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Reusing Nylon Membranes for Radioactive Hybridizations

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ABSTRACT

We describe a procedure for recycling nylon hybridization membranes, enabling their repeated use for radioactive Southern hybridization analysis of different DNA samples. Following hybridization and probe removal, nylon membranes containing covalently linked DNAs were treated with 0.55% sodium hypochlorite. This destroyed the DNA, thereby preventing it from participating in further hybridization and enabling the membranes to be used subsequently for binding new DNA samples. With this procedure, we were able to reuse a single membrane as many as 13 times, with no detectable loss in signal. This method was shown to be effective for membranes supplied by three different manufacturers.

INTRODUCTION

Nylon hybridization membranes can represent a significant recurring laboratory expense. After transfer to membranes by methods such as capillary transfer, vacuum- or electroblotting, DNA samples are usually exposed to UV light to cross-link them to the membrane surface (3). The resulting covalent linkages are strong enough to retain the immobilized DNA after boiling or alkali treatment, enabling the same DNA samples to be rehybridized with different probes. Consequently, it is natural to consider that UV cross-linking affixes DNA permanently to the membrane, and this will often result in a membrane being discarded after just one use. We considered this to be wasteful of the potential binding capacity of a typical membrane (450–600 $\mu\text{g}/\text{cm}^2$) (1,4). Therefore, in an effort to conserve these expensive resources, we sought to develop a method that would allow removal, or at least inactivation, of previously bound DNA, so that the membranes could be reused.

Sodium hypochlorite is useful for destroying DNA contaminants from sur-

faces and glassware used for PCR experiments (2). Therefore, we investigated whether it could be used to destroy DNA on hybridization membranes, without destroying the ability of the membrane to bind new DNA samples.

MATERIALS AND METHODS

DNA Preparations

Cosmid clones. Cosmid clone G27-9-E, containing DNA of the fungus *Magnaporthe grisea*, was maintained in *E. coli* strain DH5 α TM (Life Technologies, Rockville, MD, USA). DNA was prepared by alkaline lysis, followed by CsCl density gradient centrifugation.

λ HindIII dilution series. Four stock solutions of HindIII-digested λ DNA (Amersham Pharmacia Biotech, Piscataway, NJ, USA) were prepared, representing a twofold dilution series starting at 100 pg/ μL and going down to 12.5 pg/ μL . The dilutions were prepared using 1 $\times$ bromophenol blue/sucrose gel loading dye made in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). Ten microliters of each dilution were loaded in adjacent wells, providing total DNA amounts of 1 ng, 500 pg, 250 pg, and 125 pg per lane.

Solutions

Commercial bleach (Clorox, Oakland, CA, USA) was diluted tenfold to a final concentration of 0.55% sodium hypochlorite (v/v).

Electrophoresis and Southern Analysis

All electrophoresis experiments were performed using 0.7% SeaKem[®] LE agarose (BMA, Rockland, ME, USA) made in 0.5 $\times$ TBE buffer. After electrophoresis, the DNA was depurinated by shaking the gel gently in 0.25 M HCl for 20 min. The gel was rinsed with Nanopure water (Barnstead/Thermolyne, Dubuque, IA, USA) and then treated with denaturation solution (0.5 M NaOH, 1.5 M NaCl) for 30 min. After a second rinse in Nanopure water, the gel was neutralized for 30 min in a buffer of 0.5 M Tris-HCl, pH 7.5, 1.5 M NaCl. Membranes were wetted in water and then equilibrated in neutralization

buffer. Unless stated otherwise, experiments were performed using 0.22- μm MagnaGraph membranes (Osmonics, Westborough, MA, USA). Capillary transfer of DNA was performed without a wicking system, using the buffer in the gel to carry the DNA onto the membrane. After transfer, the blots were air-dried, and the DNA was cross-linked to the membranes by exposure to 1200 μJ UV light in a Stratalinker[®] (Stratagene, La Jolla, CA, USA).

Hybridization and Probe Removal

Membranes were wetted and prehybridized in 20 mL hybridization buffer [0.125 M NaHPO₄ (Na₂HPO₄ adjusted to pH 7.2 with H₃PO₄), 7% SDS, 10 mM EDTA, 50% formamide]. Prehybridization was performed for 10 min at 42°C.

The, λ HindIII probe was ³²P-labeled by oligolabeling (Amersham Pharmacia Biotech) to a specific activity of approximately 1.5×10^8 cpm/ μg . Circular cosmid DNA was labeled by nick translation (Life Technologies) to a specific activity of approximately 5.4×10^7 dpm/ μg . In both cases, unincorporated nucleotides were removed by centrifuging through a spin column of Sephadex[®] G-50 (Amersham Pharmacia Biotech). Denatured probe was added directly to the prehybridization solution. Hybridization was performed for 16 h, after which the membranes were washed at increasing stringency levels, with the final wash performed at 65°C with 0.1 $\times$ SSC; 0.1% SDS. Membranes were then blotted with paper towels and exposed to phosphor imager screens. Unless stated otherwise, exposure times were 2 h for cosmid DNA samples and 24 h for λ HindIII samples. Screens were scanned in a PhosphorImager[®] 445 SI (Amersham Pharmacia Biotech) using a pixel resolution of 176 μm . Quantitation of hybridization signals was performed using the volume quantitation function of the software program ImageQuantTM 4.0 (Amersham Pharmacia Biotech).

After imaging, probes were removed by heating for 90 min at 110°C in a preheated solution of 0.1 $\times$ standard saline citrate (SSC), 0.1% SDS (SSC/SDS), and disposed of according to the University of Kentucky's radiation safety guidelines.

Treatment of Membranes with Sodium Hypochlorite

The ^{32}P -labeled probes used previously were removed before subsequent treatment for radiation safety purposes. Membranes were washed in 0.55% sodium hypochlorite for 10 min with shaking at room temperature. The hypochlorite was then removed by washing the membrane three times by shaking for 10 min in 100 mL Nanopure water. Membranes were air-dried and stored between paper towels in plastic bags.

RESULTS

In a preliminary experiment, 0.5 μg *Bam*HI-digested cosmid (27-9-E) was loaded into each of three lanes of an agarose gel. After electrophoresis, the gel was blotted to a single MagnaGraph membrane by overnight capillary transfer. The DNA was covalently linked to the membrane, which was then cut into two pieces (Nos. 1 and 2), thus ensuring that both membranes and the DNA samples affixed to them had received equal treatment. Hybridization with a ^{32}P -labeled 27-9-E probe, resulted in very intense signals after only a 2-h exposure

(Figure 1A). As expected, the signal intensities on membrane Nos. 1 and 2 were approximately equal (Figure 1A).

The probe was then removed from both membranes by hot SSC/SDS treatment, and confirmation of probe removal was performed by phosphor imaging (results not shown). Only membrane No. 2 was subsequently treated with sodium hypochlorite, followed by rinsing in Nanopure water. Both membranes were then rehybridized in the same bottle, using a fresh 27-9-E probe. Membrane No. 1 again produced a strong hybridization signal after only a 2-h exposure (Figure 1B), thereby acting as a positive hybridization control. In contrast, membrane No. 2 yielded no signal, even after a 24-h exposure (Figure 1B). This demonstrated that the hypochlorite treatment had destroyed all DNA that had been bound to this membrane. It should be noted, however, that probe removal is not strictly necessary because we have shown that probe DNAs are also destroyed by hypochlorite treatment (results not shown).

Membrane No. 2 was then rinsed in water followed by denaturation solution and subsequently used to blot a gel

Figure 1. Inactivation of DNA bound to a nylon membrane and binding of a new sample. Cosmid G27-9-E (2.5 μg) was digested with *Bam*HI, and 0.5 μg was loaded into each of three lanes on an agarose gel. After electrophoresis, the gel was subjected to the pretransfer washes and blotted to a single membrane. After hybridization with a ^{32}P -labeled G27-9-E probe, the gel was cut into two pieces, Nos. 1 and 2 (A). The probe was removed by hot SDS treatment, and membrane No. 2 was treated with 0.55% sodium hypochlorite. (B) Membrane Nos. 1 and 2 after rehybridization with the G27-9-E probe are shown. Membrane No. 2 was then used to blot a *Pst*I digest of G27-9-E, which was then hybridized with G27-9-E (C).

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containing two lanes of *Pst*I-digested 27-9-E DNA (0.5 µg/lane). The new DNA samples were fixed to the membrane by cross-linking with UV light and then probed with the 27-9-E probe. This time, strong hybridization signals were obtained after a 2-h exposure (Figure 2C), indicating that membrane No. 2 was still able to bind new DNA samples. There was no evidence of hybridization to the previously bound *Bam*HI-digested DNA, providing additional confirmation that the original DNA was fully destroyed (Figure 1C).

These results clearly demonstrated that treatment of membranes with sodium hypochlorite effectively destroys DNA bound to a membrane, without destroying the binding capacity of the support. However, for practical application of this procedure, it is desirable that the sodium hypochlorite treatment does not result in a significant reduction in signal intensity. To address this issue, we blotted twofold serial dilutions of *Hind*III-digested λ DNA onto fresh and recycled membranes, so that the smallest fragments in the final dilution were approaching the limit of detection. This enabled the signal intensities to be visually compared. After each experiment, the probe was removed, the membrane was then subjected to hypochlorite treatment, reprobed, and the signals were compared to those obtained using a fresh membrane. In this manner, we investigated how many times a membrane could be recycled before a loss in signal intensity was detected, as compared with the results obtained with a fresh membrane.

To make the comparisons as accurate as possible, we performed the experiments in a manner that sought to minimize experimental variability. All the membranes used for this study were cut from the same roll. Fresh and reused membranes were used to blot replicated dilution series that were electrophoresed on the same gels, so that the depurination, denaturation, and neutralization washes were equivalent. The membranes were then UV-fixed and subjected to hybridization and posthybridization washes in the same bottle. Finally, they were exposed at the same time in a single phosphor imager cassette.

Figure 2 shows the results of a comparison of three membranes used 1, 5,

and 13 times. We would like to emphasize that each time a membrane was recycled, it went through all the steps of DNA binding, hybridization, probe stripping, and hypochlorite treatment. Confirmation of probe removal was not performed in these experiments. Instead, we relied on the hypochlorite to destroy any residual probe that may have remained. From the results shown in Figure 2, it is clear that hypochlorite treatment of the membranes does not affect their ability to bind DNA because the limit of detection is similar on all three membranes. For example, the 560-bp λ *Hind*III fragment in the lane containing 0.25 ng total DNA is clearly visible on all three membranes. In the original scan, this fragment was also visible on all the membranes in the lane containing 0.125 ng total DNA. Thus, the overall sensitivity of detection in these experiments was down to 1.44 pg target DNA.

Overall, the hybridization signals of comparable fragments appeared very similar among the three membranes. Quantification of hybridization signals using ImageQuant 4.0 confirmed this and revealed that recycled membranes may actually yield slightly better results than fresh membranes (results not shown). This effect was reproducible and suggested that binding capacity may actually be improved by hypochlorite treatment and/or by repeated hybridization.

We tested the efficacy of our recycling protocol on other membranes, including uncharged membranes such as Hybond™ N (Amersham Pharmacia Biotech), Pall Biodyne® B (Nalge Nunc International, Rochester, NY, USA) and NYTRAN® (Schleicher & Schuell, Keene, NH, USA), and a positively charged membrane, Gene Screen Plus™ (NEN Life Science Products, Boston, MA, USA). In each case, the

Figure 2. Comparison of hybridization signals obtained from membranes used 1, 5 and 13 times. Three replicates of a dilution series of *Hind*III-digested λ DNA were electrophoresed in the same gel. The gel was then subjected to depurination, denaturation, and neutralization washes, and the DNA was subsequently transferred onto fresh and recycled membranes. After hybridization with a ³²P-labeled λ DNA probe, the membranes were washed to high stringency, wrapped in Saran® Wrap, and exposed to a phosphor imager screen. The number of times each membrane had been recycled (blotted, hybridized, stripped, and Clorox-treated) is shown above each dilution series. The total amount of DNA loaded is shown above each lane. Fragment sizes are shown on the left.

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recycled membranes produced similar signals to fresh ones when hybridized in parallel (results not shown).

DISCUSSION

We have found that sodium hypochlorite treatment provides a simple, safe, and economical way to enable the reuse of nylon membranes. The procedure we have described is effective in regenerating membranes supplied by different manufacturers and worked with both neutral and charged nylon supports. However, we did not quantify the signals or perform multiple cycles of hypochlorite treatment on membranes from other manufacturers, so we cannot attest to their relative performance or their durability through repeated use.

We have not determined the fate of the hypochlorite-treated DNA. It is possible that it remains on the membrane in a chemically altered form that no

longer hybridizes. In that case, one might expect a membrane eventually to become saturated. However, given the high DNA binding capacity of a typical membrane (450–600 $\mu\text{g}/\text{cm}^2$) (3,4) and the fact that it has two sides, saturation is not likely to occur before the membrane is too damaged to allow good contact with the gel surface.

The potential to reuse membranes should certainly provide an economical benefit to many laboratories. In addition, we anticipate that the ability to use a single support for binding multiple DNA samples may find applications in high-throughput DNA analysis.

REFERENCES

1. **Amersham Pharmacia Biotech.** 2000. Product Catalog, p. 448-449. Amersham Pharmacia Biotech, Piscataway, NJ.
2. **Andres, L. and A.M. Prince.** 1994. PCR: how to kill unwanted DNA. *BioTechniques* 12:358-360.
3. **Church, G.M. and W. Gilbert.** 1984. Genomic sequencing. *Proc. Natl. Acad. Sci. USA* 81:1991-1995.
4. **Micron Separations.** 2000. Transfer Membranes, Blotting Paper and Protocols Catalog, p. 6. Osmonics, Minnetonka, MN.

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