Purifying Total RNA to Explore the Role of piRNAs in Zebrafish Stress Response

Alex Jakubiak, William Chen, Lily Qian, and Dr. Salvador Tarun, Jr.

Department of Chemistry, SUNY Geneseo

Geneseo, NY

Abstract

Recent studies have shown the emerging role of noncoding RNA (ncRNAs) such as piRNA in stress response and transgenerational inheritance. Zebrafish were placed under conditions mimicking global climate change stress: elevated temperature, decreased pH and reduced nutrient availability. The total RNA of zebrafish were then isolated and purified. This will be used to identify any steady-state level changes of the piRNA expressed from the genome in response to stress. Here we report the quality of the purified total RNA from the gonads of the zebrafishes.

Introduction

Significance

PIWI-interacting RNAs (**piRNAs**) belong to a broad class of regulatory RNA called non-coding RNA (**ncRNA**). piRNA are known to have transgenerational effects on gene expression, since they operate in sex cells, allowing epigenetic inheritance thru multiple generations.(1) It is anticipated that when an organism undergoes climatic stress such as heat shock, acidity, or nutrient deprivation, the piRNAs within the organism alters the expression of genes in sex cells and thus may lead to transgenerational genetic change. Intriguingly, zebrafish also rely on piRNAs for sexual determination and sex cell production. (2)

Question

Do piRNAs play a significant role in response to adaptive stressors mimicking global climate change?

Hypothesis

We hypothesize that piRNA expression has a role in regulating transgenerational adaptive stress response in Zebrafish.

Figure 1. Experimental tank after 7 days

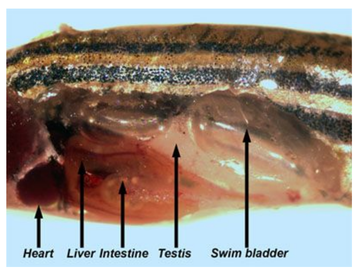
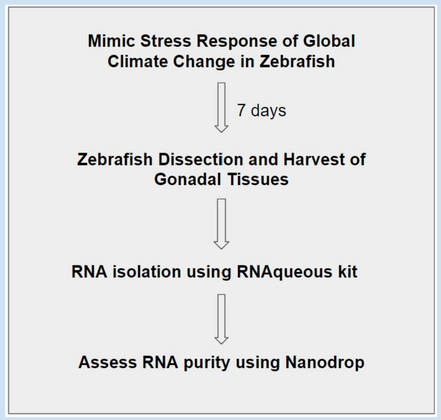


Figure 2. Example of a Zebrafish Dissection.(3)

Methods

We have purified total RNA from gonad tissues (where piRNA sequence changes will most likely be identifiable) of stressed and unstressed zebrafish for use in quantifying candidate piRNA levels by RT-qPCR method.



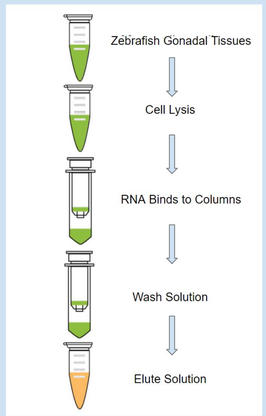


Figure 4. Total RNA isolation protocol.

Results

Table 1. Concentration of samples and absorbance ratios for purity of total RNA samples from gonadal tissues.(5)

Experimental (E)/Control (C)	Nanogram/µL	A260/A280	A260/A230
C	66.6	1.92	0.07
E	104.7	1.7	0.19
C	72.8	1.84	0.38
C	114.4	1.87	0.11
C	44.8	1.96	0.06
E	248.9	1.92	0.56

Future Plans

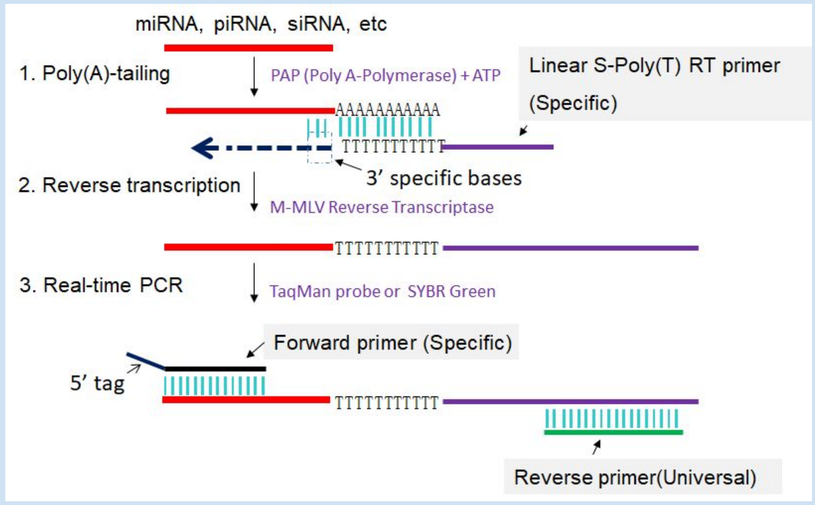


Figure 3. Scheme for future plan protocol; using isolated RNA. (4)

Discussion/Conclusions

Raising and Harvesting Zebrafish

Maintaining stressed conditions (in particular, pH) in the experimental tank proved to be difficult, as a result of the pH solution being diluted by the large volume of the tank. However, we maintained conditions of temperature and nutrient stress consistently throughout the experiment.

RNA Purity and Contamination

It seems that the quality and amount of RNA isolated was sufficient in some samples. Since the goal was to obtain A260/280 values of approximately 2.0, most of our samples came close.(6) In others, we see significantly less than 2.0, indicating a contamination of DNA in our RNA samples. This was most likely a result of ineffective DNAse treatment. However, our issue lie in the contamination of our RNA. Our goal was to have a A260/230 value around 1.8-2.2, and all of our samples reveal a significant deviation from this value, suggesting high contamination of our samples. This is most likely due to residual reagents involved in the purification process. This requires us to further improve our method of purification.

Future Plans

To remedy the contamination issue, we plan to redo the experiment and wash our RNA more extensively before the elution step.(5) Our future plan is to follow this up with RT-qPCR of our isolated RNA. By doing this, we plan to quantify candidate piRNA levels that we have already identified by bioinformatic analyses.

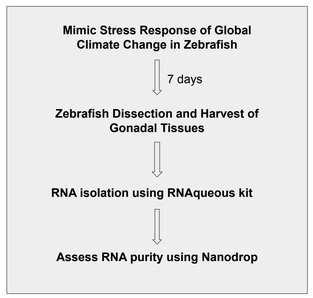
Acknowledgements

We thank the **Geneseo Foundation and Student Association** for the TRAC grant that provided funding for our research. We express our gratitude to **Dr. Travis Bailey** for donating zebrafish and **Ed Beary** for providing resources to our research. We fully thank **Dr. Salvador Tarun Jr.** for mentoring and guiding us through this research.

References

1. Kamminga, L., Luteijn, M., Broeder, M., Redl, S., Kaaij, L., Roovers, E., Ladurner, P., Berezikov, E., Ketting, R. (2010) Hen1 is Required for Oocyte Development and piRNA Stability in Zebrafish. The EMBO Journal, 29 3688-3700 doi: https://doi.org/10.1038/emboj.2010.233
2. Houwing S, Kamminga LM, Berezikov E, Cronembold D, Girard A, van den Elst H, Filippov DV, Blaser H, Raz E, Moens CB, Plasterk RH, Hannon GJ, Draper BW, Ketting RF (2007) A role for Piwi and piRNAs in germ cell maintenance and transposon silencing in Zebrafish. Cell 129: 69–82 https://doi.org/10.1016/j.cell.2007.03.026
3. Gupta, T., Mullins, M. C. Dissection of Organs from the Adult Zebrafish. J. Vis. Exp. (37), e1717, doi:10.3791/1717
4. Shengsong Xie†, Qin Zhu†, Wubin Qu†, Zhong Xu, Xiangdong Liu, Xinyun Li, Shijun Li, Wubin Ma, Yiliang Miao, Lisheng Zhang, Xiaoyong Du, Wuzi Dong, Haiwei Li, Changzhi Zhao, Yunlong Wang, Yaping Fang* and Shuhong Zhao*. sRNAPrimerDB: Comprehensive primer design and search web service for small non-coding RNAs. Bioinformatics, 2018, Doi:10.1093/bioinformatics/bty852.
5. Thermo Fisher Scientific “RNAqueous-micro Kit, Micro Scale RNA Isolation Kit”, RNAqueous- Micro Kit. Life Technologies Corporation,” *Life technologies Corporation*, 6 September 2010, bit.ly/36QK53x
6. *Nanodrop 2000 User Manual - Thermo Fisher Scientific*. https://assets.thermofisher.com/TFS-Assets/CAD/manuals/NanoDrop-2000-User-Manual-EN.pdf

GENESEO
Department of Chemistry



Simulate Global Climate Change → Zebrafish Dissection → RNA isolation using RNAqueous kit → Assess RNA purity using Nanodrop