

Staining RNA after Transfer to Nitrocellulose Filters

This procedure may be used to check the size of the RNA transferred to nitrocellulose and to estimate the efficiency of its transfer (A. Efstratiadis, unpubl.). A lane may be cut off the filter after baking, or the entire filter may be stained after hybridization and exposure to X-ray film.

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. Marker RNAs (28S and 18S ribosomal RNAs and 9S globin mRNA) appear as bands. Total poly(A)⁺ mammalian mRNA appears as a smear.