INTRODUCTION

In the past, nucleic acid studies requiring separation of macromolecules involved expensive ultracentrifuges and labour-intensive techniques. Today, life science research has been dramatically simplified with the use of electrophoresis techniques aided by UV transilluminators and crosslinkers, autoradiography, chemilluminescence and image documentation systems. Fluorescently stained gels, such as with ethidium bromide, are easily illuminated, manipulated, documented and analysed.

PROCEDURES

Preparation

Agarose gels are typically placed horizontally inside a buffer tank during electrophoresis to isolate double-stranded DNA fragments of defined sizes. Smaller fragment separations are performed in vertical gel boxes using acrylamide gels.

Visualization

To detect nucleic acids in gels, typically, ethidium bromide dye is intercalated to sample during electrophoresis or during staining after the run. Normally, the dye's natural fluorescence peaks at 590nm. However, when bound to nucleic acid, the complex is illuminated at the 300nm spectral region.

Manipulation

Using UV light to covalently bind nucleic acids to nitrocellulose or nylon membranes is an easy and effective technique to provide a permanent sample transfer for increased sensitivity in hybridization signals.
The nucleic acid is first blotted onto a membrane (Southern and northern) and then exposed to short wave UV light (254nm), activating the T or U bases to bind with the primary amine groups on the matrix. This forms a stable bond between bound molecules and the membrane, permitting use of highly stringent assay conditions. To achieve low backgrounds, concentrations of SDS at 5-7% or 20X SSC can be used in hybridization solutions or washes. Without crosslinking, target nucleic acids on blots can be removed under these conditions. For convenience and accuracy, most crosslinkers provide a preset optimal UV crosslinking dosage of 120mJ/cm2 with the touch of a button (SPECTROLINKER·, Optimal Crosslink mode). For models equipped with internal UV photosensor and microprocessor, precise dosage is assured with compensation of exposure time as UV tubes age.

Documentation and Analysis

Nucleic acids labelled with radioisotopes such as 32P, 35S, 14C, can be detected

After crosslinking, the nucleic acid blots are hybridized with radioactively labelled probes. The hybridized specimen is then exposed to X-ray film within a FOURSQUARE· stainless steel cassette stored at (-70°C) for several days. The emulsion in the film reacts to ionizing radiation particles thus forming a spatial distribution image of the labelled sample. To reduce exposure time and increase image contrast, one or two L-PLUS· intensifying screens can be used.
Recently, the use of safer and economical chemilluminescent probes have gained popularity due to their greater sensitivity, shorter exposure time and comprehensive protein probing capabilities. Much like genomic blots, western blots are exposed to chemi probes with cassettes. Our lightweight, easy to use, push-button latch-type VALUE™ cassettes are perfect for this application.

SUMMARY

High-efficiency laboratory tools such as UV crosslinkers, transilluminators and autoradiography cassettes play an important role in the rapidly expanding field of life science research. They are not only crucial for basic research but are also essential for enhancing the ability of researchers to manipulate, document, and analyse molecular samples.

Thus, Spectroline▪ 312nm UV transilluminators provide the best sensitivity while minimizing pyrimidine dimerization associated with shorter wavelength transilluminators. Available as accessories, UV-transmitting filter protectors let researchers manipulate the gels directly on the transilluminators without damaging the expensive UV filter glass.	directly using autoradiography. It is a highly sensitive yet simple method that documents radio-labelled samples by exposing them to high-speed X-ray film.