Isolation and characterization of RNA from snap-frozen tissues and cultured cells

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Overview

This paper outlines protocols for the isolation and characterization of RNA from whole tissues and cultured cells. Included are methods for eliminating RNase contamination, obtaining and storing tissues or cell pellets; and for isolating RNA from either whole tissues or cultured cells using a guanidinium thiocyanate, phenol/chloroform extraction method as modified from Chomczynski and Sacchi.[1] In addition, procedures for testing the quantity and quality of the RNA obtained are outlined.

Preventing RNase contamination

RNA is extremely vulnerable to the degradative activity of ribonucleases (RNases). In order to isolate intact RNA, it is essential to use ribonuclease-free solutions and equipment. Many laboratories working with RNA set aside certain chemicals and items of glassware and plasticware to be employed exclusively for RNA work. Sterile, disposable plasticware can be used for the isolation and storage of RNA without additional treatment to remove RNases. However, hands remain a major source of contaminating RNases. Thus, gloves should be worn at all times when working with RNA or handling items (e.g., glassware, plasticware, and solutions) that will be used for RNA work. Bacteria and molds present on airborne dust particles are another major source of RNase contamination. *Before beginning the protocol, all items needed for the RNA isolation procedure must be autoclaved, baked, treated with RNase inhibitors, or filtered.*

Autoclaving

Nalgene plasticware, foil-wrapped stir bars and spatulas, Eppendorf tubes, and disposable pipette tips can be autoclaved for 1 hour at 120° C. Most liquid solutions used for RNA work, including DEPC–free distilled, deionized water (dd H_2O), DEPC–treated water (see below), Tris buffers, EDTA, sodium chloride, and sodium acetate must also be autoclaved before use.

Baking

Autoclaving alone may not fully inactivate RNases on laboratory glassware. RNases can be removed by baking glass beakers, flasks, and bottles or packaged Pasteur pipettes in an oven at 180° C for 8 hours or overnight.

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RNase inhibitor treatment

Glassware or plasticware that cannot be sterilized by baking at high temperatures can be treated with inhibitors to remove RNases. Soak items overnight at room temperature in water containing 0.1 to 0.2% diethylpyrocarbonate (DEPC).[2] These items must then be rinsed several times with RNase-free dd H_2O and heated at 100° C or autoclaved for 30 min to remove residual DEPC that may otherwise modify purine residues in RNA by carboxymethylation.[3,4] Solutions can also be treated with DEPC (0.05% final conc.) for 12 hours or overnight at room temperature and then autoclaved for 30 min to remove residual DEPC. *Note: (a) Tris buffers cannot be treated with DEPC because DEPC reacts rapidly with amines. (b) DEPC is a possible carcinogen. It should be handled with gloves, used in a fume hood, and discarded as a toxic waste.* As an alternative to DEPC, glass or plasticware may be rinsed before use with either a 0.1 N NaOH/1 mM EDTA solution or with 3% hydrogen peroxide followed by rinsing in RNase-free dd H_2O.[5]

Filtering

Solutions that cannot be autoclaved or baked should be prepared with DEPC-treated, autoclaved water and filtered through a 0.2 micron filter (Corning or Nalgene) before use.

Sterilizing the Polytron homogenizer

Before using Polytron homogenizer: wash 3 times with 0.1%-DEPC treated water with polytron on. Then, wash 3 times with 20 ml of sterile dd H_2O with polytron on. Then, wash 3 times with 20 ml absolute alcohol with polytron on. Then, wash 3 times with 20 ml of sterile dd H_2O with polytron on.

Between samples, wash 3 times in DEPC-treated water or 0.2% SDS with polytron on. And wash with sterile dd H_2O with polytron on.

After finishing a set of tissue extractions, wash the polytron with warm soapy water, and rinse with a large volume of dd H_2O).

Reagents

Handle all reagents with RNase-free spatulas only!

Agarose, Ultrapure DNA grade	Bio-Rad, Richmond, CA, USA
Bromophenol blue	IBI, New Haven, CT, or VWR, Philadelphia, PA, USA
Chloroform	IBI or VWR
Diethyl pyrocarbonate (DEPC)	Sigma Chemical Co., St. Louis, MO, USA
EDTA, disodium salt	Sigma
Ethidium bromide	Sigma
Glycerol	IBI or VWR
Hydroxyquinoline	Sigma
Isoamyl alcohol	IBI or VWR
Isopropanol	Fisher, Springfield, NJ, USA
2-Mercaptoethanol	Sigma

10% Sarkosyl (n-Lauroyl Sarcosine) in DEPC-treated water	IBI or VWR
2 M Sodium acetate, pH 4.0	Sigma
0.75 M Sodium citrate, pH 7.0	Fisher
0.5% Sodium Dodecyl Sulfate (SDS)	BRL Inc., Gaithersburg, MD, USA
1 M Tris-HCl, pH 8.0	Sigma
0.1 M Tris-HCl, pH 8.0	Sigma
Xylene Cyanol FF	IBI or VWR

DEPC–treated water

1. Add 0.2 ml of DEPC to 100 ml of dd H_2O, and shake vigorously before overnight storage.
2. Inactivate the remaining DEPC by autoclaving for 30 min.

4M Guanidinium thiocyanate (GT stock solution)

(FLUKA Inc., Ronkonkoma, NY, USA) Gloves and goggles should be worn when handling guanidinium thiocyanate as it is a potent chaotropic agent and irritant.

1. Add 44.9 g GT to 60 ml of the following buffer: 3.1 ml. of 0.75 M Na-citrate, pH 7.0 (25 mM final conc.); 4.7 ml. 10% *n*-lauroylsarcosine (0.5% final conc.); 52.2 ml. DEPC–treated, autoclaved water.
2. Heat to 65° C to put into solution.
3. Filter sterilize through a 0.2 micron disposable filter.

This stock solution can be stored at least 3 months at room temperature.

Guanidinium thiocyanate/0.1 M 2-mercaptoethanol (GT/M solution)

1. Add 0.36 ml, of 2-mercaptoethanol to 50 ml. of GT stock solution.

This solution can be stored 1 month at room temperature.

Phenol, buffer equilibrated, pH > 7.6

1. Purchase high-quality redistilled phenol from IBI, Inc. (through VWR) and store at −20° C. Handle with caution: phenol is poisonous and can cause burns. *If a lower quality phenol is purchased, it must be redistilled at 180° C before use. See Perbal[6] for a method on redistilling phenol.*
2. Remove phenol from freezer and liquefy by warming first at room temperature and then melting at 68° C in a waterbath.[5] Transfer 100–200 ml of phenol to a sterile 500-ml beaker containing a sterile magnetic stir bar.
3. Add 8-hydroxyquinoline to a final concentration of 0.1%. This yellow compound provides a convenient way to identify the phenol phase but also acts as an anti-oxidant and partial RNase inhibitor.
4. Add an equal volume of 1.0 M Tris-HCl buffer, pH 8.0, to the melted phenol and stir for 15 min on a heater-stirrer plate set on low heat.

5. Turn off stirrer, and let phases separate for 15 min. Discard aqueous phase to a container for toxic waste.
6. Reextract phenol with 0.1 M Tris-HCl buffer, pH 8.0, stir for 15 min, and repeat steps 4, 5, and 6.
7. Remove a sample of phenol and check pH with pH paper. Repeat extraction with Tris buffer until pH is greater than 7.6.
8. To equilibrated phenol, add 0.1 M Tris-HCl buffer containing 0.2% 2-mercaptoethanol at a ratio of 1:10 (buffer:phenol).

Phenol can be stored under buffer at 4° C for up to one month.

10 times phosphate-buffered saline (PBS)

1. Weigh out the following chemicals: 11.5 g Na_2HPO_4; 2.0 g KH_2PO_4; 80.0 g NaCl; 2.0 g KCl.
2. Add DEPC–treated water to 1 L final volume.
3. Prepare a 1:10 dilution of 10 × PBS. Check that pH of 1 × PBS is 7.4.

Tris-Acetate-EDTA (TAE) buffer

1. For a final volume of 500 ml, mix together: 20.0 ml. 1.0 M Tris-HCl (pH 7.4) (40 mM final conc.); 0.83 ml. 3.0 M Na-acetate (pH 5.2) (5 mM final conc.); 2.0 ml. 0.5 M EDTA (pH 7.5) (2 mM final conc.); and 477.2 ml. DEPC–treated water.

Supplies and equipment

1. Disposable plasticware: Eppendorf centrifuge tubes (VWR); sterile 15 ml and 50 ml plastic tubes (Falcon, VWR); sterile 0.2 micron filters (Fisher); pipette tips, 250 μl and 1000 μl (Fisher).
2. Glassware or Nalgene plasticware: beakers, Erlenmeyer flasks, cylinders, and storage bottles in various sizes; Pasteur pipettes.
3. Check on the accessibility of the following pieces of equipment: low to medium speed centrifuges (for spins of 1–10,000 × *g*); Liquid nitrogen storage container (for whole tissues); autoclave or baking oven; automatic micropipettors; Eppendorf centrifuge; Polytron homogenizer; waterbath spectrophotometer (Gilford or Beckman); gel electrophoresis apparatus and power supply; UV transilluminator; camera set-up for photographing gels.

RNA isolation procedure

Preparation of whole tissues for RNA isolation

Intact RNA can be isolated successfully from autopsy or surgical specimens, but care must be taken to minimize the effect of endogenous RNases. It is often recommended that fresh tissue be transferred immediately into a deproteinizing solution (e.g., guanidinium thiocyanate) for optimal RNA isolation after surgical removal of tissues. However, it is also possible to obtain high-quality RNA from tissue samples that have been snap-frozen in liquid nitrogen after surgical removal and stored at −80° C until extraction, as described below:

1. As samples are obtained, place each tissue into a sterile labeled tube on ice.
2. Fill a 100 ml glass beaker with liquid nitrogen (LN_2).
3. Using a sterile forceps, pick up sample and submerge briefly in LN_2 until completely frozen. Return frozen sample to tube on ice. *To obtain undegraded RNA, tissue samples should be frozen in LN_2 as soon as possible after tissues are removed. If necessary, a small portable LN_2 storage container can be carried to the location of surgery.*
4. Store tissue samples at −80° C until extraction.
5. On day of extraction, remove frozen tissues one at a time, and weigh

quickly on a Mettler balance to determine sample weight. Place weighed tissue onto a sterile glass plate resting on ice.
6. Using a sterile scalpel blade, quickly cut tissue into pieces of the size needed. For extraction in 50 ml tubes, pieces may range in size from 100–1500 mg. Expect an approximate yield of 1.0–3.0 μg RNA/mg tissue.
7. Place pieces to be extracted into separate 50 ml conical centrifuge tubes on ice. Proceed quickly to step 1 of RNA isolation protocol below. Return any remaining tissue sample to −80° C freezer for subsequent extraction of DNA, RNA or protein.

Preparation of cultured cells for RNA isolation

1. Obtain 3–4 × 10^6 adherent cells in a sterile 175 cm^2 tissue culture flask or a 150 × 15 mm tissue culture dish. For cells grown in suspension, obtain culture medium containing 3–4 × 10^6 cells in a sterile conical centrifuge tube, and spin these cells out of suspension by centrifugation at 1,500 × *g* for 10 min at 4° C. Place tube on ice.
2. Wash adherent cell monolayer in culture flask or dish two times with 10 ml of autoclaved PBS buffer. Wash suspension cell pellet two times with cold PBS or isotonic saline (0.89% NaCl), centrifuging at 1500 × *g* for 10 min and decanting supernatant after each wash. (Omit steps 3 and 4 for cells grown in suspension.)
3. For adherent cells, add 10 ml of autoclaved 0.5 mM EDTA in PBS to culture dish, and incubate at 37° C for 5 min. After incubation in EDTA, rap the flask several times to dislodge cells. Confirm cell detachment by visualization under an inverted microscope. (For extracting adherent cells directly from culture dishes, see Titus[7].)
4. Remove adherent cells in EDTA/phosphate solution to a sterile 50 ml plastic centrifuge tube and pellet cells by centrifuging at 1,500 × *g* for 10 min at 4° C.
5. Carefully pour off supernatants from cell pellets. Keep pellets on ice, and proceed quickly to step 1 of RNA isolation protocol below.

RNA isolation

See *Figure 1* for an outline of this protocol.

Cell lysis, nucleoprotein denaturation, and RNA extraction

1. Add GT/M solution to sample in 50 ml plastic centrifuge tube (1 ml GT/M solution for every 100 mg tissue or every 3–4 × 10^6 cultured cells).
2. Homogenize *tissue samples* for 10 sec using a cold Polytron homogenizer with the speed set at 7. (Store homogenizer at 4° C until just before use; during homogenization steps, work with samples under a fume hood.)

   Cultured *cell pellets* do not require homogenization but should be thoroughly suspended in the GT/M solution by pipetting up and down.
3. Add each of the following solutions to the GT/M-treated samples, rehomogenizing (or pipetting thoroughly) for 10 sec after each addition: 2 M Na acetate, pH 4.0, 0.1 ml for every 100 mg tissue or 3–4 × 10^6 cultured cells; redistilled phenol [pH > 7.6], 1.0 ml for every 100 mg tissue or 3–4 × 10^6 cultured cells; chloroform : isoamyl alcohol (49:1), 0.2 ml for every 100 mg tissue or 3–4 × 10^6 cultured cells.
4. Shake suspension vigorously or vortex for 10 sec.
5. Place on ice for 15 min.
6. Centrifuge at 8–10,000 × *g* for 20 min at 4° C. (Several types of centrifuge spin are possible at this step. If sample size permits, transfer samples first to sterile 1.5 ml. Eppendorf centrifuge tubes and centrifuge at 12,000 rpm in an Eppendorf centrifuge for 20 min at 4° C. For larger samples, in 50 ml centrifuge tubes, spin for 20 min at 5,000 rpm in a Sorvall RC3B centrifuge using an H-4000 rotor.)

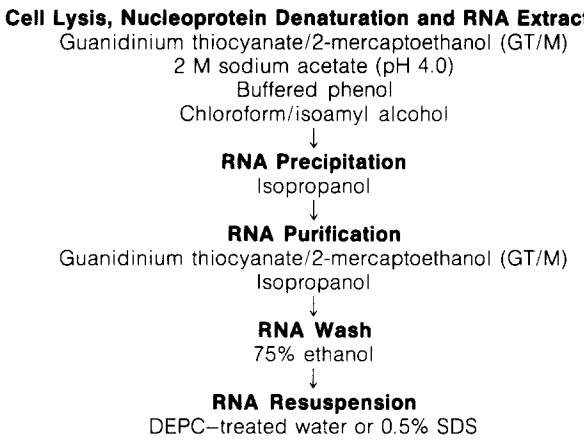

Figure 1 Outline of RNA isolation. Sequential steps to obtain pure, high-quality RNA include the disruption of tissues and lysing of cells, denaturation of nucleoprotein complexes, inactivation of endogenous ribonuclease activity, and separation of RNA from contaminating proteins and DNA. This figure outlines our protocol for accomplishing these goals with the major reagents listed for each step.

7. Remove slightly cloudy, yellow-white aqueous phase (this contains RNA), and transfer to a fresh sterile 50 ml plastic tube or 1.5 ml Eppendorf tubes. Discard into a toxic waste container the yellow organic phase (phenol/chloroform/isoamyl alcohol) (this contains DNA, proteins, and other cell constituents).

RNA precipitation and purification

8. Mix aqueous phase with an equal volume of isopropanol (or with 2 volumes of ethanol). Place tube at −20° C for at least 1 hour to precipitate RNA (at this point one may precipitate overnight).
9. Centrifuge at 8–10,000 × *g* for 20 min at 4° C to pellet RNA (see centrifuge directions outlined in step 6).
10. Carefully pour off supernatant into sterile discard beaker or 50 ml tube. (If RNA pellet is inadvertently poured off at this step, it is generally visible and can be recovered successfully from a sterile container.)
11. Dissolve white RNA pellet in 0.3 ml of GT/M solution per 100 mg of tissue or 3–4 × 10^6 cultured cells.
12. Reprecipitate with 1 volume of isopropanol (e.g., 0.3 ml for every 100 mg tissue or 3–4 × 10^6 cells), or with 2 volumes of ethanol, at − 20° C for 1 hour (at this point one may precipitate overnight).
13. Centrifuge at 8–10,000 × *g* for 20 min at 4° C (see step 6). Pour off supernatant carefully.

RNA wash

14. Wash pellet in 1–2 ml of 75% ethanol for every 100 mg tissue or 3–4 × 10^6 cells. If volume is small enough, transfer washed pellet to a sterile 1.5 ml Eppendorf tube. Otherwise, continue working with 50 ml tubes.
15. Reprecipitate RNA at −20° C for 1 hr.
16. Centrifuge at 8–10,000 × *g* for 20 min at 4° C (see step 6). Pour off supernatant carefully.
17. To remove ethanol droplets before resuspension of RNA, dry the RNA pellet briefly (*a completely dry pellet is hard to resuspend*) by one of the following methods: (a) wipe sides of tube with sterile cotton and air-dry pellet at room temperature; or (b) use a sterile Pasteur pipette to blow a gentle stream of nitrogen gas onto RNA pellet sitting on ice (about 2–3 min).

RNA resuspension and storage

18. Dissolve dried RNA in 50 μl of 0.5% SDS for every 100 mg tissue or 3–4 × 10^6 cells at 65° C for 10 min. Alternatively, RNA may be dissolved in DEPC–treated sterile distilled deionized water. (Resuspension in 0.5% SDS helps to inhibit RNases but these samples must be heated after freezing to put SDS back into solution.)
19. This RNA preparation can be stored at −20° C for several months. For long-term storage, RNA preparations should be ethanol precipitated and then stored at −20° C or −80° C (some loss occurs, so do not reprecipitate very small samples).

Checking the quantity and quality of isolated RNA

A. Determining the quantity of RNA. The concentration of RNA in solution can be measured by using ultraviolet absorption spectrophotometry.[8]

1. Clean a quartz cuvette thoroughly with dd H_2O and acetone before using.
2. Blank spectrophotometer (Beckman DU-64 or Gilford) with dd H_2O in cuvette (depending on the size of the cuvette, 100 μl to 1000 μl) at optical density (OD) 230, 260, 270, and 280 nm.
3. Add 4 μl of the RNA solution to 996 μl of dd H_2O. Repeat OD measurements at the above four wavelengths.
4. Calculate the RNA concentration based on the extinction coefficient for RNA (an OD_{260} of 1 = 40 μg RNA/ml.). If 4 μl of RNA solution are added to 996 μl H_2O, the RNA concentration in μg/μl will be 10 × OD reading at 260 nm.

Note: If the amount of RNA in the sample is less than 0.1 μg/μl, OD readings will not be reliable. Use a less dilute sample, and read again.

5. Determine the purity of the RNA. Calculating the ratios of the OD measurements at different wavelengths provides information on whether or not the RNA sample is contaminated with protein, phenol, lipids, or guanidinium.

   A_{260}/A_{280}: The ratio of absorbance at 260 nm to absorbance at 280 nm should be 1.7 to 2.0. If there is contamination with protein, this ratio will be considerably lower. A phenol/chloroform reextraction and ethanol precipitation should be performed. Some loss of RNA (up to 40%) may be expected.

   A_{260}/A_{270}: The ratio of absorbance at 260 nm to absorbance at 270 nm should be greater than 1. Otherwise, there may be phenol contamination, for which a chloroform extraction followed by ethanol precipitation should be performed.

   A_{260}/A_{230}: The ratio of absorbance at 260 nm to absorbance at 230 nm provides a measure of RNA:lipid or an indication of guanidinium thiocyanate contamination. This value should be between 2.0–2.2. If lower than this, reextract with chloroform:isoamyl alcohol, and add isopropanol or ethanol to the aqueous phase to reprecipitate the RNA.[7]

B. Determining the quality of RNA. The integrity of a small amount of purified total RNA (2–5 μg) can be examined on a 0.7% agarose minigel (50 × 75 mm; Bethesda Research Laboratories Life Technologies, Inc.) without using denaturing agents such as formaldehyde or glyoxal that are needed for Northern blot analysis.[5] Alternatively, a larger (75–100 ml. volume), firmer (0.8–1% agarose) analytical gel containing 2.2 M formaldehyde can be run in a 95 × 150 mm gel mold. Such a gel requires longer electrophoresis times (2–3 hours) and more sample[4] (10–20 mg RNA) but may be utilized directly for a Northern blot analysis if the quality and

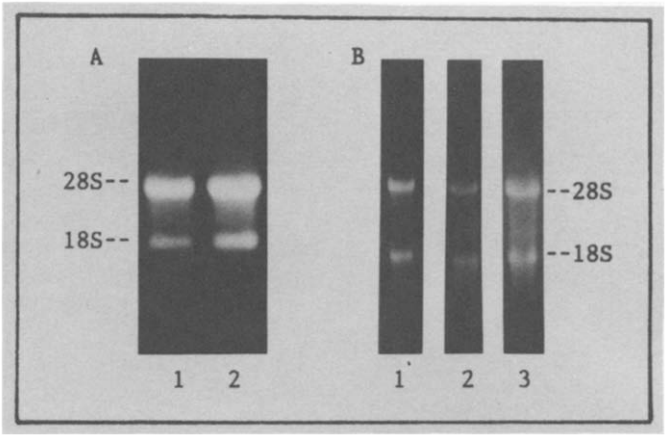

Figure 2 Analysis of isolated RNA by agarose gel electrophoresis. Preparations of RNA isolated by the described guanidinium thiocyanate method were analyzed by agarose gel electrophoresis. The 28S and 18S ribosomal RNA bands are labeled. (*A*) RNA isolated from cultured HeLa cells following electrophoresis for 1 hour on a 0.7% agarose minigel and staining with ethidium bromide as described. Lanes 1 and 2: 7.6 and 7.9 μg of HeLa cell RNA from two independent isolations were loaded, respectively. (*B*) RNA isolated from snap-frozen mammalian tissues following electrophoresis for 2.5 hours on a larger minigel of 1.0% agarose and staining with ethidium bromide. Lane 1: 15 μg rat spleen RNA extracted from tissue snap-frozen immediately after surgery. Lane 2: 20 μg normal human thyroid RNA extracted from tissue snap-frozen after autopsy. Lane 3: 20 μg of normal human thyroid RNA extracted from tissue snap-frozen after surgery.

sample loading of RNA are appropriate (see references 4, 5, 6, or 8 for Northern blot protocols). The following method is recommended as a way of checking RNA quality without sacrificing much sample.

1. Seal a gel casting mold at both ends with strips of tape. Insert a slot former close to one end.
2. Add 0.14 g of DNA grade agarose to 20 ml of Tris-acetate-EDTA buffer (0.7% final concentration). Boil the mixture until it bubbles, and allow it to cool to 60° C.
3. Pour the cooled solution into the gel mold, and let it harden for 30 min at room temperature. Carefully remove slot former and tape strips.
4. Place molded gel into buffer chamber, and slowly fill buffer chamber with Tris-acetate-EDTA buffer, roughly 400 ml, until the gel slab is covered with a 1 to 2 mm layer of buffer.
5. Heat–denature the RNA sample at 65° C for 5 min before adding 2–5 μg RNA to 5 μl of 5X loading buffer containing 25% glycerol, 0.2% bromophenol blue, and 0.2% xylene cyanol. Bring sample volume up to 25 μl with dd H_2O before loading into designated slot.
6. Apply a constant voltage of 50 to 75 volts for 1 hour. Be sure the gel loading slots are close to the negative pole with the positive electrode at the far end of the gel apparatus.
7. Follow electrophoresis of samples by observing the bromophenol blue dye front. As the bromophenol blue moves off the gel, disconnect the power supply, and transfer gel into 100 ml of water containing 0.5 μg/ml ethidium bromide. Shake for 30 min, and wash twice for 15 min with 100 ml water.
8. The ribosomal RNA bands (28 S and 18 S RNA) should be clearly visible when gel is placed on a UV transilluminator (see *Figure 2*), with the ratio of 28S to 18S band approximately 2:1. In degraded RNA samples, this ratio will be decreased or reversed since the 28S ribosomal RNA is degraded to a 18S-like species.
9. Photograph the UV-illuminated gel to record the quality of ribosomal bands. *A protective face shield should be worn when viewing and photographing bands to minimize damage to skin and eyes from the UV light.*

Discussion

The ability to isolate undegraded RNA is essential to the study of gene regulation and expression. However, mammalian tissue and cultured cells are rich in endogenous ribonucleases. Certain tissues (e.g., pancreas and spleen) are especially high producers of these degradative enzymes. Consequently, all RNA isolation steps should be done on ice to minimize the activity of these RNases.[9] To prevent degradation by RNases from other sources, all solutions, glassware, plastic tubes, pipette tips, and related materials that come into contact with RNA must be autoclaved, baked, or treated with RNase inhibitors before use, as discussed in this paper.

Over the past 20 years several methods have been developed for the isolation and characterization of RNA from cell and tissue samples. Current RNA isolation protocols employ guanidinium thiocyanate, N-lauroyl sarcosine (sarkosyl), and beta-mercaptoethanol as agents for denaturing proteins and inhibiting RNases immediately after organelle disruption during the purification process.[10-13] This guanidinium procedure works particularly well with tissues that have high levels of endogenous RNases. In this paper we have applied this technique to snap-frozen tissues and to harvested pellets of cultured cells. However, for cells grown in monolayer cultures it is also possible to lyse the cells and extract RNA directly in the culture flask using either a modification of this guanidinium thiocyanate method[7] or the detergent lysis method.[5]

RNA isolation from mammalian tissues stored at $-80°$ C is optimized by rapid snap-freezing of tissues in liquid nitrogen as soon as possible after surgery. The subsequent isolation of RNA as described in this paper depends upon first lysing the cells or tissue in a chemical environment that also inhibits RNase activity. Phenol/chloroform extraction and isopropanol precipitation are then employed to separate selectively intact RNA from contaminating DNA and proteins and to eliminate lengthy ethanol precipitation steps or overnight ultracentrifugation through cesium chloride gradients.[14] Thus, this RNA extraction procedure is fast and can be scaled up or down to isolate RNA from large or small samples. It can reproducibly yield 20 to 40 μg total RNA per 1×10^6 cells and 1–3 μg total RNA per mg of whole tissue. Total RNA isolated by this method includes intact nuclear RNA plus a mixture of cytoplasmic RNAs (ribosomal, messenger, and transfer RNA). If desired, this total RNA can be passed over an oligo-dT column to obtain purified poly-A+ mRNA (see references 4 and 9 for methodologies). However, unless an RNA transcript is extremely rare, total RNA can be analyzed successfully on Northern or slot blots by using specific molecular probes to study various RNA expression patterns.

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