ISOLATION OF POLY A+ RNA

make up all solutions with 0.1% DEPC treated dH_2O
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 H_2O 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.

14. Resuspend RNA in approximately 25 μl dH_20. Quantitate by measuring OD at 260 and 280 nm.

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