Transfer of Denatured RNA to Nitrocellulose Filters

Glyoxylated RNA may be transferred immediately after electrophoresis from agarose gels to nitrocellulose filters by capillary elution, vacuum transfer, or electroblotting (see Chapter 9, pages 9.34–9.37, for a discussion of the relative merits of these techniques). Capillary elution is carried out as described below; vacuum transfer and electroblotting should be performed according to the instructions of the manufacturer of the apparatus that is used.

Although further treatment of agarose gels before transfer is unnecessary (Thomas 1980) and may be detrimental (Thomas 1983), gels containing formaldehyde must be rinsed in several changes of diethyl pyrocarbonate (DEPC)-treated water (see page 7.4) to remove the formaldehyde. However, if the gel contains more than 1% agarose or is more than 0.5 cm thick or if the RNA to be analyzed is greater than 2.5 kb in length, soak the gel for 20 minutes in 0.05 N NaOH. This treatment partially hydrolyzes the RNA and improves the efficiency of transfer. Then rinse the gel in RNAase-free water and soak it for 45 minutes in 20 × SSC. The gel is then placed in contact with the nitrocellulose filter or nylon membrane and the RNA is transferred to the solid support (nitrocellulose or charged nylon) in an ascending flow of buffer (Figure 7.2).

Cautions: DEPC is suspected to be a carcinogen and should be handled with care.

Formaldehyde is toxic and should be handled with care in a chemical hood.

Note: Gels containing formaldehyde are less rigid than nondenaturing agarose gels, and care must be exercised in handling them.

1. Transfer the gel to a glass baking dish, and trim away any unused areas of the gel with a razor blade. Cut off the bottom left-hand corner of the gel; this serves to orient the gel during the succeeding operations.

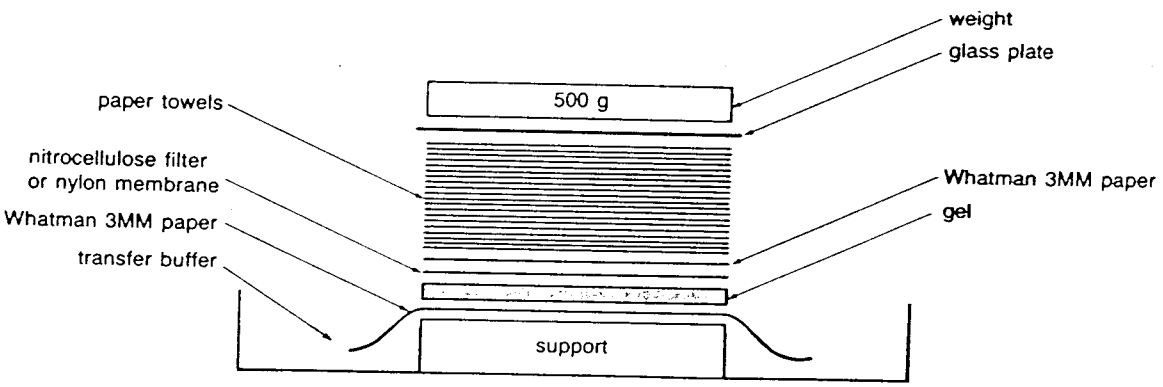

FIGURE 7.2
Capillary transfer of nucleic acids from agarose gels to solid supports. Buffer is drawn from a reservoir and passes through the gel into a stack of paper towels. The nucleic acid is eluted from the gel by the moving stream of buffer and is deposited on a nitrocellulose filter or nylon membrane. A weight applied to the top of the paper towels helps to ensure a tight connection between the layers of material used in the transfer system.

2. Place a piece of Whatman 3MM paper on a piece of Plexiglas or a stack of glass plates to form a support that is longer and wider than the gel. Place the support inside a large baking dish. Fill the dish with 20 × SSC until the level of the liquid reaches almost to the top of the support. When the 3MM paper on the top of the support is thoroughly wet, smooth out all air bubbles with a glass rod.

3. Using a fresh scalpel or a paper cutter, cut a piece of nitrocellulose filter (Schleicher and Schuell BA85 or equivalent) about 1 mm larger than the gel in both dimensions. Use gloves and blunt-ended forceps (e.g., Millipore forceps) to handle the filter. A nitrocellulose filter that has been touched by greasy hands will not wet!

4. Float the nitrocellulose filter on the surface of a dish of deionized water until it wets completely from beneath, and then immerse the filter in 20 × SSC for at least 5 minutes. Using a clean scalpel blade, cut a corner from the nitrocellulose filter to match the corner cut from the gel.

   The rate at which different batches of nitrocellulose filters wet varies enormously. If the filter is not saturated after floating for several minutes on water, it should be replaced with a new filter, since the transfer of RNA to an unevenly wetted filter is unreliable. The original filter should not be discarded but should be autoclaved for 5 minutes between pieces of 3MM paper saturated with 2 × SSC. This usually results in complete wetting of the filter. The autoclaved filter, sandwiched between the autoclaved 3MM papers saturated with 2 × SSC, may be stored at 4°C in a sealed plastic bag until it is needed.

5. Place the gel on the support in an inverted position so that it is centered on the wet 3MM paper. Make sure that there are no air bubbles between the 3MM paper and the gel.

6. Surround, but do not cover, the gel with Saran Wrap or Parafilm. This serves as a barrier to prevent liquid from flowing directly from the reservoir to paper towels placed on the top of the gel. If these towels are not precisely stacked, they tend to droop over the edge of the gel and may touch the support. This type of short-circuiting is a major reason for inefficient transfer of RNA from the gel to the filter.

7. Place the wet nitrocellulose filter on top of the gel so that the cut corners are aligned. One edge of the filter should just extend over the edge of the line of slots at the top of the gel. Do not move the filter once it has been applied to the surface of the gel. Make sure that there are no air bubbles between the filter and the gel.

8. Wet two pieces of 3MM paper (cut to exactly the same size as the gel) in 2 × SSC and place them on top of the wet nitrocellulose filter. Smooth out any air bubbles with a glass rod.

9. Cut a stack of paper towels (5–8 cm high) just smaller than the 3MM papers. Place the towels on the 3MM papers. Put a glass plate on top of the stack and weigh it down with a 500-g weight (see Figure 7.2). The

objective is to set up a flow of liquid from the reservoir through the gel and the nitrocellulose filter, so that RNA molecules are eluted from the gel and are deposited on the nitrocellulose filter.

10. Allow transfer of RNA to proceed for 6–18 hours. As the paper towels become wet, they should be replaced.

11. Remove the paper towels and the 3MM papers above the gel. Turn over the gel and the nitrocellulose filter and lay them, gel side up, on a dry sheet of 3MM paper. Mark the positions of the gel slots on the filter with a very-soft-lead pencil or a ballpoint pen.

12. Peel the gel from the filter and discard it. Soak the filter in 6 × SSC for 5 minutes at room temperature. This removes any pieces of agarose sticking to the filter.

    Remove the filter from the 6 × SSC and allow excess fluid to drain away. Place the filter flat on a paper towel to dry for at least 30 minutes at room temperature.

    To assess the efficiency of transfer of RNA, the gel may be stained for 45 minutes in a solution of ethidium bromide (0.5 μg/ml in 0.1 M ammonium acetate) and examined by ultraviolet illumination.

    Cautions: Ethidium bromide is a powerful mutagen and is moderately toxic. Gloves should be worn when working with solutions that contain this dye. After use, these solutions should be decontaminated by one of the methods described in Appendix E.

    Ultraviolet radiation is dangerous, particularly to the eyes. To minimize exposure, make sure that the ultraviolet light source is adequately shielded and wear protective goggles or a full safety mask that efficiently blocks ultraviolet light.

13. Place the dried filter between two pieces of 3MM paper, and bake the filter for 30 minutes to 2 hours at 80°C in a vacuum oven.

    The filter will become extremely brittle and may become yellow if baked too long.

    If the filter is not to be used immediately in hybridization experiments, it should be wrapped loosely in aluminum foil and stored under vacuum at room temperature.

14. *For filters containing glyoxylated RNA only:* Before hybridization, remove glyoxal from the RNA by washing the filter with 20 mM Tris · Cl (pH 8.0) at 65°C.