Open Access
CODEN: IJBNHY
ISSN: 2278-778X
Original Research Article
International Journal of Bioassays

A novel method to achieve high yield of total RNA from zebrafish for expression studies

Saurabh Shukla, Reena C. Jhamtani, M. S. Dahiya, Rakhi Agarwal*
Laboratory of Analytical & Molecular Toxicology, (Forensic Chemistry & Toxicology Laboratory), Institute of Forensic Science, Gujarat Forensic Sciences University, Sector 09, Gandhinagar- 382007, Gujarat, India.

Received: May 8, 2017; **Accepted**: May 10, 2017
Available online: 11th May 2017

Abstract: Gene expression studies require high-quality intact total RNA for real-time PCR analysis. Degraded or impure sample can provide disastrous results in the subsequence process. RNA is very unstable and can be contaminated easily by ribonuclease (RNase), therefore utmost quality control checks are required to ensure best quality RNA with high yield. Herein, we have come out with a protocol to isolate total RNA from liver, kidney and brain of adult zebrafish using a commercially available chemical denaturant and subsequent clean-up to remove traces of DNA and impurities. Kidney tissue gives maximum yield of total RNA concentration i.e. 135.8786 ng/µL. Purity of isolated RNA was observed with an absorbance ranging from 1.9 to 2.0 observed in all samples. The whole procedure was repeatedly performed to get satisfactory results. Throughout these procedures there are numerous quality control checks to ensure that the sample is neither degraded nor contaminated.

Key words: Gene Expression; Ribozol; RT-PCR; Total RNA; Zebrafish

Introduction

In molecular biology, isolation of pure Ribonucleic Acid (RNA) is an essential and first step in any type of technique such as Reverse Transcription PCR (RT-PCR) and Quantitative Real-time PCR (qPCR). Obtaining intact and pure quality of RNA for cDNA synthesis is primarily dependent on proper handling of RNA during its isolation, even more essential is to make sure that the selected protocol optimizes the best quality and quantity of RNA. Therefore, it is extremely important to use updated protocols and techniques with precautions to get better yield of pure RNA. There are different methods available for extraction and isolation of total RNA. Magnetic bead method is one such type of method used for extraction and isolation of total RNA (Ma *et al.,* 2008). Qiagen Technologies RNeasy Mini Kit also used for isolating RNA (Macedo, Nicholas J and Tracie L. Ferreira, 2014). These two techniques are costly and need a lot of precision. Amresco (Ribozol™ reagent) provides an easy and cost-effective extraction and isolation of total RNA from different tissues. Ribozol RNA extraction reagent is a single-phase phenol solution optimized for isolation of total RNA from different cell and tissue. Acid guanidinium thiocyanate phenol chloroform extraction is widely used for extraction and isolation of pure RNA from different tissue samples of rodents and humans (Ma *et al.,* 2008). Zebrafish is a powerful model organism currently used in molecular biology, toxicology, environmental monitoring and cancer research (Dai *et al.,* 2014). Methods are available for the extraction of RNA from zebrafish embryos but no specific method for obtaining RNA from tissue. Thus, the aim of the present work was to provide a new method for isolation of total RNA from liver, kidney and brain of adult zebrafish using Ribozol reagent with high yield and purity. Total 06 adult zebrafish were used for this purpose and the end product of this protocol is pure total RNA with high yield, which is suitable for cDNA synthesis and further real-time PCR analysis. The used quantity of tissues for this purpose is 15-18 mg for liver, 30-33 mg for kidney and 35-40 mg for brain.

Materials and Methods

Chemicals and Reagents

Ribozol procured from Amresco, USA, while chloroform, isopropanol and ethanol were procured from Merck, India. Plastic ware and glassware were procured from Tarson and Borosil, respectively.

Animals

Adult (4-5 months) zebrafish of length 2.8±0.5 cm and weight 0.295 to 0.395 gms were procured from pet shop certified as wild species and handled in accordance with good animal practice as defined by the animal welfare bodies and the study was approved by the university committee No. PhD/FS/RA/02. Zebrafish were kept under controlled condition with temperature (25±1°C), pH 7.0±0.2, conductance 0.2 µohm, dissolved oxygen 7.2±0.3 mg/L, hardness 110.0 mg/L, alkalinity 0.25 µg/L and 12-hour light and dark cycles.

***Corresponding Author:**
Dr. (Mrs.) Rakhi Agarwal,
Assistant Professor (Senior Grade) & In-charge,
Laboratory of Analytical & Molecular Toxicology,
(Forensic Chemistry & Toxicology Laboratory)
Institute of Forensic Science, Gujarat Forensic Sciences University,
Gandhinagar- 382007, Gujarat, INDIA.
E-mail: rakhi.gfsu@gmail.com

Brief workflow

A. Tissue Lysis: Total cellular digestion is very necessary for high quality and yield of RNA. Disruption method has been optimized and repeated thrice. Dissected liver, kidney and brain (*n*=6) were suspended separately in 200 µL Ribozol containing centrifuge tube and incubated at -20ºC for 48 hrs. At the end of 48 hrs the tissue were thoroughly homogenized with potter elvehjem homogenizer under ice cold condition to achieve complete dissociation of nucleoprotein and then again 200µL Ribozol was added.

B. Separation of Phase: Ice chilled chloroform 200µL was added, vortex for 15 seconds to mix the sample and incubate the sample for 2-3 minutes at room temperature. Samples were centrifuged at 8,000 RPM for 15 minutes at 4ºC. After centrifugation, three phases i.e. colourless pure RNA (upper), intermediate, and DNA and protein (lower) were obtained, and then the colourless pure RNA upper phase was collected in another tube.

C. RNA precipitation: Ice chilled isopropanol, 200µL was added samples were incubated for 15 minutes at room temperature and then centrifuge at 8,000 RPM for 15 minutes at 4ºC. Chloroform and isopropanol mixture were drained off, a transparent pellet of pure RNA was observed at the bottom of the centrifuge tube.

D. Washing: Supernatant was removed without disturbing the RNA pellet, pellet was washed with 75% ice chilled ethanol prepared with ice chilled RNase-free water. Centrifuged the sample at 3,000 RPM for 15 minutes at 4ºC.

E. Re-suspension the RNA Pellet: After washing carefully, ethanol was removed without disrupting the pellet. Air dried the pellet for 5-10 minutes, however complete drying of the pellet must be avoided. Pellet was dissolved in 25 µL RNase-free water.

F. Determination of Yields and Purity of RNA: RNA purity and yield was determined by measuring absorbance at Nanodrop Spectrophotometer, Mapada, Shanghai, China at 260 and 280 nm.

Note: Quality control means keeping the whole process in cold condition (Except where mentioned), regularly wiping the surface, gloves with ethanol.

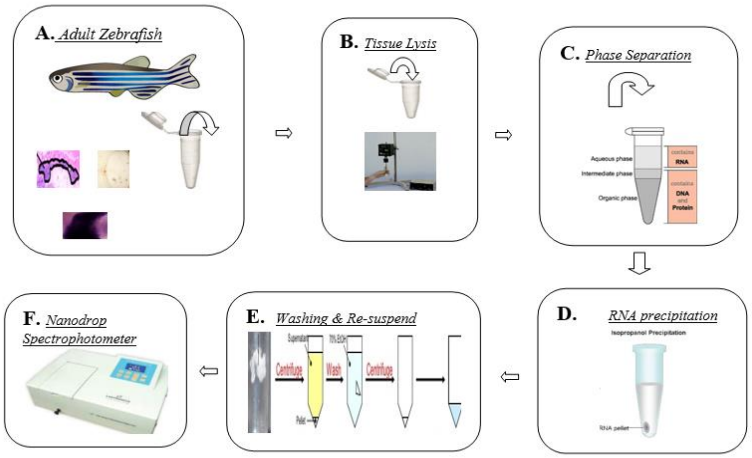

Figure 1: Schematic workflow for isolation and extraction of total RNA from zebrafish (A) Liver, Kidney & Brain of Adult zebrafish were collected in 200µL Ribozol and incubated @-20ºC for 48 hrs (B) Homogenized using potter Elvehjem homogenizer and added 200µL ice-cold Ribozol (C) 200µL ice chilled chloroform added, colorless pure RNA phase collected (D) 200 µL of ice chilled isopropanol, incubate for 15 minutes at room temperature and centrifuged @ 8,000 RPM for 15 minutes at 4ºC (E) Remove supernatant, washed with 75% ice chilled ethanol twice. Centrifuge @ 3,000 RPM for 15 minutes at 4ºC. Re-suspend RNA Pellet in 25 µL RNA's-free water. (F) Nanodrop spectrophotometer used for qualitative and quantitative analysis.

Results and Discussion

The above mentioned protocol is satisfying in comparison with the other available protocols. For example, Peterson and Freeman, 2009 have worked on the isolation of RNA from zebrafish embryos. Similarly, Claudia, 2012 has also worked on the isolation of total RNA from zebrafish embryos. Described protocol was adopted after standardization of protocol with number of trial analysis. As mentioned in Tables, during first time analysis, zebrafish were sacrificed and RNA isolation was done immediately using Ribozol as per above mentioned steps. The yield (RNA concentration) was found very less and purity (Ratio at 260:280) was also not good as the template was contaminated with DNA. The second-time trial includes the 24 hr as

incubation time at -20ºC of tissue in Ribozol for RNA isolation, the obtained results were also not satisfactory in terms of purity and yield of RNA. However, during third time, the incubation period was enhanced from 24 to 48 hrs and the results were found best with better purity and yield. Results for liver, kidney and brain are shown in table 1, 2 and 3, respectively. The same method was repeated thrice to check the reproducibility of the results and has been adopted for regular work of isolation of total RNA for further RT-PCR analysis.

Table 1: RNA Concentration and Absorbance ratio at 260:280 with reference at 320 nm in Liver with three trials.

Analysis	Purity (260:280)	Yield RNA (ng/µL)
1st time	1.4208	24.5418 ±0.02
2nd time	1.6954	74.7211 ±0.05
3rd time	2.0051	116.9499 ±0.06

Table 2: RNA Concentration and Absorbance ratio at 260:280 with reference at 320 nm in Kidney with three trials.

Analysis	Purity (260:280)	Yield RNA (ng/µL)
1st time	1.2672	18.3452 ±0.03
2nd time	1.5776	120.2712 ±0.05
3rd time	2.1041	135.8786 ±0.08

Table 3: RNA Concentration and Absorbance ratio at 260:280 with reference at 320 nm in Brain with three trials.

Analysis	Purity (260:280)	Yield RNA (ng/µL)
1st time	1.3848	26.6548 ±0.02
2nd time	1.6237	93.8397 ±0.03
3rd time	1.9912	103.8037 ±0.07

Conclusion

Extraction of pure quality as well as good quantity of RNA is the most important step in gene expression analysis. Above mentioned method of total RNA extraction from liver, kidney and brain of zebrafish need very small amount of tissue and has been found to be cost efficient.

Acknowledgments

Saurabh Shukla is grateful to University Grants Commission (UGC), New Delhi for the award of Junior and Senior Research Fellowships.

References

1. Kerstin, Howe, Matthew D Clark, Carlos F Torroja, James Torrance, Camille Berthelot, Matthieu Muffato, John E Collins, Sean Humphray, Karen McLaren, Lucy Matthews and Stuart McLaren. "The zebrafish reference genome sequence and its relationship to the human genome." *Nature* 496.7446 (2013): 498-503. Print. http://dx.doi.org/10.1038/nature12111

2. Amresco reagent manual (2017) USA.

3. Ma, Hongbao, Jenny Young, Cherng Shen. "RNA, DNA and protein isolation using Trizol reagent." *Nature and Science* 6.3 (2008): 66-75. Print.

4. Yu-Jie Dai, Yong-Fang Jia, Na Chen, Wan-Ping Bian, Qin-Kai Li, Yan-Bo Ma, Yan-Ling Chen, De-Sheng Pei. "Zebrafish as a model system to study toxicology." *Environmental Toxicology and Chemistry* 33.1 (2014): 11-17. Print. http://dx.doi.org/10.1002/etc.2406

5. Macedo, Nicholas J, Tracie L. Ferreira. "Maximizing Total RNA Yield from TRIzol Reagent Protocol: A Feasibility Study." 2014: ASEE Zone I Conference.

6. Samuel M. Peterson, Jennifer L. Freeman. "RNA Isolation from Embryonic Zebrafish and cDNA Synthesis for Gene Expression Analysis." *Jove* 30 (2009): e1470. Online. http://dx.doi.org/10.3791/1470

7. Claudia, Rodel. Total RNA isolation from 50 zebrafish embryos using Trizol; Trizol® LS Reagent protocol. (2012): (Ambion, Cat. # 10296).

Cite this article as:

Saurabh Shukla, Reena C. Jhamtani, M. S. Dahiya, Rakhi Agarwal. A novel method to achieve high yield of total RNA from zebrafish for expression studies. *International Journal of Bioassays* 6.05 (2017): 5383-5385.

DOI: http://dx.doi.org/10. 21746/ijbio.2017.05.004

Source of support: University Grants Commission (UGC), New Delhi.

Conflict of interest: None Declared