is lower than in the case of ethidium bromide staining. For example, when 60 ng of λ DNA digested with HindIII was loaded onto a 3 mm thick minigel, the 4.4 kb band (about 5 ng) was only just visible during electrophoresis. For the larger, preparative gels used routinely for isolation of vector fragments, sufficient DNA should be loaded to give 100 ng or more of the required fragment. If less is loaded it may be necessary to increase sensitivity after electrophoresis by either of two methods. The first is to simply destain in water. The second is to further stain in a large volume of 10 µg/ml crystal violet dissolved in 0.1 X TAE.

Because of the reduced sensitivity, it is not suggested that crystal violet should replace ethidium bromide for any applications other than in cloning work. UV light is well known to cause damage to DNA (Ref. 1). Improved cloning efficiencies have been achieved by staining after electrophoresis with methylene blue (Ref. 2). However, this method has not gained widespread acceptance, perhaps because of the extra time needed for the staining and destaining of gels. The finding that crystal violet can be used to detect DNA bands during the running of preparative gels allows both quicker and easier fragment isolation. I have been unable to find any conditions that allow methylene blue to be used in the same way.

DNA isolated from crystal violet gels can be ligated and transfected with the same efficiency as untreated controls (Table 1). Improvements can also be achieved by avoiding the use of UV light (Table 1). The improvement in efficiency observed when crystal violet was used was threefold when compared with ethidium bromide and a transilluminator with UV tubes that emit maximally at 320 nm and tenfold in comparison with ethidium bromide and shorter wavelength (302 nm) UV. The actual improvement that will be achieved in any particular laboratory is impossible to predict, as the degree of damage depends upon several factors. The most important is the wavelength of the UV tubes used, the shorter wavelengths being more damaging (Ref. 1). Other factors include the range of UV wavelength emitted, the type and condition of the filter and transilluminator, the time taken to cut out bands, and whether or not the gel can be left on a gel tray that partially blocks...
Twelve 100 ng aliquots of EcoRI-cut M13mp18 RF DNA were electrophoresed on 1% agarose gels, then DNA from stained bands was purified by the Qiaquick method (Qiagen). To serve as controls, three 100 ng samples that had not been run on a gel were also purified by the Qiaquick method. The ethidium-bromide-stained gels were left on the gel trays during the 45 s needed to cut out the bands. Samples of each were self-ligated and then 100 pg samples were used to transfect competent XL1Blue cells (Ref. 4). Dilutions of the transfected cells were plated on lawns of XL1Blue and plaques counted after overnight incubation. The number of plaques resulting from the ligation of 5 pg of DNA is recorded.

UV. By avoiding the use of UV it may be possible to achieve more consistent results.

Crystal violet is usually readily available in labs as it is a component of the Gram stain, and is also used in a number of other staining protocols. Although it has been used in medicine for many years, for example as an antiseptic for external use, oral administration can have harmful effects (Ref. 3). Reduced use of UV transilluminators for isolating DNA bands should decrease the chance of inadvertent exposure of experimenters to UV light. Table 1.

Acknowledgements
I thank Geoff Grigg, Trevor Lockett, Julie Kelly, Peter Molloy and Garry Hannan for useful comments.

References

Table 1. Number of plaques obtained after transforming Escherichia coli with DNA from gels stained with ethidium bromide or crystal violet

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of plaques</th>
<th>Mean</th>
</tr>
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<tbody>
<tr>
<td>Crystal violet</td>
<td>188, 211, 220, 276</td>
<td>224±37</td>
</tr>
<tr>
<td>Ethidium bromide, UV 302 nm</td>
<td>5, 5, 6, 6</td>
<td>6±1</td>
</tr>
<tr>
<td>Ethidium bromide, UV 320 nm</td>
<td>66, 79, 86, 95</td>
<td>82±12</td>
</tr>
<tr>
<td>Control (not isolated from gel)</td>
<td>210, 212, 255</td>
<td>226±25</td>
</tr>
</tbody>
</table>

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References