6. DNA transfer buffer for Zeta-Probe membrane[36]
   20x TAE (0.8 M Tris, 0.4 M sodium acetate, 20 mM EDTA, pH 7.8)
   Dissolve 96.9 g Tris, 32 g sodium acetate and 7.45 g EDTA in dd H_2O; pH to 7.4 with concentrated glacial acetic acid. Adjust volume to 1 liter with dd H_2O. For the working solution, dilute to 0.5x by adding 25 ml of 20x TAE to 975 ml of dd H_2O. Pre-chill the buffer before use.

   DO NOT ADJUST THE pH WITH HCl

7. 0.7% acetic acid for transfer of native gels[7]
   Add 7 ml concentrated glacial acetic acid to 993 ml dd H_2O. Pre-chill the buffer before use.

Section 5
Examples of Specific Protocols

Note: In order to determine the optimum conditions for a particular sample, a time course of transfer should be performed. Since many factors affect transfer, e.g., molecular weight, pI, porosity of the gel, it may not be necessary to transfer for the full time or to use high field intensity transfer conditions. Final transfer conditions for any protein should be determined empirically.

5.1 SDS-Protein Blotting

Standard Blot to Nitrocellulose

1. Equilibrate the gel in 500 ml of Towbin transfer buffer (Section 4.2) for 15 minutes.
2. Pre-chill buffer prior to transfer.
3. Assemble the sandwich as described in Section 3. Place into the middle slot of the tank and add 2.5-3.0 L of buffer.
4. For **standard electrode cards, transfer for 3 hours at 70 V (~0.25 A). Cooling is not required.**
5. For the **plate electrodes, transfer for 30 minutes to 1 hour at 50 V (~0.5 A). Cool to 4 °C with the Super Cooling Coil and a refrigerated recirculator.**

High Intensity Transfer

1. Follow steps 1 and 2, as above.
2. Assemble the sandwich and insert it into the slot closest to the cathode, as described under the high field intensity instructions, Section 3.4.
3. For standard electrode cards, transfer or 1 to 2 hours at 100 V (~0.36 A). Cool to 4 °C with the Super Cooling Coil and a refrigerated recirculator.
4. For plate electrodes, transfer 15-30 minutes at 100 V (~1.0 A). Cool to 4 °C with the Super Cooling Coil and a refrigerated recirculator.

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 $\mu g/cm^2$. Nonspecific protein binding sites are easily and rapidly