SELECTION OF POLY(A)$^+$ RNA

Several techniques have been developed to separate polyadenylated RNA from nonpolyadenylated RNAs. The method of choice is chromatography on oligo(dT)-cellulose (Edmonds et al. 1971; Aviv and Leder 1972), which can be prepared as described by Gilham (1964) or obtained commercially. Up to 10 mg of RNA can be processed per ml of oligo(dT)-cellulose.

1. Equilibrate the oligo(dT)-cellulose in sterile loading buffer. To sterilize the loading buffer, mix appropriate amounts of RNase-free stock solutions of the Tris, sodium chloride, and EDTA and autoclave. Add SDS from a 20% stock solution that has been treated at 65°C for 1 hour. Alternatively, 0.05 M sodium citrate can be substituted for Tris, and the loading buffer and SDS can then be treated with diethylpyrocarbonate.

   Loading buffer

   20 mM Tris · Cl (pH 7.6)
   0.5 M NaCl
   1 mM EDTA
   0.1% SDS

2. Pour a 1.0-ml column in a Dispocolumn or pasteur pipette. Wash the column with 3 column-volumes each of:

   a. sterile H_2O
   b. 0.1 M NaOH and 5 mM EDTA
   c. sterile H_2O

3. Check that the pH of the column effluent is less than 8.

4. Wash the column with 5 volumes of sterile loading buffer.

5. Dissolve the RNA in sterile water and heat to 65°C for 5 minutes. Add an equal amount of 2× loading buffer, cool the sample to room temperature, and apply to the column. Collect the flow-through, again heat to 65°C, cool, and reapply to the column.

6. Wash the column with 5–10 column-volumes of loading buffer, followed by 4 column-volumes of loading buffer containing 0.1 M NaCl.

   Note. Read the OD_{260} of each column-volume fraction collected. Initially, the OD_{260} will be very high as the poly(A)$^-$ RNA comes through the column. The later fractions should have no or very little OD_{260} absorbing material.

7. Elute the poly(A)$^+$ RNA with 2-3 column-volumes of sterile

   10 mM Tris-Cl (pH 7.5)
   1 mM EDTA
   0.05% SDS

   If desired, the eluted poly(A)$^+$ mRNA can be selected again by oligo(dT)-cellulose chromatography by adjusting the sodium chloride concentration of the eluted mRNA to 0.5 M and repeating steps 5, 6, and 7.

8. Add sodium acetate (3 M, pH 5.2) to a final concentration of 0.3 M. Precipitate the RNA with 2.2 volumes of ethanol at −20°C. Rinse the pellet in 70% ethanol.

9. Dissolve the pellet in sterile water. The yield from 10^7 cells should be 1-5 μg of poly(A)$^+$ RNA. Regenerate the column by sequential washing in sodium hydroxide, water, and loading buffer as described in steps 2, 3, and 4 above.

Notes

i. When many RNA samples are to be processed, it may be more efficient to carry out a batch absorption and elution with oligo(dT)-cellulose. After dissolving the RNA in loading buffer (step 5), add 0.3 g (dry weight) of oligo(dT)-cellulose for each 0.5 mg of RNA. Centrifuge at 1500*g* for 4 minutes at 15°C and wash the oligo(dT)-cellulose four to five times with 5 ml of loading buffer at room temperature. Elute the poly(A)$^+$ RNA with four 1-ml washes of sterile

   10 mM Tris · Cl (pH 7.5)
   1 mM EDTA
   0.05% SDS

   Continue with step 8 above.

ii. The sodium salt of SDS is relatively insoluble and therefore may tend to impede the flow of the column. This can be avoided by using lithium chloride instead of sodium chloride in the loading buffer.

iii. RNA should be stored in 70% ethanol at −70°C.