

Transfer of Denatured RNA to Nylon Membranes

The methods used to transfer RNA from gels to nylon membranes are similar to those used for transfer to nitrocellulose filters and include vacuum transfer, electroblotting, or capillary elution (see Chapter 9, pages 9.34–9.37, for a discussion of the relative merits of these techniques). Vacuum transfer and electroblotting should be carried out according to the instructions of the manufacturer of the apparatus that is used. Capillary elution is carried out essentially as described on pages 7.46–7.48. Gels containing formaldehyde must be rinsed in several changes of DEPC-treated water to remove the formaldehyde before transfer. Because charged nylon membranes retain nucleic acids in alkaline solution (Reed and Mann 1985), glyoxylated RNA can be eluted from agarose gels in 7.5 M NaOH (Vrati et al. 1987). This partially hydrolyzes the RNA and thereby increases the speed and efficiency of transfer of large (>2.3 kb) RNAs. In addition, alkali removes glyoxal adducts from the mRNA (Thomas 1983), eliminating the need for postfixation stripping (step 14, page 7.48). Finally, because RNA transferred in alkaline transfer buffers becomes irreversibly fixed to the charged nylon membrane, there is no need to bake the membrane or to expose it to ultraviolet irradiation before hybridization.

The sole disadvantage of nylon membranes is a tendency to give increased levels of background hybridization, especially with RNA probes. The level of background hybridization is almost always significantly higher when the membrane has been exposed to high concentrations of alkali for extended periods of time. In many cases, this problem can be overcome by using increased amounts of blocking agents in the prehybridization and hybridization steps.

Alkaline transfer of glyoxylated RNA to charged nylon membranes is carried out essentially as described on pages 7.46–7.48, except that the transfer buffer is 7.5 M NaOH. After transfer is completed (4.5–6.0 hours), the membranes should be rinsed briefly in $2 \times$ SSC, 0.1% SDS and allowed to dry at room temperature.

When transfer is carried out with a neutral transfer buffer, nylon membranes must be treated to immobilize the nucleic acid after transfer is complete. RNA becomes fixed to the nylon membrane if it is thoroughly dried or if it is exposed to low doses of ultraviolet irradiation. To fix the RNA to the membrane either place the dried membrane between two pieces of 3MM paper and bake the membrane for 30 minutes to 2 hours at 80°C in a vacuum or conventional oven, or expose the side of the membrane carrying the RNA to a source of ultraviolet irradiation (254 nm). The latter method, although a nuisance to set up, is preferred because it greatly enhances the hybridization signal obtained with some brands of positively charged nylon membranes (Khandjian 1987). However, for maximum effect, it is important to make sure that the membrane is not overirradiated. The aim is to form cross-links between a small fraction of the bases in the RNA and the positively charged amine groups on the surface of the membrane (Churn and Gilbert 1984). Overirradiation results in the covalent attachment of a higher proportion of thymines, with a consequent decrease in hybridization signal. Most manufacturers advise that damp nylon membranes should be exposed to a total of 1.5 J/sq. cm. and that dry membranes should be exposed to 0.15 J/sq. cm.

However, we recommend carrying out a series of preliminary experiments to determine empirically the amount of irradiation required to produce the maximum hybridization signal. In addition, the system should be recalibrated routinely.

Caution: 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.

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