Staining RNA Before and After Transfer to Nitrocellulose Filters

Prolonged staining with ethidium bromide is not recommended before RNA is transferred from agarose gels to nitrocellulose filters because saturation of the nucleic acid with the dye appears to reduce the efficiency of transfer. However, brief staining with ethidium bromide does not detectably inhibit transfer and has the advantage of allowing RNA to be detected both in the gel and on the filter.

METHOD 1

1. After electrophoresis is completed, gels containing glyoxal/DMSO should be immersed in 10 mM sodium phosphate (pH 7.0) containing ethidium bromide (0.5 μg/ml). Gels containing formaldehyde should be washed in RNAase-free water and immersed in 20× SSC before staining in 20× SSC containing ethidium bromide (0.5 μg/ml).

2. After staining for 5–10 minutes at room temperature, examine and photograph the gel in ultraviolet light as described in Chapter 6, page 6.19.

3. Transfer the RNA from the gel to a nitrocellulose filter as described on pages 7.46–7.48. After transfer, the stained RNA is usually visible when the filter is examined by ordinary illumination.

Note

This method works only when each lane of the gel contains considerable quantities of RNA (e.g., 5 μg or more of mRNA).

METHOD 2

RNA may also be stained on the nitrocellulose filter as follows: A lane may be cut from the filter after it has been baked or the entire filter may be stained after hybridization and exposure to X-ray film (A. Efstratiadis, unpubl.).

1. Soak the dried filter in 5% acetic acid for 15 minutes at room temperature.

2. Transfer the filter to a solution of 0.5 M sodium acetate (pH 5.2) and 0.04% methylene blue for 5–10 minutes at room temperature.

3. Rinse the filter in water for 5–10 minutes. RNAs used as molecular-weight standards should appear as sharp bands. Total poly(A)$^+$ mRNA appears as a smear composed of many individual species whose sizes range between <500 bases and >5 kb. The median size of mRNA is approximately 2 kb.

Note

Staining with ethidium bromide is not recommended when RNA is to be transferred from gels to nylon membranes. RNA may be stained with methylene blue after transfer from agarose/formaldehyde gels to certain types of nylon membrane. For details of this method, see Herrin and Schmidt (1988).