ISOLATION OF TOTAL CELLULAR RNA

The two methods given below are used to isolate RNA from cells that cannot be fractionated easily into cytoplasm and nuclei (e.g., frozen fragments of tissue) or from cells that are particularly rich in RNase (e.g., pancreatic cells).

Guanidinium/Hot Phenol Method[1]

1. Add 4 M guanidinium isothiocyanate mixture (prepared as described on page 189) to a tissue fragment or washed cell pellet in a plastic, disposable centrifuge tube. Use 1 ml of guanidinium isothiocyanate mixture for 10^7 cells or 5 ml for every gram of tissue.

   Note. Tissue fragments may require disruption by homogenization in an omnimixer or polytron mixer.

2. Bring the mixture to 60°C and, while maintaining this temperature, draw the slimy suspension into a syringe fitted with an 18-gauge needle. Forcefully eject the suspension back into the tube. Repeat until the viscosity of the suspension is reduced by shearing of the liberated chromosomal DNA.

3. Add an equal volume of phenol preheated to 60°C and continue to pass the emulsion through the syringe.

4. Add 0.5 volume of:

   0.1 M sodium acetate (pH 5.2)
   10 mM Tris · Cl (pH 7.4)
   1 mM EDTA

5. Add an equal volume of a 24:1 solution of chloroform and isoamyl alcohol and shake vigorously for 10–15 minutes while maintaining the temperature at 60°C.

6. Cool on ice and centrifuge at 2000*g* for 10 minutes at 4°C.

7. Recover the aqueous phase and reextract with phenol/chloroform.

8. Centrifuge and recover the aqueous phase and reextract twice with chloroform.

9. Add 2 volumes of ethanol. Store at −20°C for 1-2 hours.

10. Recover the RNA by centrifugation at 12,000*g* for 20 minutes at 4°C.

[1] Feramisco et al. (1982)

11. Dissolve the pellet in the original starting volume (step 1) of:

    0.1 M Tris · Cl (pH 7.4)
    50 mM NaCl
    10 mM EDTA
    0.2% SDS

    Add proteinase K to a final concentration of 200 μg/ml. Incubate for 1–2 hours at 37°C.

12. Heat to 60°C. Add 0.5 volume of phenol preheated to 60°C and mix. Add 0.5 volume of chloroform and mix vigorously at 60°C for 10 minutes.

13. Cool in ice and centrifuge at 2000*g* for 10 minutes at 4°C.

14. Extract once more with phenol/chloroform at 60°C and then extract twice with chloroform at room temperature.

15. Precipitate the nucleic acids with ethanol by centrifugation and rinse the pellet in 70% ethanol. Store the RNA in 70% ethanol at −70°C.

16. Dissolve the RNA in sterile water. 5×10^8 cells should yield approximately 5–10 mg of RNA.

Guanidinium/Cesium Chloride Method[2]

1. To a fragment of tissue or a cell pellet, add 5 volumes of:

   4 M guanidinium isothiocyanate
   5 mM sodium citrate (pH 7.0)
   0.1 M β-mercaptoethanol
   0.5% Sarkosyl

   Disperse the tissue by homogenization or the cell pellet by vortexing.

2. Add 1 g of cesium chloride to each 2.5 ml of homogenate.

3. Layer the homogenate onto a 1.2-ml cushion of 5.7 M CsCl in 0.1 M EDTA (pH 7.5) in a Beckman SW50.1 polyallomer tube (or its equivalent). (Other types of centrifuge tubes can be used; see Chirgwin et al. [1979]).

4. Centrifuge at 35,000 rpm for 12 hours at 20°C. This procedure takes advantage of the fact that the buoyant density of RNA in cesium chloride is much greater than that of other cellular macromolecules. During centrifugation, the RNA forms a pellet on the bottom of the tube while most of the DNA and protein floats upward in the cesium chloride solution.

5. Discard the supernatant, dry the walls of the centrifuge tubes thoroughly, and dissolve the pellet of RNA in:

   10 mM Tris · Cl (pH 7.4)
   5 mM EDTA
   1% SDS

6. Extract once with a 4:1 mixture of chloroform and 1-butanol and transfer the aqueous phase to a fresh tube. Reextract the organic phase with an equal volume of:

   10 mM Tris · Cl (pH 7.4)
   5 mM EDTA
   1% SDS

   Combine the two aqueous phases.

7. Add 0.1 volume of 3 M sodium acetate (pH 5.2) and 2.2 volumes of ethanol. Store at −20°C for at least 2 hours. Recover the RNA by centrifugation.

8. Dissolve the pellet in 1 ml of H_2O and reprecipitate with ethanol. Store the RNA in 70% ethanol at −70°C.

[2]Glisin et al. (1974); Ullrich et al. (1977).