Improved Purification and Yields of RNA by RNeasy®

BioTechniques 21:57-60 (July 1996)

Many relatively rapid methods are presently available for isolation of total RNA from tissues or cultured cells, most of which are reported to yield RNA of sufficient purity for Northern blotting, cDNA synthesis, cloning and reverse transcription-polymerase chain reaction (RT-PCR). One such recent method is in the form of a commercial kit called RNeasy® (Qiagen, Chatsworth, CA, USA) and is based on the selective binding of RNA in crude guanidinium isothiocyanate (GIT) extracts to silica beads within microcentrifuge spin columns. In this method, cells are lysed with a buffered solution of GIT, lysate-mixed with an equal volume of 70% ethanol and then spun through a silica column by centrifugation at 10000× *g* for 20 s. Impurities are washed through the column by centrifugation once with a solution containing GIT and twice with a solution of 80% ethanol. RNA is then eluted with a relatively small volume (30–60 µL) of nuclease-free water. We find RNeasy to be a very economical and effective way of rapidly purifying multiple samples (12 samples/h) suitable for Northern blotting, RT-PCR and other common applications. However, RNeasy is suitable for relatively small amounts of RNA (<50 µg). In this report, we describe a simple and rapid addition to this procedure that results in a 3-fold to 60-fold increase of RNA yield, while significantly improving RNA purity. Furthermore, this additional step shortens the total hands-on time required for RNA purification by RNeasy when greater than 1.8 mL per sample of extract is processed, by reducing the number of additions of extract to the column (each following a centrifugation step) from ≥6 to only 1.

This improvement of the RNeasy method consists of extracting cells or

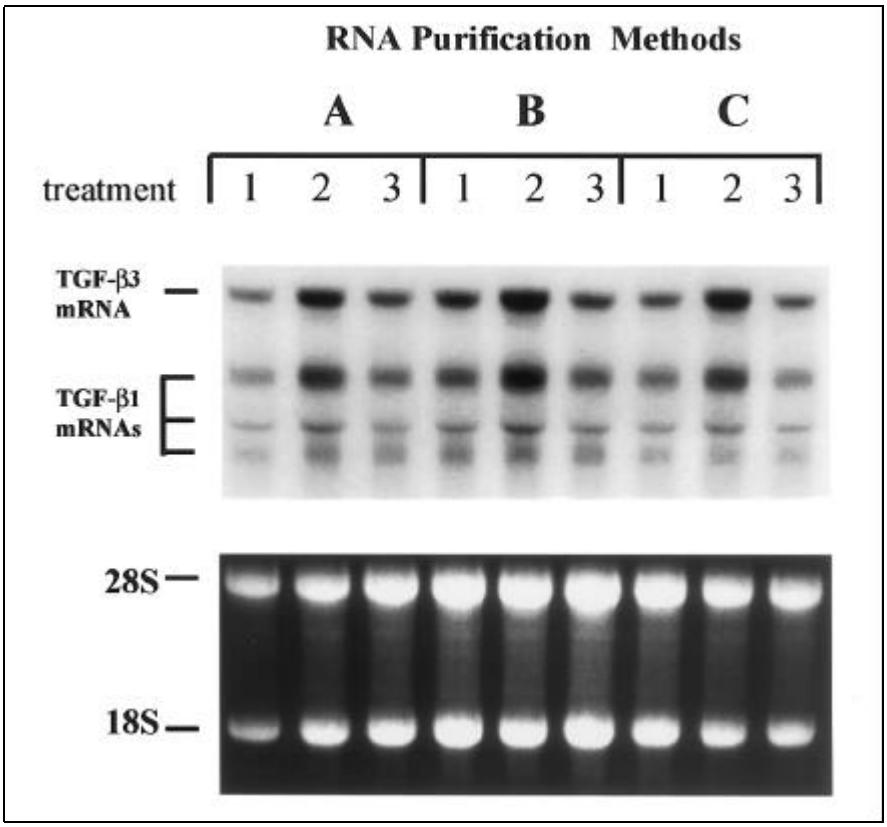

Figure 1. Autoradiogram of a Northern blot as described in Table 1. RNA was purified by IRNeasy (A), RNeasy (B) and AGPC (C). The treatments are: 1 = control, 2 = TGF-β1 and 3 = TGF-β1 latency associated protein. Bottom panel shows the fluorescence of 28S and 18S ribosomal RNAs.

Table 1. Comparison of Yield and Purity of Three Methods For RNA Purification

Methods	μg RNA (by A_{260})	Densitometric Scan (volume* × 10^{-3}) 260/280	TGF-β1 mRNA	TGF-β3 mRNA
Control				
RNeasy (A)	59	2.01	7.8	3.8
IRNeasy (B)	122	1.94	13.4	6.6
AGPC (C)	108	1.67	10.7	4.4
Treatment 2				
RNeasy (A)	46	2.10	13.9	8.6
IRNeasy (B)	161	1.85	18.4	11.3
AGPC (C)	67	1.60	13.8	8.0
Treatment 3				
RNeasy (A)	69	2.05	9.8	4.1
IRNeasy (B)	166	1.84	13.2	4.9
AGPC (C)	109	1.72	9.4	3.4

RNA was purified by three different methods (described in text) from equal numbers of NRP-152 cells (ca. 3×10^6 cells) cultured in Dulbecco's modified Eagle medium (DMEM)/F12 + 1% calf serum for 2 days for the controls or those treated with either 5 ng/mL TGF-β1 (treatment 2) or 250 ng/mL TGF-β1 latency-associated protein (treatment 3). 10 mg of RNA (measured by A_{260}) were electrophoresed, blotted and hybridized with rat TGF-β1 and mouse TGF-β3 [^{32}P]cDNAs, as described by Danielpour et al. (2). Intensity of the signal, which is directly proportional to the amount of message, was determined by phospho imager scanning of the average of two blots and is expressed in volume*.

tissues according to the first step in the procedure by Puissant and Houdebine (4) in 13- × 100-mm polypropylene round-bottom tubes, as follows: Cells from 100-mm and 150-mm dishes are extracted with 1 mL or 2 mL solution D (1), respectively, and then treated by vortex mixing sequentially with 0.2 vol of 4 M sodium acetate (pH 4.0), 1.0 vol of phenol (water-saturated) and 0.05 vol of chloroform. Following a 10-min centrifugation at 10 000× *g*, the RNA in the top (aqueous) phase is transferred to a tube containing an equal volume of isopropanol. RNA is pelleted by a 10-min centrifugation at 5000× *g*. The supernatant is carefully decanted, and the RNA pellet is dissolved by gentle pipetting in 350 μL solution D. After the addition of 350 μL 70% ethanol, the solution is mixed by gentle pipetting and transferred to an RNeasy column. All subsequent steps are done as described in the RNeasy kit.

We compared the yield and quality

Table 2. RNA Recovery from Tissues by IRNeasy

Method	Source	mg Tissue	µg RNA Recovered
A	liver	480	298
B	liver	480	26.4
A	heart	240	126
B	heart	240	2
A	lung	480	136
B	lung	480	11.4
A	spleen	400	230
B	spleen	400	78
A	kidney	240	230
B	kidney	240	31
A	testis	400	254
B	testis	400	36

Adult Lobund-Wistar rat tissues were homogenized in 2.5 mL of solution D or RNeasy lysis buffer, and RNA was purified by either IRNeasy (A) or RNeasy (B). Purified RNA was quantitated by absorbance at 260 nm.

of RNA from NRP-152 rat prostatic epithelial cells (2) purified by RNeasy, improved RNeasy (IRNeasy), and improved modification (4) of the acid guanidinium-phenol-chloroform (AGPC) method of Chomzynski and Sacchi (1). The RNA was quantified by absorbance at 260 nm, and the quality of RNA was determined by Northern blot analysis of TGF-β1 and TGF-β3 mRNAs (Table I), by loading 10 µg RNA (based on A_{260}) per lane. As shown by RNA quantitation at A_{260}, the improved RNeasy (IRNeasy) method gave greater RNA yields than AGPC and 2-fold to 3-fold more RNA than the standard RNeasy method. When TGF-β1 and 3 mRNAs were quantitated by densitometric scanning, an average (from 12 determinations) of over 44% more specific transcript were found in IRNeasy lanes than in RNeasy or AGPC lanes. This implies that the IRNeasy method yields 44% more RNA per unit of A_{260} than the unmodified RNeasy method, and therefore the high 260/280 absorbance ratios of RNA obtained by RNeasy seriously overestimates RNA purity. Based on the µg RNA/A_{260} correction, the actual yield of RNA by the IRNeasy method is about 3-fold to 4-fold greater than by the unmodified RNeasy method.

The RNAs purified by all three methods were equally stable, even after incubation for 3 h at 37°C in the absence of RNase inhibitor (Figure 1). DNA contamination was far less in the RNA preparation from the IRNeasy method than from the other two methods, as assessed by DNA amplification with differential display methodology (3) without reverse transcription of RNA into cDNA (data not shown).

We compared the yield and quality of RNA from rat tissues (240–480 mg tissue in 2.5 mL extract) prepared by IRNeasy and RNeasy procedures (Table 2). The improved procedure gave a yield that was about 3-fold to 60-fold greater than the standard procedure (Table 2). As assessed by Northern blot analysis, the purity of RNA by IRNeasy was significantly better than by RNeasy (data not shown). Although we have not determined the upper limit of RNA recovery by IRNeasy purification, we have obtained as much as 400 µg of high-quality RNA from a single purification (1 column). The phenol-chloroform extraction in the IRNeasy method significantly reduces the amount of protein and DNA loaded on the column. It is thus likely that the increased recovery of RNA in the RNeasy method may be attributed to the removal of such impurities, which interfere with the binding of RNA to the resin.

Because of the greater time required, the improved RNeasy method may not be advantageous for all applications. We recommend using the improved protocol if *(i)* >50 µg RNA is needed, *(ii)* RNA is extracted from tissues, *(iii)* >1.8 mL lysate is prepared or *(iv)* cleaner RNA than that generated directly by RNeasy is required.

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Address correspondence to David Danielpour, Laboratory of Chemoprevention, Building 41, Room C629, National Cancer Institute, Bethesda, MD 20892-5005, USA.

Received 9 August 1995; accepted 10 January 1996.

Michael J. Bonham and David Danielpour
National Cancer Institute
Bethesda, MD, USA