UVP
ULTRA-VIOLET PRODUCTS

Corporate Headquarters: UVP, Inc.
2066 W. 11th Street, Upland, CA 91786
Telephone: (800)452-6788 or (909)946-3197
E-Mail: uvp@uvp.com

Internet: http://www.uvp.com

European Operations: Ultra-Violet Products Ltd.
Unit 1, Trinity Hall Farm Estate, Nuffield Rd.
Cambridge CB4 1TG UK * Tel: +44(0)1223-420022
E-Mail: uvpuk@uvp.com

Application Bulletin
UVP-AB-209

APPLICATION: DNA/RNA Cross-linking to nylon membranes.

WAVELENGTHS/ LAMPS USED: Shortwave (254nm)/TS Series Transilluminators, UVG-54, R-52G, C-50 Eprom Eraser

FIELDS OF USE: Biotechnology

BACKGROUND: The process of blotting nucleic acids onto nylon membranes from agarose and acrylamide gels induces the formation of ionic bonds between the membrane and nucleic acids. Subsequent hybridization of the nucleic acid containing membrane in this form (ionic bonds between nucleic acids/membrane) will result in a large loss of nucleic acid due to the ionic bond breaking nature of the chemicals used to perform the hybridization. Exposing the nucleic acid containing membrane to shortwave (254nm) ultraviolet eliminates such loss because the shortwave UV induces irreversible cross-linking of the nucleic acid to the nylon membrane.

PROCEDURE: Crosslinking simply involves exposing the nucleic acid side of the nylon membrane to shortwave (254m) ultraviolet. In the case of UVP's line of shortwave transilluminators and lamps this means laying the nucleic acid side of the nylon membrane on the filter itself. Crosslinking with the C-50 Eprom Eraser is performed by placing the nylon membrane on the tray with the nucleic acid side up. Exposure times vary from 25-45 seconds with UVP's C-50 Eprom Eraser, about two minutes with UVP's line of shortwave transilluminators, and, up to several minutes with the hand held lamps. Exposure time is determined by the intensity of the UV source and type of hybridization being performed.

PRIMARY ADVANTAGES OF THIS METHOD: UVP's C-50 Eprom Eraser, shortwave transilluminators and UVG-54 and R-52G hand held lamps save the researcher valuable time because their high intensities yield short exposure times.

The dipping method introduces the dye in a weak ethanol solution into which the part is dipped. After exposure the part is washed with water, dried and then examined under ultraviolet radiation.
The dusting technique involves mixing the dry dye with a finely divided diatomaceous earth and applying it to the parts with a "puffer" or dusting on with a camel hair brush. Dye not held by the contaminating film is then removed by washing with a weak ethanol solution and the contamination on visualized by the presence of fluorescence under ultraviolet illumination.
As a side benefit, the presence of other fluorescent organic and inorganic contaminants will be indicated in the examination, the intensity of fluorescence in all cases indicating the magnitude of the contamination.

EQUIPMENT NEEDED

For maximum contrast and visibility, the inspection should be done either in a darkened booth with a powerful ultraviolet lamp such as the UVP B-100A, or within a C-71 CHROMATO-VUE Cabinet which is a portable "darkroom" provided with ultraviolet lamps. A suitable fluorescent dye is the DF-502 Fluorescent blue, also made by UVP, Inc.