Isolation of Total RNA from Cultured Cells

I. Solutions

A. GIT buffer
   1. 47.26g guanidine isothiocyanate (4M)
   2. 1.67ml 3M sodium acetate, pH 6.0 (50mM)
   3. dH₂O 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. dH₂O 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. dH₂O to 45ml
   6. pH to 7.5 with HCl
   7. 1ml 10mg/ml protease K (200ug/ml)
   8. dH₂O to 50ml
   9. store at -20°C

II. Procedure

A. Pellet 400-500ml of cells (10⁵-10⁶ cells/ml) in the Sorvall T600B centrifuge, 5 min., 4°C, 1000rpm.
B. Decant supernatant and wash cell pellets with 10ml cold (4°C)
   Dulbecco's PBS. Pellet cells as above.
C. Resuspend cells in 18ml GIT buffer and vortex vigorously.
D. Shear the chromosomal DNA by transferring the cells to a 60ml
   syringe and shearing through a 20 gauge needle (3X).
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°C. The temperature is critical, since the
   CsCl will precipitate at lower temperatures.
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°C) 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 then re-suspend in 1X PK buffer
   (directly) + 0.1% SDS

Use protease K
   Use at 100 ug/ml

Andrew has used brake (no setting)
   Shouler"s better to use cry brake (3/10 setting)
H. Air dry the pellet and then add 1 ml of 1X PK buffer and incubate at 50° for 30-60 min. to completely solubilize the RNA.
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., 

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

Tissues

- See index
- (Resuspend in 100 µl H2O)
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