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A useful procedure to isolate simultaneously DNA and RNA from a single tumor sample

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Method Article

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Abstract

Introduction

The advances in high throughput techniques for analyzing DNA and RNA have the potential to make a major impact on prevention, diagnosis, prognosis and treatment of many human cancers \(1). One of the major challenges is to obtain high-quality DNA and RNA molecules. Although several RNA and DNA isolation methods for frozen tissues have been used \(2-6), few efforts have been conducted to optimize these existing methods in order to isolate simultaneously high-quality RNA and DNA in an easy-to-use manner. In an effort to establish an efficient, cost-effective and simultaneous isolation of RNA and DNA from single human tumor sample, it was used RNeasy® spin columns with an unique modification of Qiagen protocol \(7-8). That allows isolating simultaneously genomic DNA and totaling RNA from a single sample, with low cost and time-consuming less than 30 minutes. In contrast to other procedures where either the biological sample or the purified total nucleic acids is divided into two before being processed separately. The resulting high-quality DNA has an average length of 15-30 kb depending on homogenization and storage conditions. And it is ready to use in any downstream application, including: PCR; Southern Blot; Comparative Genome Hybridization, Methylation-specific PCR and SNP analysis.

Reagents

Biological sample \(Tumor specimens) RNAlater™ \(Ambion, Austin, TX). RNeasy® Midi and, or Mini spin columns \(Qiagen, Valencia, CA) NaOH 8.0mM HEPES 0.10M or 1.0M EDTA 1.0mM Tris-HCl 10mM pH 8.0

Equipment

POLYTRON® \(Kinematica AG) Centrifuge Spectrophotometer Gel eletrophoresis apparatus

Procedure

Procedure to simultaneous RNA and DNA isolation: 1) Fresh, frozen, or RNAlater™ stabilized tissues can be used in this protocol. Note: For optimal results, stabilize harvested tissues immediately in RNAlater RNA Stabilization Reagent is recommended. Tissues can be stored in the reagent for up to 1 day at 37°C, 7 days at 15-25°C, or 4 weeks at 2-8°C, or archived at -20°C or -80°C. 2) Excise the tissue sample from storage. If the tissues were stored in RNAlater™ at -20°C, be sure to remove any crystals that may have formed. 3) Place the tissue on a clean surface and cut it in small peaces. Next, place them directly into a suitably sized vessel for disruption and homogenization. 4) Disrupt the tissue and homogenize the lysate in Buffer RLT \(RNeasy® Mini or Midi kits, Qiagen, Valencia, CA) as recommended by instructions of manufacturer \(7-8). Immediately disrupt and homogenize the tissue using a conventional rotor-stator homogenizer \(Polytron) until it is uniformly homogeneous \(at least 20s). 5) Follow all steps described in RNeasy® Mini or Midi Handbook for total RNA extraction. 6) After RNA isolation, place each RNeasy®

spin column in a new 1.5mL \(mini) or 15mL \(midi) collection tube and add the appropriate volume of DNA nuclease-free isolation buffer \(8.0mM NaOH) \(see **Table 1**) directly onto the RNeasy® silica-gel membrane. 7) Next, close the tube gently and let it stand for 5-10 min at 55°C, and then centrifuge for 3 min at 3000-5000 x g at room temperature to elute genomic DNA. 8) If the expected DNA yield is high, repeat step 7 using another DNA nuclease-free isolation buffer \(see **Table 1**). Note: Reuse the collection tube from step 7. 9) After elution, DNA samples in 8.0mM NaOH can be stored overnight at 4°C. Nevertheless, for prolonged storage, adjustment of samples with HEPES to pH 7-8 and supplement with 1.0mM EDTA is required. Use the following amounts of 0.10M or 1.0M HEPES \(free acid) per 1.0mL of 8.0mM NaOH \(**Table 2**). Once pH was adjusted, DNA could be stored at 4°C or -20°C. 10) In order, to determine the amount and purity of DNA, read samples in spectrophotometer at wavelengths of 230, 260 and 280 nm. The reading at 260 nm is used to calculate concentration \(yield). The ratio of the readings at 260 and 230 nm \(OD260/OD230) and 260 and 280 nm \(OD260/OD280) provides an estimate of the purity of the DNA. 11) Finally, perform the DNA integrity check by an electrophoresis running of 300ng DNA sample on agarose 0.8%\(w/v) Ethidium Bromide stained gel.

Timing

Time-consuming less than 30 minutes.

Troubleshooting

1) RNA/DNA degradations: β-Mercaptoethanol \(β-ME) must be added to Buffer RLT before use \(7-8). 2) Less DNA quantities and impurity: All centrifugation steps are performed at 20-25°C in a standard laboratory centrifuge with a swinging bucket rotor capable of ≥ 3000 x g. Ensure that the centrifuge does not cool below 20°C \(7-8). 3) DNA degradation: The disruption and homogenization of tissue sample with Polytron must not exceed 20s. 4) DNA degradation: The adjustment of DNA samples with HEPES to pH 7-8 must be made for prolonged storage. 5) RNA/DNA degradation: Be sure that all homemade solutions are nuclease-free. 6) Ratios < 1.8 fold: Use Tris-HCl 10mM pH 8.0 for spectrophotometric determination of the amount and purity of DNA.

Anticipated Results

After RNA isolation using either RNeasy®Mini or Midi spin columns was possible to isolate high-quality and integrity genomic DNA \(**Figure 1**). For all DNA samples the ratios OD260/OD230nm and OD260/OD280 were at least >1.8 fold.

References

1) Rangel MC, Silva SD, Castro NP, Valentin MD. Essentials of Molecular Biology in Cancer Research. Applied Cancer Research 2008;28\(1):2-10. 2) Krieg P, Amtmann E, Sauer G. The simultaneous extraction of high-molecular-weight DNA and of RNA from solid tumors. Anal Biochem. 1983;134\(2):288-94. 3)

Meese E, Blin N. Simultaneous isolation of high molecular weight RNA and DNA from limited amounts of tissues and cells. Gene Anal Tech. 1987;4\(3):45-9. 4) Raha S, Merante F, Proteau G, Reed JK. Simultaneous isolation of total cellular RNA and DNA from tissue culture cells using phenol and lithium chloride. Genet Anal Tech Appl. 1990;7\(7):173-7. 5) Chomczynski P. A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. Biotechniques 1993; 15 \(3): 532-4, 536-7. 6) Merante F, Raha S, Reed JK, Proteau G. The simultaneous isolation of RNA and DNA from tissues and cultured cells. Methods Mol Biol. 1996;58:3-9. 7) RNeasy® Mini Handbook. Qiagen, Fourth Edition, 2006. p.39-44. 8) RNeasy® Midi/Maxi Handbook. Qiagen, Second Edition, 2001.p.26-31.

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Figures

RNeasy® column	Expected total RNA yield	DNA nuclease-free isolation buffer
Mini ○	10-30µg	30µL
Mini ○	≥ 30µg	50µL
Midi ●	≤150µg	150µL
Midi ●	150µg-1.0mg	250µL

Figure 1

Table 1 DNA nuclease-free isolation buffer volumes for RNeasy Mini/Midi elution.

Final pH	0.10M HEPES (µL)	Final pH	1.0M HEPES (µL)
8.4	86	7.2	23
8.2	93	7.0	32
8.0	101		
7.8	117		
7.5	159		

Figure 2

Table 2 Adjustment of DNA solution to a desired pH by the addition of HEPES.

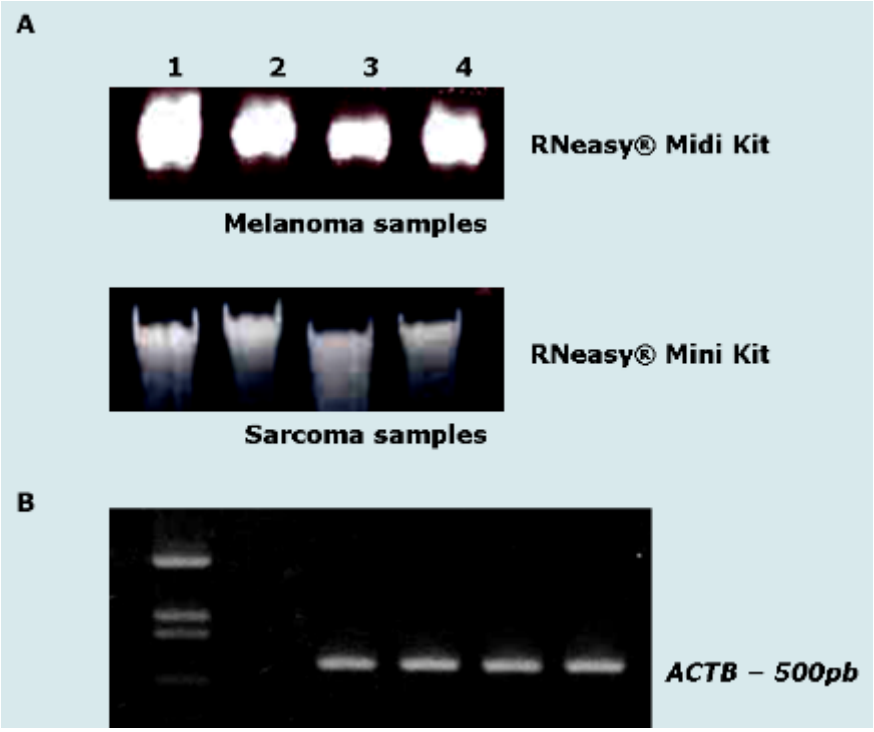

Figure 3

Figure 1 Integrity of purified genomic DNA. A) Genomic DNA isolated from tumor samples (1-4) by using optimized RNeasy® Mini or Midi kits. B) PCR (30 cycles) for ACTB gene, using genomic DNAs (<100ng) from sarcoma samples as template.