PolyA RNA Purification via Oligo-dT Spin Column

Chris Seidel May 1999

The protocol below utilizes the Amersham Pharmacia mRNA Purification Kit (Cat. 27-9258-01) to purify mRNA from Total RNA.

Prepare Spin Columns (takes about 45 min)

- invert column several times to resuspend resin
- Remove Cap, place in 15 ml centrifuge tube, allow to drain under gravity
- Add 1 ml **High Salt** Buffer, allow to drain under gravity
- Add 1 ml **High Salt** Buffer, allow to drain under gravity
- Remove cloumn, discard buffer, replace column and apply RNA

Prepare 1.25 mg RNA in 1 ml **TE**

Prepare RNA for Column

- Heat to 65 ˚ C, 5 min.
- Place on ice
- add 200 ul sample buffer

Purify Poly A RNA with Spin Column

- place elution buffer at 65 ˚ C to pre-warm
- add RNA to Column, allow to soak in under gravity
- Spin Column 1400 RPM, 2 min.
- add 250 ul **High Salt** Buffer, spin 1400 RPM, 2 min.
- Repeat
- add 250 ul **Low Salt** Buffer, spin 1400 RPM, 2 min.
- Repeat twice
- Remove Column, discard buffer, place 1.5 ml tube inside column
- Replace column with its end inside the 1.5 ml tube
- add 250 ul **Elution** Buffer at 65˚ C
- Spin Column 1400 RPM, 2 min.
- Repeat elution 3 more times

Notes

Ethanol Precipitation: add 1/10th volume 3M NaOAc
Add 10-20 ug linear acrylamide as carrier
Add 2.5 volumes 100% ethanol

Store at -20 C indefinately

The oligo-dT columns tend to leave a small amount of resin fines with the polyA RNA. Once the RNA is resuspended, the fines can be pelleted by a brief spin.

The proper RCF for these spin columns is 350g. To calculate the RCF for your centrifuge measure the radius (r) of your rotor in mm from spindle center to bucket bottom and use the following formula: RCF = $(1.12)\ (r)\ (rpm/1000)^2$

RNA is sensitive to degradation by high pH. (I often use 1/2x TE to resuspend RNA, or 5 mM Tris pH 7.5)

RNA is very sensitive to contamination and degradation by nucleases. Always wear gloves when dealing with RNA. Keep RNA on ice as much as possible. Be very careful with all reagents that you use for RNA work. A primary source of conatimination is inadvertant touching of the lips of tubes. Most plastics are RNAse free. Dust is not RNAse free so be wary of leaving racks of pipet tips open to collect dust.