Biochemistry & Physiology: Open Access

Salehi and Najafi, Biochem Physiol 2014, 3:1
DOI: 10.4172/2168-9652.1000126

Mini Review Open Access

RNA Preservation and Stabilization

Zahra Salehi[1] and Mohammad Najafi[2]*

[1]Medical Biotechnology Department, School of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran
[2]Biochemistry Department, Iran University of Medical Sciences, Tehran, Iran

Abstract

RNA quality and integrity is a prominent issue in gene expression analysis which emerged as a critical tool in life science researches, drug discovery and optimization of bioproduction. Handling and preservation methods including formalin fixation, flash freezing and chemical preservatives (sulfate solution and TRIzol) and commercial compounds (RNAlater, Allprotect and PAXgene) are widely applied to keep high quality RNA within fresh tissue samples. In this article, we tried to give a general idea on basic aspects of the above methods.

Keywords: RNA; RNAlater; TRIzol; RNase; Allprotect; PAXgene

Introduction

Gene expression analysis provides information at which a particular gene pattern may be expressed by cellular responses. Gene expression is evaluated by means of reverse transcription polymerase chain reaction (RT-PCR), quantitative real-time RT-PCR (qRT-PCR) as well as multiplex gene expression quantification arrays and, data analysis depends on the RNA integrity and stability isolated from cells [1]. RNA quality is influenced by warm and cold ischemic durations, cellular stress responses, tissue processing protocols and storage conditions [2,3]. Isolation of highly pure intact RNA is vital for successful quantification of gene expression [1,4] so that RNA preservation is an essential subject during handling processes [5]. Since RNA degradation takes place by cytoplasmic RNase [4] thus, RNase inhibition is a main approach in RNA extraction and subsequently gene expression analysis [1,4].

Current methods

Formalin fixation

RNA degradation correlates with the length of delay in sample fixation or preservation. Most pathology departments still rely on tissue preservation with formalin fixation followed by paraffin embedding step [3]. Although it is suitable for morphologic assessment, it is unable to keep intact RNA [5].

In fact, formalin fixative keeps tissue structure via cross-linking of cellular proteins. In addition, there also happen cross-links between proteins and nucleic acids. Mono-methylol groups (-CH_2OH) added to all four bases of RNA create methylene bridges between two amino groups. Although it makes formalin an ideal fixative hardening agent for histopathological analysis, cross-linking of proteins and nucleic acids causes intact mRNA extraction from formalin-fixed tissues to be difficult [3,4,6,7]. RNA extracted from formalin-fixed paraffin-embedded (FFPE) tissues is chemically modified and dramatically degraded. It is often present in fragments less than 300 bases in length [4,5,8,9] and may produce reliable data if selected genomic sections be heavily amplified using specific primers and short amplicons (<300 bps) [4,10]. In addition, RNA chemical modifications in FFPE tissues can be reversed with proteinase K digestion and preheating in guanidinium or TE (10 mM Tris-HCl, pH 7.0, 1 mM EDTA) or citrate (pH 4.0) buffers making it more amenable to RT-PCR yet, the major drawback of formalin fixation (i.e. fragmentation) remains uncorrected [4,9,10]. On the other hand, labile and low mRNA transcripts of specific genes may also remain unamplifiable [4]. Therefore, biopsy tissues have to be preserved with alternative methods providing higher-quality RNA for gene expression analysis. It should be noted that small RNA molecules (such as mature mi RNA) are less affected by formalin and can be recovered more easily and efficiently in the extraction process [8]. This highlights the FFPE archived tissues are valuable resources for miRNA expression analysis [8,11].

Formalin can be substituted with other fixatives such as ethanol, methanol, Carnoy fixative (60% ethanol, 30% choloroform and 10% glacial acetic acid) and methacarn solution (60% methanol, 30% choloroform and 10% glacial acetic acid).These fixatives would avoid hydroxymethylene cross-linking between proteins and nucleic acids and, keep tissue morphology and RNA integrity [5,12,13].

Flash freezing

Flash freezing (also called snap freezing) is a better method for providing high quality RNA for high throughput expression analysis as compared to formalin fixation [4]. RNA stability in intact fresh frozen tissue is due to both preserved cellular structure [14] and RNase inactivation [4]. In this method, small fragments of fresh tissue (approximately 0.1 cm3) are immediately frozen (after half an hour of surgical resection) using immersion within liquid nitrogen (-196°C) or on dry ice (-78°C) and finally stored at -80°C or below and, never allowed to thaw [4,14-17].

Although optimal RNA quality is preserved using flash freezing, this method also has a few limitations. It may not be practical if there is not access for freezing facilities and sample collection is decentralized (i.e. samples are gathered from medical services in different locations) [4]. To reach the place where freezing facilities are available, transportation of fresh tissues on ice would be a practical solution, as it does not compromise RNA integrity and gene expression profiles in tissue [4,18]. Even brief thawing of flash frozen sample before homogenization in a guanidinium-based lysis solution (containing 4 M guanidine thiocyanate, 25 mM sodium citrate, 0.5% Sarkosyl and 0.1 M 2-ME) [19] can lead to RNA degradation [5,15] so that, it has to be grounded at cryogenic temperatures(below -150°C) [15]. In

***Corresponding author:** Mohammad Najafi, Department of Biochemistry, Iran University of Medical Sciences, Tehran, Iran Tel: +985118673107; E-mail: nbsmmsbn@iums.ac.ir

Received January 02, 2014; **Accepted** February 04, 2014; **Published** February 10, 2014

Citation: Salehi Z, Najafi M (2014) RNA Preservation and Stabilization. Biochem Physiol 3: 126. doi: 10.4172/2168-9652.1000126

general, flash freezing disadvantages including availability, expense of cold shipping and storage as well as risk of RNA degradation prior to homogenization [3,5,6,12,18] impose great needsfor applying other preservative methods.

Chemical preservatives

Aqueous sulfate solutions (containing 25 mM sodium citrate, 10 mM EDTA and 70 g ammonium sulfate/100 ml solution, pH 5.2) precipitate degenerative RNases and other solubilized proteins resulting in preserving tissue with intact RNA. In this way, tissue can be stored at room temperature for weeks and at -60°C for long-term periods [18,20]. Other chemical reagent called TRIzol, consisting of phenol and guanidine isothiocyanate can preserve high RNA quality and quantity [21]. However, it destroys tissue structure, making it unamenable for histomorphological studies [22].

Commercial compounds

RNAlater (Ambion, Austin, TX, USA) is an available preservative in which small tissue pieces (less than 0.5 cm thickness) are immediately submerged to stabilize cellular RNA without the need to freeze samples. This solution preserves intact RNA by precipitating out RNases into an aqueous sulfate salt solution [4,18,20]. With RNAlater, tissue can be stored at room temperature for up to 1 week, at 4°C for up to 1 month and at -80°C for a long time. Based on the passive diffusion of reagent, thin tissue pieces must be prepared to penetrate rapidly into the tissue before RNA destruction by RNases. Moreover, aggregation of fragments may prevent fine diffusion that can be obviated either by gentle agitation or placement in a large container [18].

Allprotect tissue reagent (Qiagen) is another commercial stabilizer designed to preserve DNA, RNA and proteins in tissue samples concurrently [23-25]. DNA, RNA and proteins can be stabilized in freshly harvested tissues if the tissues are immediately submerged in Allprotect at room temperature, eliminating the need for liquid nitrogen and dry ice. It is claimed that stabilized tissues can be transported at 15-25°C for up to 7 days, or stored at 2–8°C for up to 12 months. Long term storage of tissues is also possible at–20°C or –80°C [25].

PAXgene (Paxgene Tissue System, PreAnalytix GmbH, Hombrechtikon) is other commercial product used to improve tissue molecular quality and morphological analysis. PAXgene fixation reagent is a non-carcinogenic, non-cross-linking mixture of different alcohols and other chemical compounds that can rapidly preserve tissue morphology and bio-molecules up to 26 months at –20°C [26]. It has been reported that fixed, paraffin embedded (PFPE) tissues with PAXgene are morphologically similar to those preserved in neutral buffered formalin and can be used for immunohistochemical staining. In addition, RNA quantity and quality extracted from PFPE tissues are almost similar to that from flash frozen tissues and, is significantly better than what obtained from their FFPE counterparts [3,27,28].

PAXgene Blood RNA System (PreAnalytiX GmbH, Hombrechtikon, Switzerland) is a well-described product for RNA stabilization in human blood [29]. PAXgene Blood RNA tubes are prefilled with a RNA stabilization reagent that stabilizes intracellular RNA for 3 days at 18-25°C and, 5 days at 2-8°C. Additionally, it can be kept at -20 to -70°C for long terms (Table 1).

Methods		Sample	Period	Authors (year)/[Ref]/General description
Fixation	Formalin Method	Human cell line	-	Li et al. [8] miRNA analysis
		-	-	Shi et al. [9] Antigen retrieval (AR) technique
		Human cell line	Overnight	Evers et al. [10] Temperature, time, buffer, and pH Effects
	Modified Method	Mouse / Human tissues	Up to 2 months	Vincek et al. [5] Fixed with UMFIX
		Rat tissue	3 days	Cox et al. [12] Nine different fixatives
		Human tissue	-	Gillespie et al. [13] Fixed with ethanol
Flash Freezing	Flash Freezing	Human tissue	Up to 16 hour	Micke et al. [14] Time and transport effects
		Human tissue	-	Wang et al. [17] Flash freezing vs. other methods
Chemical Preservative	TRIzol	Animal tissues	-	Pérez-Portela et al. [21] TRIzol vs. other methods
Commercial compounds	RNAlater	Human tumor tissues	-	Hatzis et al. [2]
		Human tissues	1 day	Florell et al. [22]
		Human tissues	-	Sherker et al. [23] RNAlater vs. other methods
		Blood	6 months	Weber et al. [29]
		Human tumor tissue	1 week	Grotzer et al. [31]
	Allprotect	Human tissue	-	Sherker et al. [23] Allprotect vs. other methods
		Human normal/tumor tissues	-	Mee et al. [24]
	PAXgene	Rat tissues	3 days	Groelz et al. [3] PAXgene vs. other methods
		Human tissues	Up to 1 week	Kap et al. [26] PAXgene vs. formalin fixation
		Blood	6 months	Weber et al. [29] PAXgene vs. RNAlater

Table 1: Studies on current methods for RNA preservation.

RNA later versus other preservations

Some studies suggested that RNAlater (AmbionInc, Austin, TX) improves RNA integrity and yield in comparison to flash freezing method [2]. Florell et al. showed that RNAlater (AmbionInc, Austin, TX) not only preserves RNA integrity but also keeps histologic structure [22]. On the contrary, other studies reported that results of using RNA later and flash freezing in short and long term periods are comparable [17,30,31]. Another study even had shown large variability in gene expression level and RNA integrity in immersed samples within RNAlater (Ambion, Austin, USA) [14]. However, ease of use and handling of decentralized samples are considered with RNAlater. Moreover, it is a safe, non-toxic solution that can be stored at ambient temperature precluding the need for specialized equipment such as liquid nitrogen and thawing RNA-preserved tissues [18,22,30].

RNAlater is also compared with other commercially available RNA preservatives. In a study assessing stabilization of mRNA and miRNA, RNAlater (Ambion, Austin, TX, USA) and PAXgene (Pre AnalytiX, Hombechtikon, Switzerland) were shown to be suitable for RNA stabilization in blood and, isolation of good quantity and quality RNA which was well suited for qRT-PCR [29]. Another study showed no significant differences in RNA, DNA and protein qualities and yields between RNAlater (Qiagen, Cat. No. 76104) and Allprotect (Qiagen, Cat No. 76405) reagents. They also noted a modest trend towards better yields of RNA when Allprotect was used. This was in contrast with the results of another study demonstrating higher yields of RNA in samples immersed in RNAlater [23]. In addition, it is noteworthy that using RNAlater would be more desirable since Allprotect is more expensive than RNAlater and more difficult to work due to its viscosity [23,32].

Conclusion

In general, decision on the use of RNA preservatives is based on availability of required equipment, expenses, ease of work, handling and preservation periods. If freezing facilities are available and sample collection is centralized, flash freezing as a suitable method for tissue RNA stabilization is preferred. Otherwise, the use of chemical preservatives such as sulfate solution or TRIzol may be advisable. In this circumstance, if preserved tissue is intended for both molecular and histopathological studies, the commercial compounds such as RNAlater, Allprotect and PAXgene would be recommended.

However, apart from preservation methods, other parameters such as timing of tissue collection and preservation, use of different fixatives, RNA extraction procedures, tissue quantity and checking methods for RNA quantity and quality would also directly or indirectly influence RNA integrity and gene expression.

References

1. Williams MA (2010) Stabilizing the code-methods to preserve RNA prove their worth. Bio mark Insights 5: 139-143.
2. Hatzis C, Sun H, Yao H, Hubbard RE, Meric-Bernstam F, et al. (2011) Effects of tissue handling on RNA integrity and microarray measurements from resected breast cancers. J Natl Cancer Inst 103: 1871-1883.
3. Groelz D, Sobin L, Branton P, Compton C, Wyrich R, et al. (2013) Non-formalin fixative versus formalin-fixed tissue: a comparison of histology and RNA quality. Exp Mol Pathol 94: 188-194.
4. Medeiros F, Rigl CT, Anderson GG, Becker SH, Halling KC (2007) Tissue handling for genome-wide expression analysis: a review of the issues, evidence, and opportunities. Arch Pathol Lab Med 131: 1805-1816.
5. Vincek V, Nassiri M, Nadji M, Morales AR (2003) A tissue fixative that protects macromolecules (DNA, RNA, and protein) and histomorphology in clinical samples. Lab Invest 83: 1427-1435.
6. Ribeiro-Silva A, Zhang H, Jeffrey SS (2007) RNA extraction from ten year old formalin-fixed paraffin-embedded breast cancer samples: a comparison of column purification and magnetic bead-based technologies. BMC Mol Biol 8: 118.
7. Russell JN, Clements JE, Gama L (2013) Quantitation of gene expression in formaldehyde-fixed and fluorescence-activated sorted cells. PLoS One 8: e73849.
8. Li J, Smyth P, Flavin R, Cahill S, Denning K, et al. (2007) Comparison of miRNA expression patterns using total RNA extracted from matched samples of formalin-fixed paraffin-embedded (FFPE) cells and snap frozen cells. BMC Biotechnol 7: 36.
9. Shi SR, Shi Y, Taylor CR (2011) Antigen retrieval immunohistochemistry: review and future prospects in research and diagnosis over two decades. J Histochem Cytochem 59: 13-32.
10. Evers DL, Fowler CB, Cunningham BR, Mason JT, O'Leary TJ (2011) The effect of formaldehyde fixation on RNA: optimization of formaldehyde adduct removal. J Mol Diagn 13: 282-288.
11. Liu A, Xu X (2011) MicroRNA isolation from formalin-fixed, paraffin-embedded tissues. Methods Mol Biol 724: 259-267.
12. Cox ML, Schray CL, Luster CN, Stewart ZS, Korytko PJ, et al. (2006) Assessment of fixatives, fixation, and tissue processing on morphology and RNA integrity. Exp Mol Pathol 80: 183-191.
13. Gillespie JW, Best CJ, Bichsel VE, Cole KA, Greenhut SF, et al. (2002) Evaluation of non-formalin tissue fixation for molecular profiling studies. Am J Pathol 160: 449-457.
14. Micke P, Ohshima M, Tahmasebpoor S, Ren ZP, Ostman A, et al. (2006) Biobanking of fresh frozen tissue: RNA is stable in nonfixed surgical specimens. Lab Invest 86: 202-211.
15. Top Ten Ways To Improve Your RNA Isolation.
16. Tumor Analysis Best Practices Working Group (2004) Expression profiling--best practices for data generation and interpretation in clinical trials. Nat Rev Genet 5: 229-237.
17. Wang SS, Sherman ME, Rader JS, Carreon J, Schiffman M, et al. (2006) Cervical tissue collection methods for RNA preservation: comparison of snap-frozen, ethanol-fixed, and RNAlater-fixation. Diagn Mol Pathol 15: 144-148.
18. Mutter GL, Zahrieh D, Liu C, Neuberg D, Finkelstein D, et al. (2004) Comparison of frozen and RNALater solid tissue storage methods for use in RNA expression microarrays. BMC Genomics 5: 88.
19. Kingston RE, Chomczynski P, Sacchi N (1996) Preparation and analysis of RNA. Curr Protoc Mol Biol 36: 4.2.6.
20. Bradley BJ, Pastorini J, Mundy NI (2005) Successful retrieval of mRNA from hair follicles stored at room temperature: implications for studying gene expression in wild mammals. Molecular Ecology Notes 5: 961-964.
21. Pérez-Portela R, Riesgo A (2013) Optimizing preservation protocols to extract high-quality RNA from different tissues of echinoderms for next-generation sequencing. Mol Ecol Resour 13: 884-889.
22. Florell SR, Coffin CM, Holden JA, Zimmermann JW, Gerwels JW, et al. (2001) Preservation of RNA for functional genomic studies: a multidisciplinary tumor bank protocol. Mod Pathol 14: 116-128.
23. Sherker AR, Cherepanov V, Alvandi Z, Ramos R, Feld JJ (2013) Optimal preservation of liver biopsy samples for downstream translational applications. Hepatology International 7: 758-766.
24. Mee BC, Carroll P, Donatello S, Connolly E, Griffin M, et al. (2011) Maintaining Breast Cancer Specimen Integrity and Individual or Simultaneous Extraction of Quality DNA, RNA, and Proteins from Allprotect-Stabilized and Nonstabilized Tissue Samples. Biopreserv Biobank 9: 389-398.
25. Allprotect Tissue Reagent – Qiagen.
26. Kap M, Smedts F, Oosterhuis W, Winther R, Christensen N, et al. (2011) Histological assessment of PAXgene tissue fixation and stabilization reagents. PLoS One 6: e27704.
27. Viertler C, Groelz D, Gündisch S, Kashofer K, Reischauer B, et al. (2012) A new technology for stabilization of biomolecules in tissues for combined histological and molecular analyses. J Mol Diagn 14: 458-466.

28. Grölz D, Gündisch S, Viertler C, Dettmann N, Blassnig I, et al. (2012) PAXgene® Tissue Fixation Technology for Simultaneous Preservation of Morphology and Biomolecules. European Congress of Pathology.

29. Weber DG, Casjens S, Rozynek P, Lehnert M, Zilch-Schöneweis S, et al. (2010) Assessment of mRNA and microRNA Stabilization in Peripheral Human Blood for Multicenter Studies and Biobanks. Biomark Insights 5: 95-102.

30. Storage of tissue over extended periods prior to RNA extraction can degrade RNA resulting in altered gene expression patterns.

31. Grotzer MA, Patti R, Geoerger B, Eggert A, Chou TT, et al. (2000) Biological stability of RNA isolated from RNAlater-treated brain tumor and neuroblastomaxenografts. Med Pediatr Oncol 34: 438-442.

32. Terri G, Nina G, Tony SM, Sunil MK, Kim MO, et al. Comparison of RNA, DNA, small RNA (including miRNA) and protein yields from liver biopsy samples using two commercially available tissue preservation reagents: RNALater and AllProtect