ISOLATION OF POLY A+ RNA

make up all solutions with 0.1% DEPC treated dH2O
use filter tips if RNA is to be used for PCR
keep all solutions on ice and collect all eluates in tubes on ice

1. Equilibrate 0.25g oligo d(T) cellulose in 1.5 - 2 ml 1 x loading buffer
   overnight at 4°C.

NOTE: steps 2-12 can take up to 12 hours depending on column flow

2. Prepare two columns by adding equilibrated cellulose to sterilized
disposable columns until there is approximately 0.5 ml bed volume of
   cellulose in each.

3. Warm elution buffer to 45°C.

4. Sterilize the column with 5 - 10 ml 0.1 M NaOH then fill the column
   with 0.1 M NaOH, plug and let stand for 20’.

5. Wash the column with 5 ml H2O and then approximately 6 ml 1 x
   loading buffer or until the eluate is pH 8.0.

6. Adjust the flow rate to approximately one drop every twenty seconds.

7. Adjust RNA volume to 1 ml (2 - 5 mg) and heat to 65°C for 15’. Snap
   cool on ice for 15’.

8. Before loading RNA on column, add 0.25 ml 5 x loading buffer.

9. Apply RNA to 1st column. Collect the eluate in a falcon tube. Re-apply
   to the column three times.

10. Wash the column with 5 - 10 ml ice cold 1 x loading buffer.

11. Elute the RNA with 3 ml elution buffer at 45°C. Collect six 0.5 ml
    fractions. Identify the poly A containing fractions by mixing 3 µl of
each fraction with 20 µl 1 µgml⁻¹ ethidium bromide. Mix and spin and then spot onto saranwrap on the transilluminator. Pool the RNA containing fractions (generally 1-4).

12. Run this pool through the second column as before.

13. Precipitate overnight with ethanol. Pellet RNA, wash with 70% ethanol
   and dry in speedvac.


**5 X loading buffer (200ml)**

0.1 M KCl 37.28 g

0.05 M Tris-HCl pH7.5 5 ml 2M

**Elution buffer**

0.01M Tris-HCl pH7.5