ULTRA-VIOLET PRODUCTS

Application Bulletin
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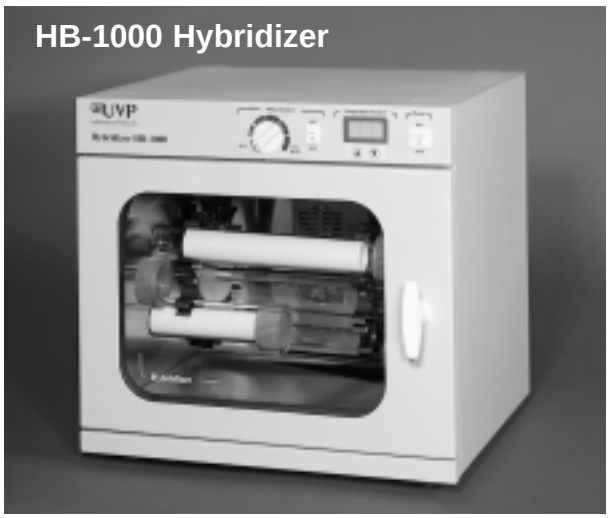
USE OF HYBRIDIZATION OVENS IN THE LABORATORY

The HB-1000 Hybridizer is a high-performance instrument for procedures involved in Southern, Northern, and *in situ* hybridization. UVP's state-of-the-art design includes precise microprocessor temperature controls with rotation speed control and various bottle positioning for dynamic mixing capability.



INTRODUCTION

Hybridization is the annealing of nucleic acids or proteins to other nucleic acids, proteins, or specific probes. With the HB-1000 Hybridizer, the process of covalently binding the nucleic acids to probes is easy and effective. Various applications can be performed with the Hybridizer, including Southern, Northern, Slot and Dot blotting, and RNA primer extension.

Western blotting is the localization of the DNA fragment of interest. The DNA strand is first digested by restriction enzymes and then separated by electrophoresis. The DNA fragments are denatured and transferred to a solid support, usually nylon or nitrocellulose membranes [1]. The fragments are fixed onto the membrane with the CL-1000 UV Crosslinker. The DNA attached to the membrane is then hybridized to radiolabeled probes (may be DNA or RNA). Using autoradiography, the position of the fragment of interest is located.

Northern blotting is the localization of the RNA of interest by the hybridization of the RNA with radiolabeled DNA or RNA probes. RNA is first separated by electrophoresis and then transferred to cellulose, nylon, or nitrocellulose. The membrane is then fixed using the CL-1000 Crosslinker. Like Western blotting, the RNA is hybridized to probes and detected by autoradiography.

Dot and Slot blotting is performed by spotting a small sample of RNA onto nitrocellulose and then hybridized to a specific RNA or DNA probe. The membrane is then exposed to X-ray film. It provides a way quantitate the intensity of specific gene expression [2].

Primer extension is the hybridization of the RNA of interest with a single stranded DNA primer, radiolabeled at the 5' end. Using reverse transcriptase, the primer extends to form a complementary strand to the RNA template, creating a cDNA.

MATERIALS AND METHODS

A. SOUTHERN BLOTTING

Digest 10pg to 10µg of DNA with restriction enzymes then add 10µl of loading buffer to the sample. Perform agarose gel electrophoresis. Transfer the fragments from the gel to nylon or nitrocellulose membrane filters with capillary, electrophoretic, or vacuum transfer. Capillary transfer is the most popular method [3]. DNA is then fixed onto the membrane by various applications: UV irradiation, baking, or vacuum [4].

The filter is the placed in the hybridization tube with 0.2 ml of prehybridization solution for each square cm of filter. Incubate the tube for 1-2 hours at 40-68°C. Add the probes to the solution, once again incubate the tube in the Hybridizer at the prehybridization temperature for 6 hours to overnight. Wash the filter and view it using autoradiography.

B. NORTHERN BLOTTING

Run denatured RNA electrophoresis using glyoxal and dimethyl sulfoxide or gels containing formaldehyde [5]. Transfer RNA to nitrocellulose membrane using capillary transfer method for about 6 to 24 hr. Fix the membrane by UV irradiation, baking, or vacuum. Prehybridization, hybridization, and washing methods are the same as in Southern blotting but with different solutions [6]. View with autoradiography.

C. DOT AND SLOT BLOTTING

Put a piece of wet nitrocellulose filter on the bottom of the sample wells of the filtration manifolds. Connect the vacuum. Mix RNA with solution and load the samples into the wells. Apply gentle suction. Remove filter and dry it at room temperature. Fix by UV irradiation, baking, or vacuum method [7]. Perform hybridization and autoradiography using the methods described in Northern blotting.

D. PRIMER EXTENSION

Mix radiolabeled DNA primer with 0.5-150 µg of the RNA of interest. Add sodium acetate and ethanol and precipitate. Add hybridization buffer after precipitation. Incubate the mixture at 85°C for 8-12 hr in the Hybridizer. Extract the nucleic acids and dissolve primer [8]. Analyze by electrophoresis and autoradiography.

REFERENCES

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8. Short Protocols in Molecular Biology. “Primer Extension”.