Isolation of Total RNA from Cultured Cells

I. Solutions — make up fresh each time

A. GIT buffer

9.452 g
.334 ml
19.66 ml
.334 ml

1. 47.26g guanidine isothiocyanate (4M)
2. 1.67ml 3M sodium acetate, pH 6.0 (50mM)
3. dH_2O to 98.33ml
4. filter through 0.2um filter
5. 1.67ml 2-mercaptoethanol
6. store in the dark at RT

B. CsCl buffer

1. 47.98g CsCl (5.7M)
2. 0.415ml 3M sodium acetate, pH 6.0 (25mM)
3. dH_2O to 50ml
4. filter through 0.2um filter
5. store at RT

C. 2X PK buffer

1. 1.21 g Tris (0.2M); 10ml 1M Tris, pH 7.5
2. 0.46g EDTA (25mM); 2.5ml 0.5M EDTA
3. 0.88g NaCl (0.3M); 3.75ml 4M NaCl
4. 1.0g SDS (2%)
5. dH_2O to 45ml
6. pH to 7.5 with HCl
7. ~~1ml 10mg/ml proteinase K (200ug/ml)~~ → Use proteinase K at 100 µg/ml
8. dH_2O to 50ml
9. ~~store at -20°~~

II. Procedure

A. Pellet 400-500ml of cells (10^5-10^6 cells/ml) in the Sorvall T600B centrifuge, 5 min., 4°, 1000rpm.

B. Decant supernatant and wash cell pellets with 10ml cold (4°) Dulbeco's PBS. Pellet cells as above.

C. Resuspend cells in 18ml GIT buffer and vortex vigorously.

D. Shear the chromosomal DNA by transfering the cells to a 60ml syringe and shearing through a 20 gauge needle (3X).

Use 2N NaOH to wash sludge well of acid

E. Into 2 SW41 centrifuge tubes add 3ml CsCl buffer and layer 9ml of the GIT-cell mixture. Pellet the RNA by centrifuging 21 hr., 32K rpm, 20°. The temperature is critical since the CsCl will precipitate at lower temperatures. Andrew has used brake (no setti… may be better to … use any brake (g… setting)

F. Remove the GIT buffer layer and part of the CsCl layer using a Pasteur pipette and house vacuum. Decant the remaining CsCl layer using a Pasteur pipette being careful not to disturb the RNA pellet. The pellet should appear clear.

G. Rinse the ~~pellet with 1ml cold (-20°) 80% EtOH by swirling gently.~~ Do not rinse the portion of the tube that contained the GIT buffer. Carefully remove the EtOH with a Pasteur pipette.

Dry the pellet + resu… in 2ml T.E + .1% SDS
Add NaOAc + EtOH, …
rinse, dry, then + resuspend in 1X PK bu…

~~$dd H_2O$~~ + .1% SDS to resuspend originally from pelleted T.E.

g transfer to eppendorf

H. Air dry the pellet and then add 1ml of 1X PK buffer and incubate at 50° for 30-60 min. to completely solubilize the RNA. then split into 2 eppendorfs to do phenol

I. Add an equal volume of buffered phenol (see Maniatis p 438), mix by vortexing and separate the phases by spinning in the microfuge, 2 min, RT, full speed.

J. Transfer the upper aqueous phase with a P-200 and re-extract 2-3X until the interphase is clear.

K. Add 1/10 volume 3M sodium acetate, pH 6.0, and 2.5 volumes cold (-20°) EtOH. Mix by inverting several times and place on dry ice for 20 min. Pellet the RNA by spinning in a microcentrifuge, 15 min, 4°, full speed.

L. Decant the EtOH and dry the pellet in the speed-vac for 5 min., ~~heat on~~. Resuspend the dried pellet in 200-500ul dH_2O and store at -70°. For long term storage, pellet the RNA as above and store as is at -70°.

III. References

A. Basic Methods in Molecular Biology, Section 11-1, Davis, L.G., Dibner, M.D., and Battey, J.F. (1986).

B. Molecular Cloning, A Laboratory Manual, Chapter 6, p. 196, Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982)

C. Current Protocols in Molecular Biology, Vol. 1, Section 4.2.1-4.2.5

Tissues

- see notes
- resuspend in 100) d_2O