Zeta-Probe® Blotting Membranes for Northern, Southern, Alkaline Southern, and Western Blotting

1. INTRODUCTION

Zeta-Probe blotting membranes are high tensile-strength cationized nylon membranes which carry a high density quaternary amine charge. Zeta-Probe membranes have unique binding and handling characteristics, making them a useful complement to nitrocellulose membranes for both nucleic acid and protein blotting applications. Many of the important attributes of Zeta-Probe membranes are only now being recognized,[30,31] and are presently being exploited in both novel[18,23,24,30,44] and routine[35-38] nucleic acid blotting applications. These applications and benefits are detailed in this bulletin. Zeta-Probe membranes are among the most widely used nylon-based blotting membranes for nucleic acids research.

2. HISTORY OF NYLON MEMBRANES

Uncharged nylon membranes (Nylon-66) were first introduced, and applied to the Southern blot hybridization method,[11] in 1981. These membranes generated much interest due to their superior physical characteristics, such as resistance to cracking, shrinking, and distortion, compared to nitrocellulose. Furthermore, it became obvious that RNA could be stably bound in low ionic strength buffers, which is a clear advantage compared to the popular method described by Thomas.[13] However, hybridizations using uncharged nylon membranes had the disadvantage of high non-specific backgrounds following reactions with radioactive probes. Binding of nucleic acids to uncharged nylon was also known to be pH dependent, as the net charge of Nylon-66 varies from positive at pH 4.0 to negative at pH 7.0.

Bio-Rad introduced Zeta-Probe positively charged nylon membranes in early 1982. Zeta-Probe membranes were developed in order to overcome the deficiencies inherent in uncharged nylon. Zeta-Probe membranes allow the advantages of the physical and chemical characteristics of nylon membranes to be exploited.

Zeta-Probe membranes were created by modifying Nylon-66 to contain a high density positive charge. This modification allows better standardization and reproducibility of binding and detection of nucleic acids in Southern[11] and Northern[13] procedures. In addition, the high density, positively charged surface of Zeta-Probe membranes increases the intensity of the binding of native or denatured nucleic acids and assures their firm attachment throughout hybridizations, washes, and stripping procedures. In contrast, nitrocelluiose and uncharged nylon surfaces carry low density net negative charges at the neutral pHs typically used in transfers.

3. FEATURES AND BENEFITS OF ZETA-PROBE MEMBRANES

The features of Zeta-Probe membranes are summarized in Table 1.

Table 1. Key Features of Zeta-Probe

Physical and Chemical Properties	Strong, flexible, not brittle Heat resistant, non-flammable, autoclavable Two-sided, no curls, no additives Optimal 0.45 micron pore size Resistant to most organic chemicals Stable at room temperature, user ready
Transfer and Binding Properties	5–10 × capacity of nitrocellulose 2–4 hour transfers possible Binds native as well as denatured DNA Binds ≥ 6 bases H_2O, NaOH, HCl, TAE, or TBE OK for binding NaOH or UV-crosslinking OK
Hybridization and Detection Properties	Low background with BLOTTO Sensitivity up to ten times greater than conventional Southern blots Greater resolution than conventional Southern blots Bound nucleic acids more hybridizable RNA, DNA, multiple hybridizations – up to 12 × Dual labeling from single blot High stringency hybridizations are possible

A. Physical and Chemical Properties

- **High tensile strength.** Pliable and flexible, Zeta-Probe membranes won't shrink, tear, or become brittle during transfer, baking, hybridization, or reprobing. Their strength allows vigorous agitation, which improves hybridization efficiency and background control.
- **Heat resistant, non-flammable, and autoclavable.**
- **One optimal pore size (0.45 μ).** Smaller sizes are unnecessary, since they increase background without enhancing signal. Larger sizes decrease resolution and binding of oligonucleotides.
- **Two-sided, non-laminated, non-curling.** Both sides equal, unlike other nylon-based membranes.
- **No detergents added.** Thoroughly wettable without additives.

- **Stable and user ready.** Stable dry at room temperature. No pre-activation or salt pretreatment required.
- **Chemically resistant.** Not affected by strong organic reagents used in probe stripping or hybridizations, i.e. 100% formamide, 2M NaOH, 4M HCl, acetone, most alcohols, DMSO, DMF, and chlorinated aliphatic hydrocarbons.

B. Transfer and Binding Properties

- **Rapid transfers.** Alkaline blots complete in 2–4 hours, no pre-treatments required.[30,32,33]

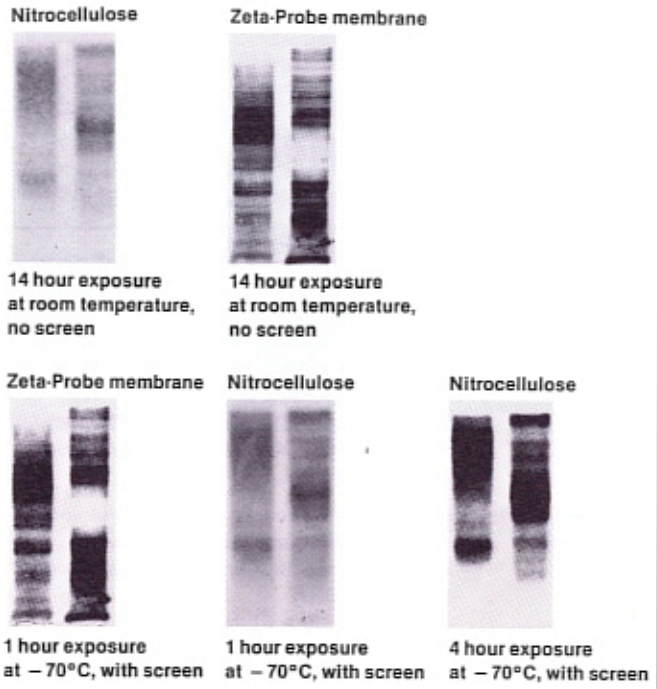

Fig. 1. Alkaline Southern Blotting. Genomic DNA was digested with restriction enzymes, electrophoresed in agarose gels, then blotted to nitrocellulose using standard Southern blotting procedures, or to Zeta-Probe membrane using alkaline transfer. Data provided by Dr. Ken C. Reed, Australian National University, Canberra, Australia.

- **High capacity.** 5–10 fold greater than nitrocellulose. DNA remains firmly bound by the strong positive charge throughout hybridization, washing, and reprobing.
- **Native and denatured DNA bound.**[30,32,33]
- **Low ionic strength binding and transfer solutions used.** Zeta-Probe membranes bind nucleic acids independent of buffer pH and salt concentration, unlike nitrocellulose, diazotized-papers, and uncharged Nylon-66 membranes. Binding is efficient in common electrophoretic buffers, dH_2O, 0.4M NaOH, 0.2M HCl.[30] 20× SSC is not required and, in fact, transfer efficiency of DNA greater than a few hundred bases appears inversely proportional to the ionic strength of transfer buffer. Reed observed a two-fold increase in signal for 0.8–3 kb DNA transferred in dH_2O vs. 20× SSC.[30] Zeta-Probe membrane remains positively charged between pH 2–14, unlike uncharged nylon. The net charge of Nylon-66 may vary from positive at pH 4.0 to negative at pH 7.0.
- **Small oligonucleotides bound.** Qualitative retention of labeled DNA as small as six bases, and detection, by hybridization, of bound fragments as small as 12 bases[24] (Figure 2). DNA greater than 40 bases is quantitatively retained with baking fixation, eliminating the need for ultraviolet crosslinking. Quantitative retention of DNA less than 40 bases following UV-crosslinking.[24, and Gross, personal communication]

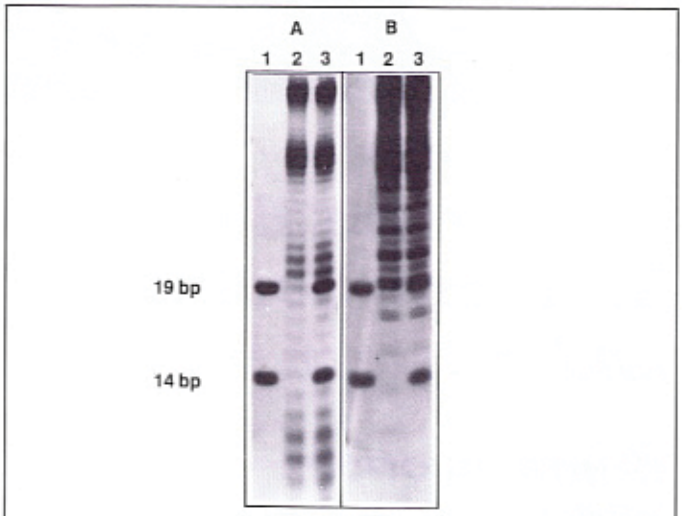

Fig. 2. Detection of oligonucleotides on Zeta-Probe membrane. Panel A is a fluorogram of ^{3}H-DNA isolated from DNase I digested mouse nuclei, with ^{32}P-labeled 14-mer and 19-mer standards included separately (lane 1), or mixed with mouse DNA (lane 3). Panel B is an autoradiogram of the same blot following hybridization to a ^{32}P-labeled poly (dT-dG) probe. Data provided by Dr. David S. Gross, UTHSC, Dallas, Texas.

- **Crosslinking DNA.** DNA can be fixed to Zeta-Probe membrane by baking, alkaline treatment, or ultraviolet crosslinking.[18,33] Residues of the nylon membrane "backbone" may interact with DNA thymidine dimers formed upon illumination with 254 or 300 nm UV-light. The polyamide structure of nylon can also be acid or base hydrolyzed to form highly reactive carboxyl and primary amino groups. Covalent binding of DNA to Zeta-Probe membranes in 0.4M NaOH is found to be superior.[30] Reed observed a two-fold to four-fold reduction in hybridization signal when alkaline crosslinked blots were further treated for 5 minutes under a 300 nm UV-light (at a distance of 60 cm).[30] Gross also observed a loss of hybridizability when using short A–T rich probes to mouse genomic DNA following UV-crosslinking. He further found no increase in sensitivity when detecting DNA larger than 80 bases whether UV-crosslinking was used or not. (personal communication)

C. Hybridization and Detection Properties

- **Low backgrounds.** Highest sensitivities and very low non-specific backgrounds are possible with proper blocking.[34] With Zeta-Probe membrane's high capacity, blocking is more critical than with nitrocellulose. In some applications, Denhardt's solution plus carrier DNA has been found to be inadequate as a blocking agent. Church replaced Denhardt's and carrier DNA with 7% SDS in both prehybridization and hybridization solutions;[18] however, caution must be exercised with this procedure, as quantitative DNA binding and hybridization may be affected by this treatment. Reed found that a 0.5% BLOTTO (non-fat dried milk)[5], plus 1% SDS completely eliminated non-specific background even when self-complementary probes were used in hybridization solution containing 10% dextran sulfate.[30,34] Use of mini gels and mini blots further helped control background, as larger blots are more difficult to keep bathed continuously in blocking solution when contained in plastic bags. Recent publications discuss practical aspects of nucleic acid hybridizations[7,15,24,34,38] and preparation and use of DNA and RNA probes.[7,23,34,36,38] Some of these discussions are particularly relevant to high capacity nylon-based membranes.

- **Sensitivity.** Improved resolution from alkaline blots[30,32] plus increased binding capacity, may generate up to 10-fold greater detection signals on Zeta-Probe membranes, as more DNA is bound and available for hybridizations. In standard Southern blots,[11] DNA may partially renature following gel neutralization and not be retained by the nitrocellulose. Homologies are more clearly and definitively detected, and more information has been obtained upon analysis of genome organization.[32] High salt concentrations (low stringency hybridizations) are not essential, unlike nitrocellulose, due to the strong binding of DNA to Zeta-Probe membranes (Figure 1).
- **Resolution.** Alkaline transfers eliminate the need for gel pretreatments (depurination, denaturation, and neutralization) and reduce transfer time.[30,32] The resulting decrease in diffusion of DNA results in sharper bands on blots (Figure 1). As transfers are more sensitive, intensifying screens may be eliminated from autoradiographic procedures, further improving resolution. Zeta-Probe membrane surfaces are smooth, not highly textured like diazotized papers.
- **Hybridizability.** DNA is stably bound and has been observed to be more readily hybridized when compared to nitrocellulose (Figures 1 and 6).
- **Dual-label experiments possible.** [^{3}H]-labeled DNA can first be detected by fluorography, the fluor can then be removed by acetone washes, and hybridization with [^{32}P]-labeled probes performed followed by autoradiography[24] (Figure 2).
- **Multiple probing.** DNA has been rehybridized for as many as 12 consecutive probings (Figures 3 and 4).[23] Others have reprobed Zeta-Probe membrane bound nucleic acids[22,29,34,37,48,51,54,57] and RNA has been reprobed up to 6 times.[44] Small DNA is efficiently retained throughout stripping in boiling 0.5% SDS plus 0.1% SSC.

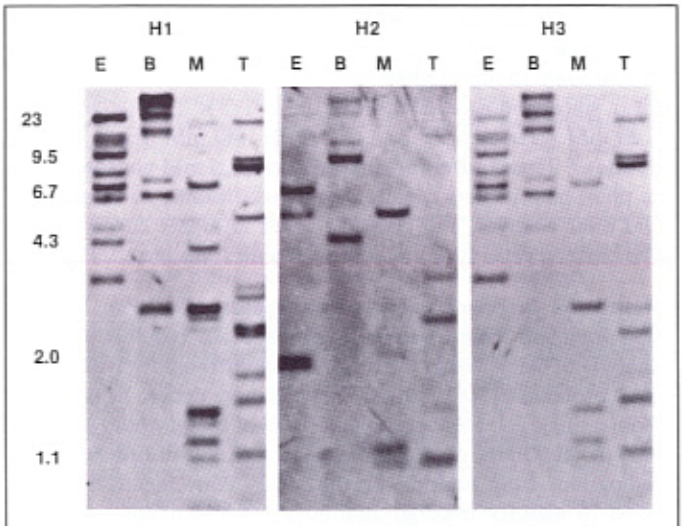

Fig. 3. Sequential hybridization to the same genomic Southern blot. Total human DNA was digested with one of 4 restriction enzymes, E, EcoRI; M, MspI; B, BamHI; T, TaqI. After Southern blotting to Zeta-Probe membranes, the blots were hybridized sequentially with three different probes, H1, H2, H3. Data provided by Dr. Richard A. Gatti, UCLA.

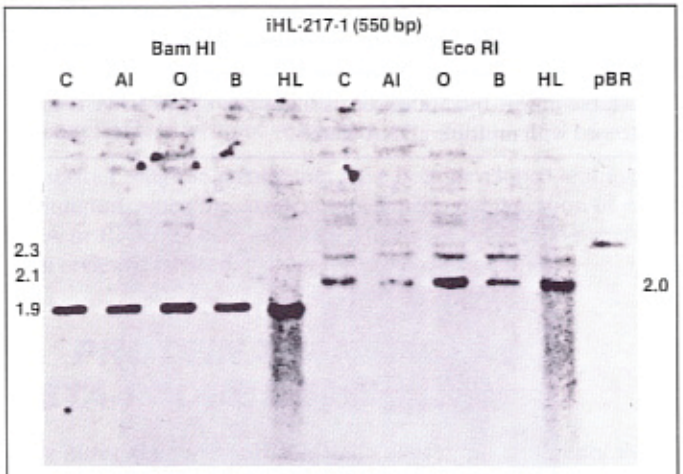

Fig. 4. Rehybridization on Zeta-Probe membranes. DNA transfer to Zeta-Probe membranes was followed by hybridization. This figure shows the autoradiogram prepared after the eleventh round of hybridization. After each cycle of hybridization the radiolabeled probe is selectively removed by treatment with SDS. Data provided by Dr. Richard A. Gatti, UCLA.

4. NUCLEIC ACID BLOTTING APPLICATIONS AND BENEFITS OF ZETA-PROBE MEMBRANES

Applications and methods are summarized in Tables 2 and 3.

Table 2. Zeta-Probe Membrane Applications

	References
Southern Blotting	
Genomic organization/function	16, 17, 18, 19, 24, 26, 27, 29, 30, 36, 37, 38, 39
Restriction fragment polymorphisms	20, 22, 23, 25, 39, 40, 41
Restriction enzyme mapping of clones	16, 27, 35, 39
Genetic recombination	28, 42
Footprint analysis of native genes	18
Homologies and plasmid typing	21
S_1 nuclease transcript maps	29
Northern Blotting	
Homologies	43, 44, 53, 58
Expression and transcript abundance	45, 50, 51, 52, 54, 55, 57
Transcript characterization	35, 48, 56, 58
Synthesis and processing of transcripts	47, 49
Characterization of cloned probed	48, 54
Dot Blotting	
DNA sequence abundance	15, 24, 26
RNA transcript abundance	46
Probe cross-hybridization	48
RNA processing	47

- **Southern blotting.** Zeta-Probe membrane is ideal for single copy detections of genes within complex eucaryotic genomes[36,38] and demonstrates superior sensitivity and resolution.[30,31] Denatured DNA may be bound quantitatively and is thus more accessible to hybridization.[30] Small oligonucleotides are bound, as well as large DNA.[24,30] Multiple probings may be conveniently performed.[22,23]
- **Northern blotting.** RNA is efficiently and directly bound to Zeta-Probe membrane in low ionic strength solutions including water, and in all commonly used denaturants, i.e. glyoxal,[30,35,50,51,54,57] formaldehyde,[46,48,55,56,58] NaOH,[29,30,32,43–45] or

urea (Figure 5).[49,53] RNA blots have been reprobed as many as six times[44] demonstrating the usefulness of Zeta-Probe membrane for experiments involving rare species of RNA, RNA available in low quantities, or in situations where RNA is to be screened with multiple cDNA clones.

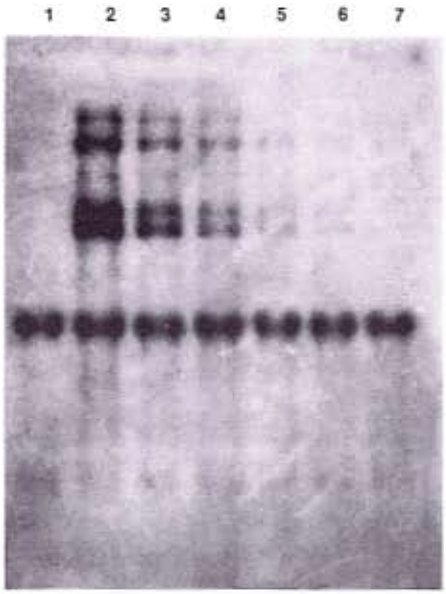

Fig. 5. DNA/RNA Hybridization on Zeta-Probe membranes. RNA was transferred to Zeta-Probe membrane followed by hybridization with a nick translated DNA probe. Exposure - 72 hours. Lanes 2 through 7: 2-fold dilutions of RNA from NIH 3T3 cells transformed with HMSV, from 0.5 μg (lane 2) to 0.016 μg (lane 7). Lanes 1 through 7: NIH 3T3 cell RNA to make a total of 4 μg RNA per well. Photo courtesy of Dr. Eric Westin, NIH.

Table 3. Zeta-Probe Membrane Methods

	References
Methods	
Single copy gene detection and probes	15, 26, 27, 34, 36, 38
Alkaline blots and DNA crosslinking to blots	30, 39
RNA probes and reprobing	15, 34, 43–45, 47
DNA reprobing of Northerns and Southerns	22, 23, 29, 37, 46, 48, 51, 57
Blotting oligonucleotides	24
Probing with oligonucleotides	42, 49, 53
Binding native DNA	30, 33
Blocking with BLOTTO and SDS	15, 18, 30, 34
Hybridization with dextran sulfate	15, 17, 20, 23, 30, 34, 39, 40, 46, 48
Hybridization with polyethylene glycol – single-stranded probes	15
Dual-label detection from one blot	24, 26
Genomic DNA Blots	
Plant	15, 16, 27
Yeast	28, 42
Eucaryote	17–20, 22–26, 30, 35, 36, 38–40
Glyoxyl transfers	30, 35, 50, 51, 54, 57
Formaldehyde transfers	46, 48, 55, 56, 58
Urea transfers	17, 24, 33, 53
Electrophoretic transfer from agarose	15, 17, 21, 24, 29, 36, 45, 48, 49, 58
Electrophoretic transfer from polyacrylamide	17, 19, 24, 28, 33, 36, 42–44, 46, 49, 53
Western blots and Immunoassays	1
Peptide blots	59, 66, 67
Antibody purification using blots	61

- **Dot blots.** Denatured or native nucleic acids may be bound quantitatively[15,24,26,46–48] in low salt solutions or dH_2O. Binding in 0.4M NaOH assures 100% denaturation, and, in combination with Zeta-Probe membrane's high capacity, more nucleic acid is retained in a hybridizable state.[30]

- **Alkaline Southern blots.** Alkaline blotting is the direct transfer and covalent binding of DNA to Zeta-Probe membrane in 0.4M NaOH (Figure 1).[30,32] Depurination is completed and denaturation accomplished simultaneously with transfer. Since depurination, denaturation, and neutralization pretreatments are eliminated, transfer is considerably simplified and more rapid (2–4 hours). Diffusion of DNA within the gel matrix and during the transfer is minimized, resulting in sharper bands detected on the blot and improved resolution. Sharper bands in combination with Zeta-Probe membrane's increased capacity for nucleic acids further results in the generation of signals of up to 10-fold greater than those seen in classical Southern hybridizations using nitrocellulose. Assuming equivalence to nitrocellulose, these results would allow: 1. one-tenth as much DNA to be used per experiment, 2. the same amount of DNA, but one-tenth as much autoradiographic exposure time with an intensifying screen, or 3. longer autoradiographic exposure, but without intensifying screen. Elimination of intensifying screens further improves resolution. Alkaline blotting has allowed single copy gene detection using 0.5 μg total mammalian genomic DNA and overnight autoradiograms with intensifying screen. Finally, NaOH covalently fixes DNA to Zeta-Probe membrane, thereby eliminating other fixation procedures such as lengthy baking or UV-crosslinking which may render DNA less hybridizable.[30, Gross, personal communication]

- **Electrophoretic transfers.** These transfers are useful for genomic blotting from DNA sequencing gels,[18,24] (Figure 2) for transfer of nucleic acids from any polyacrylamide or composite gel[17,19,24,26,28,33,36,42,43,44,49,53] and for agarose gel blots[15,21,24,29,36,45,48,58] especially when transferring large RNAs[Eric Westin, personal communication] (Figure 6). Electrophoretic transfer can only be performed in low ionic strength buffers.[8,11] Zeta-Probe membrane is thus ideal, since binding is efficient in 1× or 1/5× TAE (40 mM Tris - 20 mM acetate)[23,24,44] or 1/2× TBE (40 mM Tris - 40 mM borate).[33] These buffers are preferred over 25 mM phosphate,[2,12] as phosphate buffers rapidly degenerate, leading to drastic pH and ionic changes, and generally result in higher currents than Tris buffers, generating considerably more Joule heat.

- **Restriction fragment length polymorphisms (RFLPs).** Restriction fragment length polymorphism analysis requires multiple analysis of single blots. Gatti[23] has demonstrated twelve consecutive probings of RFLP blots, allowing more data to be obtained from a single gel. He suggests that 15 ml of blood would allow 60 tests per blot in the clinical screening for genetic disorders. Autoradiograms from multiple probings may conveniently be overlayed to identify missing, additional, and identical restriction fragments, thus simplifying the analysis.

- **Genomic sequencing.** Genomic sequencing allows the footprinting of native chromatin at single nucleotide resolution.[18] Chromatin is sequenced, transferred to a nylon membrane, and probed multiple times with various specific sequences. Zeta-Probe membrane is ideal, since it binds oligonucleotides as small as six bases,[24] and may be reprobed up to twelve times.[23] DNA can be UV-crosslinked as originally described[18,30,32] for improved retention of DNA <40 bases.[24] However, improved retention and hybridizability have been observed upon base catalyzed fixation.[30,31]

5. NITROCELLULOSE AND DIAZOTIZED CELLULOSE PAPER FOR NUCLEIC ACID BLOTTING

Nitrocellulose has been the membrane of choice for most DNA[11] and RNA[13] blots since Nygaard and Hall [*B.B.R.C.*, **12**, 98–104 (1963)] first described its use for adsorption of single stranded DNA. A comprehensive review by Meinkoth and Wahl[7] summarizes nucleic acid blotting to nitrocellulose, hybridization parameters, and probe strategies and preparations. While generally useful, nitrocellulose is not ideal for all applications.

Zeta-Probe cationized nylon membranes have been found useful in applications poorly suited to the use of nitrocellulose.[1] The following shortcomings of nitrocellulose have been observed:

- Small DNA fragments are not well retained on nitrocellulose following transfer, hybridization, or rehybridization reactions (Figure 6).[7,8,11]

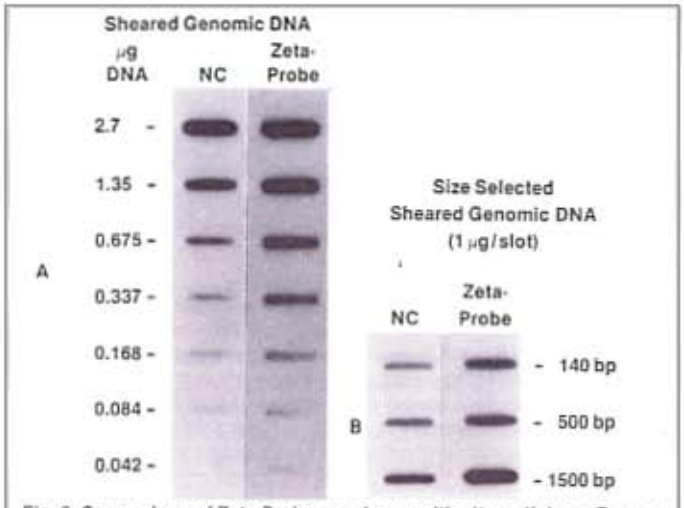

Fig. 6. Comparison of Zeta-Probe membrane with nitrocellulose. Genomic DNA was applied to either Zeta-Probe membrane or nitrocellulose membrane (NC) using a microfiltration apparatus. The membranes were hybridized with a ^{32}P-labeled probe. Panel A compares the membranes for detection limits. Panel B compares the signal obtained after probing different sizes of genomic DNA. Data provided by Drs. William Garrard and Stephen Rose, UTHSC, Dallas, Texas.

- Multiple hybridizations may be difficult, as nitrocellulose membranes become brittle after baking and probe stripping.

- Binding of DNA and RNA is susceptible to variations in experimental conditions such as buffer composition, salt concentration, and pH.[3,4,8,11,13] For example, Nagamine[8] demonstrated the selective loss of DNA from nitrocellulose with use of salt less than 20× SSC, thus verifying Southern's original data. Thomas[13] and Bresser and Gillespie[4] noted that RNA is best bound under conditions of high salt or NaI. Kafatos[6] demonstrated improved retention of DNA on nitrocellulose upon application in ammonium acetate rather than in sodium chloride.

- Nitrocellulose is not a suitable medium for electrophoretic transfer of nucleic acids because the high concentration of salts required for efficient binding causes high currents at very low voltages, resulting in poor transfers and excessive heating.[8] This is an important limitation, since electrophoretic transfer has several advantages: 1. molecules are efficiently eluted from polyacrylamide gels without the aid of soluble crosslinkers or the need for depurination reactions; 2. transfer of high molecular weight DNA and RNA is facilitated; 3. digests of whole eucaryotic genomes can be quantitatively eluted from gels, resulting in increased detection sensitivity for low copy genes at lower sample loadings; 4. transfers are rapid; and 5. multiple transfers may be produced simultaneously, are exactly reproducible, and are quantifiable in terms of volt-hours.

Diazotized cellulose membranes have been developed and applied in situations when the covalent attachment and detection of either DNA or RNA has been required. The use of diazotized-papers has been reviewed by Seed.[10]

6. PROTEIN TRANSFERS TO ZETA-PROBE MEMBRANES

Pure nitrocellulose membranes and Zeta-Probe membranes should always be compared first when blotting a new type of protein.[1,14] Aside from the obvious advantages in the use of nitrocellulose, there are various circumstances when an alternative membrane is desirable. Zeta-Probe cationized nylon membranes have been found useful in such cases.

- **Retention of small proteins.** There have been several reports of loss of proteins smaller than 20,000 daltons from 0.45 micron pore size nitrocellulose.[1] In these cases, 0.2 micron pore size nitrocellulose or Zeta-Probe membranes have been successfully used to retain proteins which would otherwise be lost. This is demonstrated in the detection of antibodies to HTLV III virus in serum from AIDS patients. The 18,000 dalton HTLV III diagnostic band is seen on 0.2 micron nitrocellulose but lost from 0.45 micron nitrocellulose.

  Alternatively, Zeta-Probe membrane has been observed to retain small proteins more efficiently than nitrocellulose either with or without gluteraldehyde fixation.[66] French[59] has reported good resolution and retention of low molecular weight highly sulfated keratin proteins on Zeta-Probe membranes when compared with 0.1, 0.2, and 0.45 micron nitrocellulose. It has been observed (Bio-Rad Laboratories) that hemoglobin may be detected by immunoperoxidase following transfer and binding to Zeta-Probe membranes, but not to nitrocellulose (0.45 micron) (Figure 7). Zeta-Probe membrane has been observed to efficiently retain peptide fragments throughout competitive ligand binding experiments in the structural mapping of the α-bungarotoxin binding domain of acetylcholine receptor protein subunits.[67]

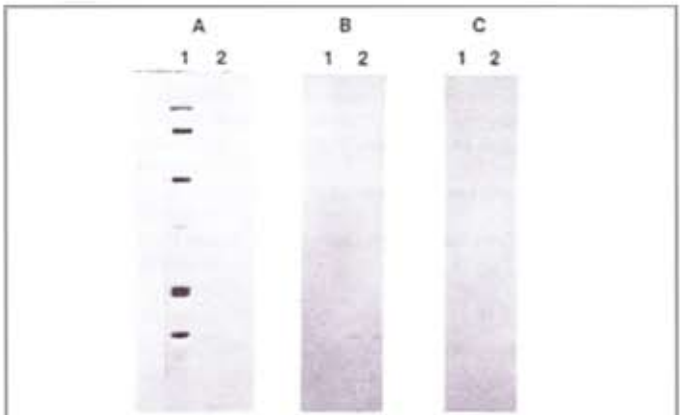

Fig. 7. Immun-Blot® detection of human hemoglobin on Zeta-Probe membrane. Primary antibody: monoclonal anti-human hemoglobin. Secondary antibody: goat anti-mouse horseradish peroxidase conjugate. A, silver stained polyacrylamide gel; B, Zeta-Probe membrane; C, nitrocellulose membrane. Lane 1, SDS-PAGE standards; Lane 2, human RBC lysate, 1/10,000 dilution.

- **Transfer of high molecular weight SDS-denatured proteins.** It is often observed that elution of large proteins (greater than 100,000 daltons) which have been denatured by SDS is poor when methanol is included in the transfer buffer. However, elimination of alcohol results in considerably diminished binding capacity of nitrocellulose.[1,60] Zeta-Probe membranes bind SDS-proteins to high capacity in Western blots without methanol.[60] Transfers to Zeta-Probe membranes may be performed without methanol in order to diminish both gel shrinkage and protein precipitation within the gel matrix, thereby improving transfer.

- **High binding capacity for protein.** Zeta-Probe membrane will bind 480 $\mu g/cm^2$ [60] unlike pure nitrocellulose which binds up to 80 $\mu g/cm^2$ or mixed ester nitrocellulose which binds up to 20 $\mu g/cm^2$. This may be important when detecting protein in low concentration or protein contaminated by excesses of other co-migrating proteins. Proteins are tightly bound and stable to post-transfer washes.

  It may be possible to use Zeta-Probe membrane for multiple protein "probings"; much the same as has been demonstrated on diazotized-papers[2,65] and with DNA on Zeta-Probe membrane.[23] The high binding capacity has its disadvantages. It may be inconvenient when blocking non-occupied protein binding sites. Typically, efficient blocking requires overnight incubation with 10% BSA at 50°C.[60] More recently, it has been observed that blocking Zeta-Probe membrane twice, thirty minutes each with 5% BLOTTO[5] at room temperature efficiently quenches non-occupied protein binding sites on the membrane.

- **Blot purification of monospecific antibodies.** Monospecific antibodies can be purified as originally described by Olmsted,[9] but with the substitution of Zeta-Probe membrane for diazotized-paper.[61] Protein antigens are separated by SDS-PAGE, transferred to Zeta-Probe membrane, and located. Bands of interest are excised and reacted with crude antisera. After washing, specifically bound antibody is released with 0.2M glycine-HCl (pH 2.8).

- **High density positive charge.** The strong positive charge of Zeta-Probe membrane may be advantageous when binding highly acidic proteins. French[59] observed superior retention of highly-sulfated keratin proteins and Arnaud[(personal communication)] observed superior retention of alpha 1 acid glycoprotein on Zeta-Probe membrane compared to nitrocellulose.

Conversely, the binding of highly basic proteins to Zeta-Probe membrane may be weak. While this may be a disadvantage for immunodetection procedures, such weak binding may be exploited for preparative recovery of basic proteins. Gershoni[60] noted that the net positive charge of Zeta-Probe membranes prevented the staining of total blot-bound proteins by classical organic dyes (Coomassie Blue R-250, Amido Black, etc.). Kittler, et al., [62] developed a non-specific immunoperoxidase stain for total proteins bound to Zeta-Probe membrane. An improved, rapid, and simple non-antibody biotin-avidin peroxidase stain has been developed by Bio-Rad and shown to stain Zeta-Probe membrane bound total protein to 20 ng (Figure 8).[1]

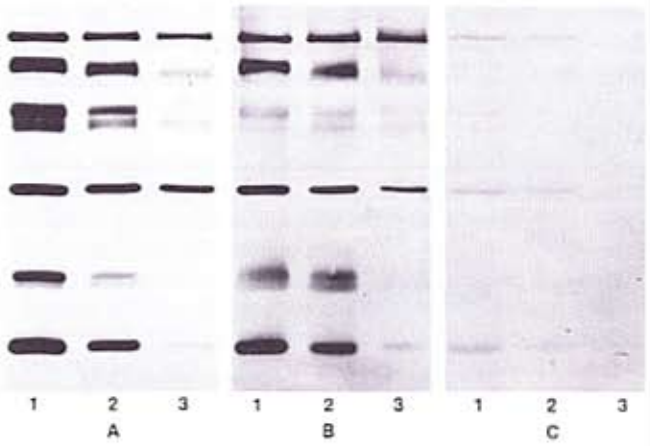

Fig. 8. Total protein detection of low molecular weight standards after electrophoretic transfer onto nitrocellulose and Zeta-Probe membranes. In panels (A) and (B), the Biotin-Blot method was used to detect serial dilutions of low molecular weight standards (Lane 1, 1:40 dilution; Lane 2, 1:80; Lane 3, 1:160; 10 µl sample load) transferred onto (A) nitrocellulose and (B) Zeta-Probe membranes, respectively. Panel (C) shows amido black staining of a comparable protein transfer onto nitrocellulose.

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Ordering Information

Blotting Media

Catalog Number	Description
Zeta-Probe Blotting Membranes	
162-0153	Sheets, 9 × 12 cm, pkg 15
162-0154	Sheets, 10 × 15 cm, pkg 15
162-0155	Sheets, 15 × 15 cm, pkg 15
162-0156	Sheets, 15 × 20 cm, pkg 15
162-0157	Sheets, 20 × 20 cm, pkg 15
162-0158	Sheets, 20 × 25 cm, pkg 3
162-0159	Roll, 30 cm × 3.3 m, pkg 1
162-0165	Roll, 20 cm × 3.3 m
162-0166	Roll, 30 cm × 30 m
Nitrocellulose Membrane (0.45 micron)	
162-0115	Roll, 33 cm × 3 m, pkg 1
162-0113	Sheets, 20 × 20 cm, pkg 5
162-0116	Sheets, 15 × 15 cm, pkg 10
162-0114	Sheets, 15 × 9.2 cm, pkg 10
170-3202	Disks, 82 mm diameter, pkg 50
Nitrocellulose (0.2 micron)	
162-0112	Roll, 33 cm × 3 m, pkg 1
Blot Absorbent Filter Paper (Thin)	
162-0118	Roll, 33 cm × 3 m, pkg 1
Blot Absorbent Filter Paper (Thick)	
165-0921	Sheets, 18 × 34 cm, pkg 25
165-0962	Sheets, 35 × 45 cm, pkg 25

Blotting Apparatus

Catalog Number	Description
170-3910	**Trans-Blot® Electrophoretic Transfer Cell,** includes 1 gel holder, cell with safety lid and power cables, fiber pads, sponge pads, and free blotting media selection kit.
165-4761	**Model 200/2.0 Constant Voltage Power Supply,** 100/120 V
165-4762	**Model 200/2.0 Constant Voltage Power Supply,** 220/240 V
170-3912	**Super Cooling Coil** (required for all high intensity transfers)
170-6542	**Bio-Dot SF Apparatus,** includes Bio-Dot SF sample template, vacuum manifold base, membrane support, gasket, filter paper, and free sample package of nitrocellulose and Zeta-Probe membrane.
170-6545	**Bio-Dot Apparatus,** includes Bio-Dot sample template, vacuum manifold base membrane support, gasket, and free Nitrocellulose and Zeta-Probe membrane.
170-3930	**Mini Trans-Blot® Electrophoretic Transfer Cell,** includes gel holder cassettes (2), fiber pads (4), modular electrode assembly, Bio-Ice cooling unit, lower buffer chamber, safety lid with power cables, and instruction manual.

Total Protein Detection Kit

170-6512	**Biotin-Blot Protein Detection Kit,** includes NHS-Biotin, 4 ml; avidin-HRP, 2 ml; Tween-20, EIA grade, 100 ml; Tris, 100 g; HRP color development reagent, 5 g.

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