5.2 DNA Blotting

(For acrylamide or agarose gels with DNA from 120 bp to 23 kb)

Standard Blot to Zeta-Probe

1. Prepare the stock electrophoretic transfer buffer, 20x TAE or 5x TBE. Dilute the stock to 0.5x and pre-chill 3 L of buffer.
2. Prepare gels for transfer immediately after electrophoresis:
   A. Electrophoresis under denaturing conditions:

      If gel electrophoresis was done under denaturing conditions (e.g., agarose/formaldehyde, etc.) equilibrate the gel in 0.5x transfer buffer for 10–15 minutes prior to electrophoretic transfer.

   B. Electrophoresis under non-denaturing conditions:
      1. Soak the gel in 0.2 N NaOH, 0.5 M NaCl for 30 minutes. For polyacrylamide gels, be sure not to exceed 30 minutes, since limited gel hydrolysis may occur with subsequent swelling during transfer.
      2. After base treatment, neutralize the gel by washing in 5x transfer buffer two times, 10 minutes each. Then, wash the gel once in 0.5x transfer buffer for 10 minutes.

   Note: Zeta-Probe membrane will bind non-denatured nucleic acids. Therefore, denaturing is not mandatory before transferring. If non-denatured nucleic acids are transferred, the blotted Zeta-Probe membrane must be treated with NaOH prior to hybridization. Refer to the Zeta-Probe Membrane Instruction Manual.
3. While gels are being equilibrated, soak the Zeta-Probe membrane at least 10 minutes in 0.5x transfer buffer.
4. Assemble the sandwich as described in Section 3.
5. For the standard electrode cards, transfer at 80 V (~0.8 A) for 2 hours. Cool to 4 °C with the super cooling coil and a refrigerated recirculator.
6. After transfer, separate the membrane from the gel, rinse the membrane briefly in 1x transfer buffer, and air dry the membrane. Dried membranes are stable at room temperature, and can be stored dry between two pieces of filter paper in plastic bags at room temperature.

Section 6
Choice of Blotting Membranes

6.1 Properties of Blotting Media

Table 6.1 summarizes the physical properties of the most commonly used blotting media.

6.2 Protein Blotting Membranes

Nitrocellulose membranes have been used extensively for protein binding and detection.[7,20,23,24,27] They can be easily stained for total protein by a dye stain (Amido Black, Coomassie Blue, Ponceau S, Fast Green FCF, etc.),[27] or the more sensitive Colloidal Gold Total Protein Stain, and also allow either RIA, FIA or EIA.[7] Nitrocellulose has a high binding capacity of 80-100 μg/cm^2. Nonspecific protein binding sites are easily and rapidly

blocked, avoiding subsequent background problems. No pre-activation is required. Low molecular weight proteins (esp. <20,000 daltons) may be lost during post transfer washes, thus limiting detection sensitivity.[19] However, use of glutaraldehyde fixation, smaller pore size nitrocellulose membrane (0.2 µm), or a combination of these techniques has been shown to be effective in eliminating this loss.[37] Large proteins (≥ 100,000 daltons) denatured by SDS may transfer poorly due to the addition of alcohol to the transfer buffer. Alcohol increases binding of SDS-proteins to nitrocellulose, but decreases pore sizes in the gel. Elimination of alcohol from SDS-protein transfers results in considerably diminished binding; [redacted] to 0.1%) to the transfer buffer increases the transfer efficiency of proteins, but reduces the amount of binding to the membrane. Also, SDS increases the [redacted]

Zeta-Probe membrane, a positively charged nylon membrane, allows binding of SDS-protein complexes in the absence of alcohol.[26,27] This membrane binds proteins very tightly and is stable to post transfer washes. The binding capacity of Zeta-Probe membrane is ~480µm/cm^2. Reprobing, after stripping of prior probes, may be performed without significant loss of primary bound protein. Even small proteins appear to bind stably. Zeta-Probe membrane may not by dye-stained, as destaining is impossible. Instead, the Biotin-Blot Total Protein Stain should be used on Zeta-Probe membrane. This assay uses NHS-Biotin (N-hydroxysuccinide-biotinate) to biotinylate all the proteins on the membrane surface, and a combination of an avidin-horseradish peroxidase or avidin-alkaline phosphatase and a color development reagent to detect these biotinylated proteins.[39,40] The large capacity for molecules allows sensitive detection of small amounts of protein in a complex mixture. This high capacity requires more stringent blocking conditions than nitrocellulose.[26]

6.3 DNA and RNA Blotting Membranes

Nitrocellulose is not a suitable medium for electrophoretic transfer of nucleic acids, as high concentrations of salt (≥ 10 x SSC) are required for efficient binding.[13] Molecules ≤ 500 bp are not bound at all, even at high salt. Low resistance results when an electric current is passed through a solution of high salt. This causes potentially damaging high currents (and power) at very low voltages. Since V/cm is the eluting force, inefficient transfer occurs under conditions required for proper binding. Zeta-Probe membrane allows efficient binding of all sizes of single stranded DNA and RNA in the presence of low ionic strength buffers.[13] Zeta-Probe membrane is an ideal alternative to nitrocellulose for the analysis of nucleic acids. Binding is more stable through post transfer washes, and reprobing may be performed as many as 10 times.