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Research Paper

Rapid and Reliable Method For Extraction of Total Rna From Blood/ Tissue

Biology

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ABSTRACT

To study the function of any cell/ tissue, detect certain diseases, for expression studies, it is highly desirable to obtain RNA/mRNA from a homogeneous source. In this method pure RNA was isolated manually, without trizol or use of PBS or any extraction kit. In this, Denaturing solution is been used, at first it denatures the cell membrane and protein. In this method β- mercaptoethanol is added separately so as to reduce disulphide bonds more efficiently and denature the protein. It eliminates the ribonuclease released during cell lysis and prevents the RNA from getting digested during RNA extraction. Total RNA is extracted by this process with a very high intensity and purity. This process is completed within 2 hours. Intense RNA extracted was further used for cDNA synthesis by RT-PCR.

Introduction

The study of RNA has an immense importance in the field of biochemistry, microbiology, molecular evolution, and structural biology, so it is important to understand the changing roles of RNA within the cell. The role of RNA within the cell has changed from catalysis to endogenous RNA interface to non-coding RNAs. The field of RNA is defined by many different species that exist in nature. The most known and diverse species is messenger RNA (mRNA's). These mRNA's are further translated into proteins. There are many different types of RNA's that have different functions and roles in signaling of the cell. The genetic information resides in the sequence of mRNA and the phenotype is derived from catalytic properties of RNA (Gerald.F.Joyce, July 2002). It has been observed that 70% of genome is transcribed into mRNA but only 2% of it serves as blueprints of protein (Bertone.P, 2004; Berney.E, 2007; Carninci.P, 2005; Cheng.J, 2005; Kapranov.P, 2007). RNA sequencing is now an approach to transcriptome profiling (Wang Z, Jan 2009). Long non coding RNA's (ncRNA) are deregulated in different human cancers and also show tissue specific expression (Tony Gutschner, Sven Diederichs, 2012). Micro RNA's (miRNA) play an important role in cancer (Calin GA, 2006; Trang P, 2008; Winter J, 2009.) The extraction of RNA is complicated by the ubiquitous presence of RNases that may degrade RNA samples, thus making its use for molecular studies difficult. RNases are quiet hardy and it's difficult to inactivate them.

The aim of current study was to determine the optimal extraction of RNA from the blood/tissue samples.

Materials and Methods

Blood and Tissue samples

Twelve different blood samples of diabetic and cancer tissue were collected with the help of fine sterile syringe and were then transferred into vacuum tube containing EDTA. These fresh blood samples are collected from Local Hospital, Delhi.

Solutions

Denaturing solution was composed of 4M guanidium thiocyanate(sisco research laboratories) , 0.75 M sodium citrate(sigma) (pH-7.0) and 26.4 ml of 10% sodium dodecyl sulphate(srl pvt. Ltd) and chloroform: isoamyl(49:1) and saturated phenol (pH4-5) (saturated by either chloroform or milliQ or DEPC treated water, and β mercaptoethanol 0.72µl.

RNA isolation protocol

- Fresh or frozen blood/ tissue samples were collected from different cancer and diabetic patients in vacuum tubes.
- Take 0.5ml of blood samples into RNase free eppendoff.
- Centrifuge (thermo centrifuge) these samples in normal centrifuge at 3000 rpm for 10 minutes to extract other components of blood.
- Discard the supernatant and to pellet add equal proportion of denaturing solution and keep at room temperature for 20 minutes to homogenize the sample.
- Add 0.1 ml of 2M sodium acetate pH 4.0, inverse mix gently.
- Place the tubes in ice for 10 minutes.
- Add 300µl or maximum quantity of 500 µl phenol, inverse mix and place in ice for 5 min.
- Add 0.2 µl (49:1) ratio of CIA.
- Vortex and immerse pellet, mix thoroughly and incubate the suspension for 15 minutes at 0º-4º C.
- Centrifuge (thermo refrigerator centrifuge) at 20 minutes for 9000 rpm at 4º C.
- Three phases are formed.
- Transfer upper aqueous phase into new vial.
- Precipitate RNA by adding 1µl of 100% isopropanol.
- Incubate for 30 minutes at -20º C.
- The mix was centrifuged at 9000 rpm for 10 minutes.
- Discard the supernatant and air dry the pellet for 10 minutes on ice packs to inactivate RNase.
- Resuspend in TE buffer (70µl or 1ml) and incubate at -20 C for overnight for proper expression of RNA.

Amount and purity of RNA

The yield of RNA for 0.5ml of blood sample extracted was recorded using a UV-double beam spectrophotometer (thermo) at ʎ 260nm.

The purity of RNA was carried by determining absorbance ratio between ʎ 260 and ʎ 280nm. 1 OD of RNA at 260/280 absorbance give 40-50 ng/ µl, OD recorded has given value at maximum of 2.0 and at minimum of 1.46 of RNA in blood samples.

RNA concentration and purity was also determined by running samples on 0.5 – 1.5 % agarose based gels, intensity of band along with the Lambda DNA marker.

The concentration of nucleic acid was calculated as following:

RNA quantification by spectrophotometric method:

The quantification of RNA was done by recording its absorbance at ʎ260 & ʎ280 nm using UV double beamed spectrophotom-

eter as follows:

1. 200µl TE buffer was taken in a cuvette and spectrophotometer was calibrated at ʎ 260 nm to ʎ 280nm wavelength. 5µ of RNA mixed in 1500 µl of TE buffer properly and the absorbance (A) at 260 nm to that of 280 nm was recorded.
2. RNA concentration was calculated by using the formula:

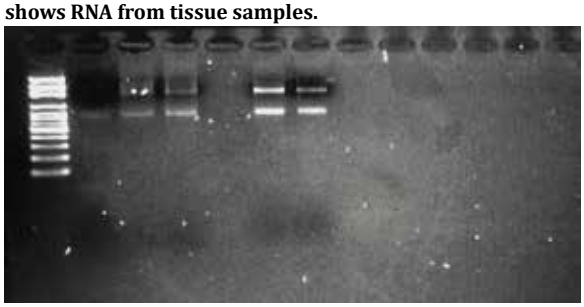
$$\text{Amount of RNA (µg/µl)} = \frac{\text{A260 X 50 X dilution factor}}{1000}$$

3. Quality of RNA depends upon the ratio of absorbance recorded at ʎ 260 nm and 280nm.

RNA quantification by agarose gel method:
1% agarose gel was prepared (5µg/µl ethidium bromide was added). The gel was submerged in running buffer, 4µl loading dye and 6µl RNA sample was loaded into wells 20 voltage was applied to electrophoresis apparatus till dye migrated one third distance in gel. RNA bands can be under transilluminator and quantified in comparison to standards.

Results & Discussion
It was observed that RNA extraction from tissue / blood samples was improved by modifying denaturing solution method done manually, without use of trizol reagents. High quality RNA was isolated from both tissue as well as blood samples, with low protein and RNase contamination. The quality and quantity of RNA was checked by running 1% agarose gel in electrophoretic unit viewed under UV transilluminator.

Figure 1: Results of agar gel electrophoresis performed. First well has 1kb DNA ladder. Second , third and fourth well shows RNA from blood samples. Sixth and seventh well shows RNA from tissue samples.



RNA isolated from different blood samples of diabetic patients both fasting and post-parodial.

In present study, denaturing solution was added to remove the cell membrane and components and yield pure RNA. The extracted RNA is highly quality from 1.57-1.96. In all blood samples and obtained yield range from 56-76 µg/µl. We have found change in yield of RNA for manual and kit method.

Table 1:Results of RNA quantification by spectro-photometric analysis.

Samples	Absorbance	Yield (µg/µl)
Blood		
1.	1.65	63 µg/ul
2.	1.72	69.25µg/µl
3.	1.92	76 µg/µl
Tissue		
4.	1.45	56 µg/µl
5.	1.57	60 µg/µl

RNA obtained from different blood samples

Various methods of RNA isolation like trizol method, kit method was tested to obtain excellent quality of RNA for RT-PCR reaction.

In present study RNA was isolated from denaturing solution method without use of any addition PBS and yield of RNA obtained is good.

We successfully isolated RNA from tissue and blood samples of diabetic patients within 2 hours. The RNase free RNA produced 44 -45 µg/ gM.. The present optimized protocol for RNA isolation can serve as efficient tool for optimizing good yield of RNA and produce efficient amount of cDNA libraries.

Conclusion
We conclude that RNA can be easily extracted in 2 steps & within 2 hours without any trizol reagents or extraction kit with good quality from tissue or blood samples for cDNA libraries.

REFERENCE

Gerald F. Joyce. The antiquity of RNA based evolution. Nature 418, 214-221 (11 July 2002) doi: 10.1038/418214a | Wang Z, Gerstein M, Snyder M. RNA- Seq: a revolutionary tool for transcriptomics. Nat Rev Genet, 57-63 10 Jan 2009, doi: 1038/nrg2484. | Tony Gutschner, Sven Diederichs. The hallmarks of cancer: A long non coding RNA point of view. RNA biology 9:6. 703-719 June 1, 2012. | Bertone P, Stolc V, Royce TE, Rozowsky JS, Urban AE, Zhu X, et.al. Global identification of human transcribed sequences with genome telling arrays, Science 2004 ; 306: 224-6. | Birney C, Stamatoyannopoulos JA, Dutta A, Guigo R, Gingeraas Tr, Margulies Eh, et.al. Identification and analysis o functional elements in 1% of human genome by the ENCODE pilot project. Nature 2007; 447:779-816. | Carninci P, Kasukawa T, Katayama S, Gough J, Frith MC, Maeda N, et al. The transcriptional landscape of the mammalian genome. Science 2005 ; 309:1559-63. | Cheng J, Kapranov P, Drenkow J, Dike S, Brubaker, Patel S, et.al. Transcriptional maps of 10 human chromosomes at 5 nucleotide resolution. Science 2005, 308: 1149-54. | Kapranov P, Cheng J, Dike S, Nix DA, Duttagupta R, Willingham AT, et.al. RNA maps reveal new RNA classes and possible function for pervasive transcription. Science 2009; 316:1484-8. | Calin GA, Croce CM. Micro RNA signatures in human cancer. Nat Rev Cancer 2006; 6:857-66. | Trang P, Weidhaas JB, Slack FJ. Micro RNA's as potential cancer therapeutics. Oncogene2008; 27:552-7. | Winter J, Jung S, Keller S et.al. Many roads to maturity: miRNA Biogenesis pathways and their regulation. Nat Cell Biol 2009; 11:228-34.