Developing and Optimizing RNA Isolation Methods from Avian Tissues

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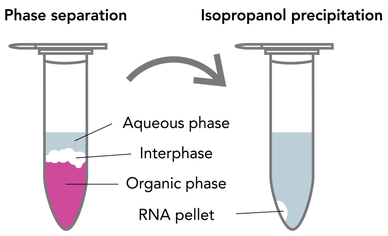
Introduction

- Investigating tissue-specific gene expression first requires RNA isolation.
- We optimized and evaluated multiple methods of RNA extraction from blood and gonadal tissues.
- Our study species is the house sparrow (*Passer domesticus*), a widespread songbird.

Materials and Methods

- We used a Trizol-based phenol-chloroform protocol



- We compared the following:
  - Tissue: blood vs gonad
  - Storage: flash frozen vs. RNALater
  - Homogenization: Tissue Tearor vs. bashing beads

Acknowledgments

Thanks to Dr. Johanna Harvey for assistance troubleshooting RNA extraction from blood in RNALater

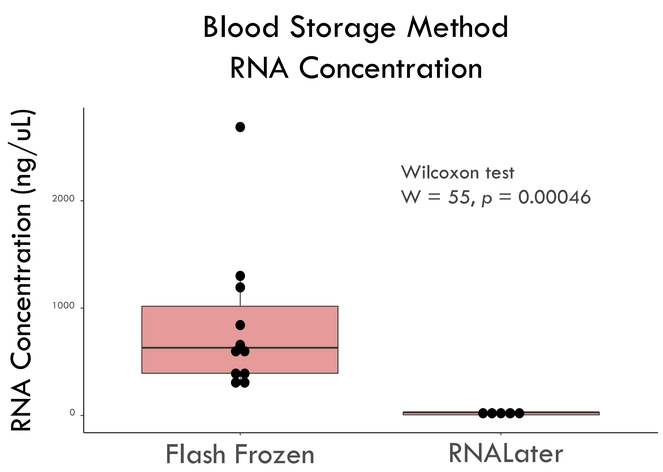
Harvey Protocol

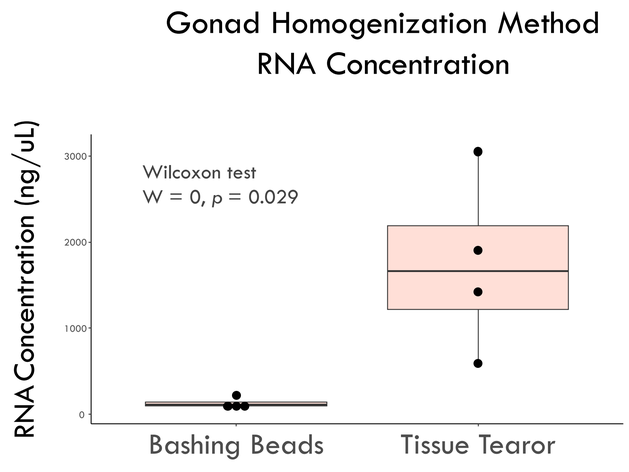
Citations

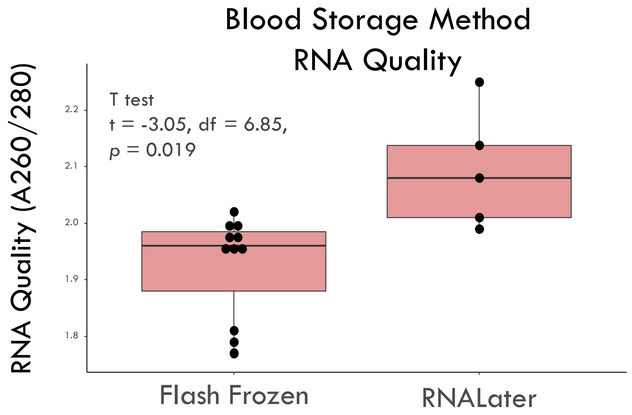
Harvey JA & Knutie SA. 2023. Effect of RNA preservation methods on RNA quantity and quality of field-collected avian whole blood. *Avian Biology Research*

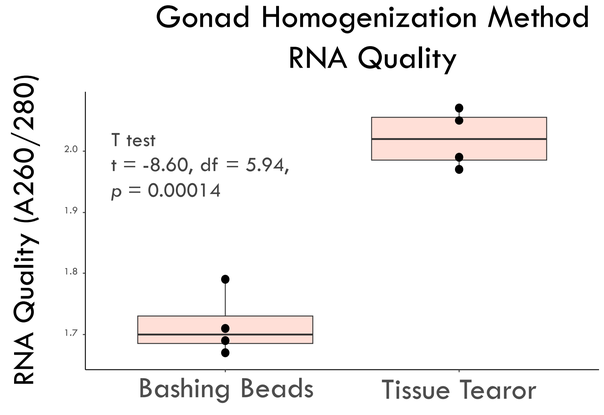
https://www.zymoresearch.com/pages/what-is-trizol

Tissue, Storage, and Homogenization Comparisons









Main Issues Encountered

Tissue	Method	Issue	Solution
Blood	Flash Frozen	Bloodcicle hard to remove from tube before thawing	Work quickly to avoid thawing
Blood	RNALater	Thawed blood too viscous to pipette out completely	Avoid thawing
Gonad	Tissue Tearor	Gonad tissue stuck inside Tissue Tearor	Clean Tissue Tearor between samples
Gonad	Bashing Beads	Bashing beads did not break up tissue samples sufficiently	Use Tissue Tearor

Conclusions

- Blood that was flash frozen produced higher RNA quantity and quality (A260/280 closer to 2.0) than blood stored in RNA Later.
- Flash frozen blood may be a more reliable storage method than RNALater.
- Gonadal tissue homogenized with Tissue Tearor produced higher RNA quantity and quality than with bashing beads.
- Future research will use qPCR to measure differential gene expression in relation to variation in phenotypic behaviors.