Hybridization and Autoradiography

The conditions for prehybridization, hybridization, and washing of RNA immobilized on filters are essentially the same as those used for DNA. These are described in detail in Chapter 9, pages 9.47–9.55. In brief:

1. Prehybridize the filter for 1–2 hours in

   either (at 42°C)
   50% formamide
   5 × SSPE
   2 × Denhardt's reagent
   0.1% SDS

   or (at 68°C)
   6 × SSC
   2 × Denhardt's reagent
   0.1% SDS

2. Add the denatured radiolabeled probe directly to the prehybridization fluid. To detect low-abundance mRNAs, use at least 0.1 μg of probe whose specific activity exceeds 2×10^8 cpm/μg (see Chapter 10). Continue incubation for 16–24 hours at the appropriate temperature.

   It is important to remember that RNA is complementary to only one of the two DNA strands. Therefore, if a single-stranded probe is utilized, it must be complementary to the RNA strand.

3. Wash the filter for 20 minutes at room temperature in 1 × SSC, 0.1% SDS, followed by three washes of 20 minutes each at 68°C in 0.2 × SSC, 0.1% SDS.

4. Establish an autoradiograph by exposing the filter for 24–48 hours to X-ray film (Kodak XAR-2 or equivalent) at −70°C with an intensifying screen (see Appendix E).