Optimizing RNA Isolation and Histology Protocols for Characterization of Preclinical Models of Tendon Disease

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INTRODUCTION

- Tendons join muscle to bone and are essential for posture and movement.
- Tenocytes are the resident tendon cells.

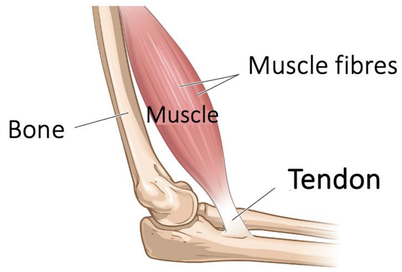


Fig. 1. Schematic diagram depicting tendon-bone anatomy.

- Isolating RNA from small animal models such as mice can be difficult, especially from tendon, as much of the time, they are extremely small samples
- Downstream analysis such as qPCR and especially RNA sequencing require greater quantity and quality of RNA than is often achievable
- RNA degradation is extremely rapid following tendon isolation, with RNA quality decreasing as time increases between the collection of tendons and RNA isolation. Even tendons whose RNA was isolated immediately following tendon isolation had imperfect quality (*PeerJ* 2018. 6:e4664).

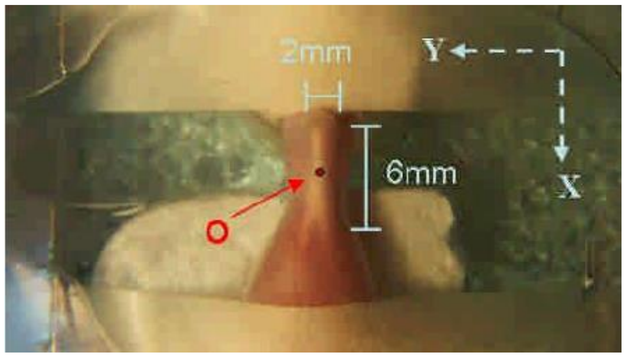


Fig. 2. Scale of Achilles tendon (*IEEE Ultrasonics Symposium* 2008. (pp. 1230-1233).

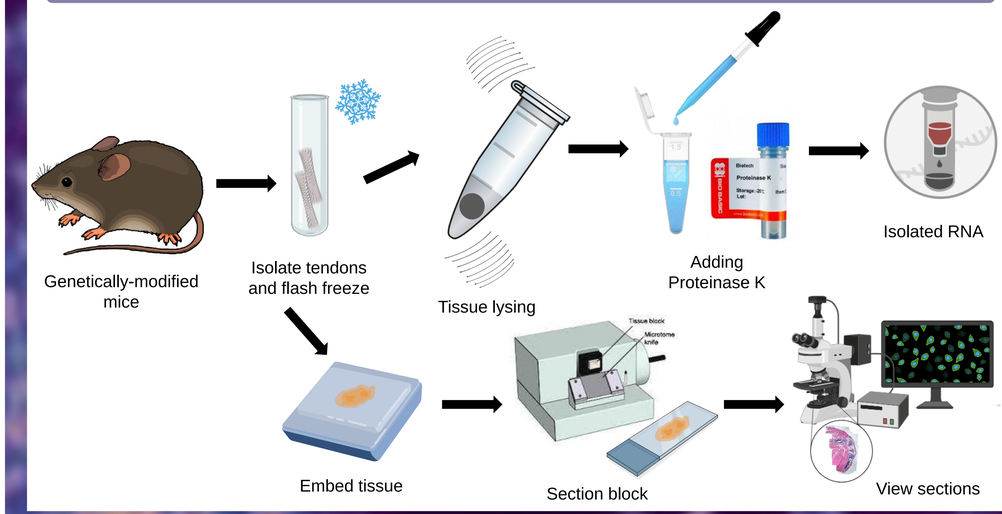
OBJECTIVES

1. Find and optimize a reproducible method for isolating RNA from mouse tendons for downstream qPCR and next generation sequencing that is replicable for people with varying degrees of experience
2. Optimize a method of sectioning mouse joints for the examination of tendon tissue structure

Fig. 3. QIAGEN RNeasy Fibrous Tissue Mini Kit Used for RNA Isolations

METHODS

Tendon Collection for RNA Isolation and Histology



RESULTS

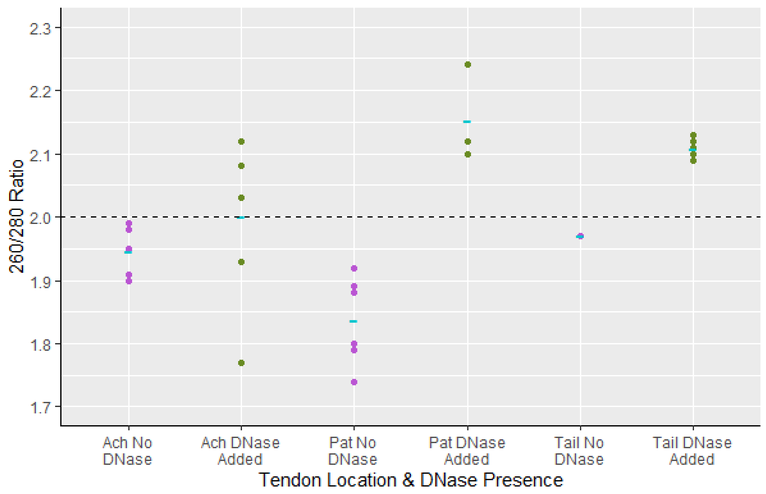


Fig. 4. 260/280 ratios for Achilles, patellar, and tail tendons before and after performing the isolation protocol with DNase using the QIAGEN RNeasy Micro Kit. Absorbance measured at 260 and 280 nm (A260/280). Ideal ratio is 2.0 for pure RNA (represented by the dotted line) and 1.8 for pure DNA. Turquoise lines represent the mean for each group, with individual points representing tissue isolated from a single one-month-old mouse. Ratios after adding a DNase step were greatly improved and closer to the ideal 2.0 ratio for RNA.

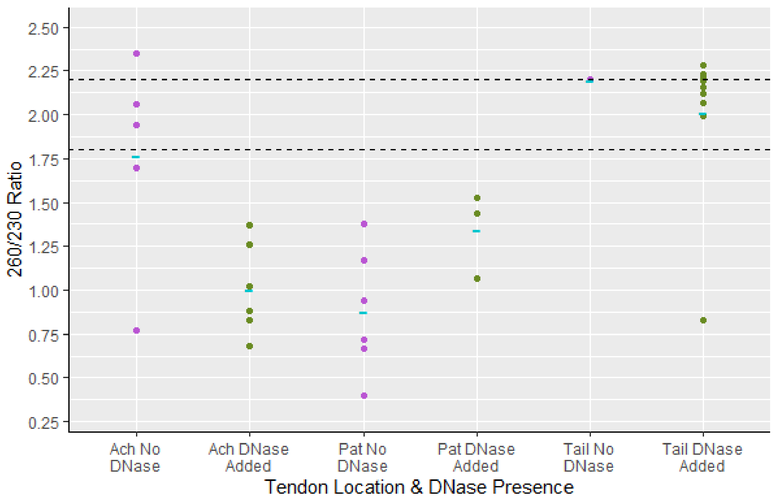


Fig. 5. 260/230 ratios for Achilles, patellar, and tail tendons before and after performing the isolation protocol with DNase using the QIAGEN RNeasy Micro Kit. Absorbance measured at 260 and 230 nm (A260/230). Ideal ratio is 2.0-2.2 for RNA, with 1.8 being the lowest acceptable limit. Dotted lines represent the lower and upper limits of 1.8 and 2.2, respectively. Only the tail tendon consistently resulted in quality RNA, likely due to its size; any contamination is less concentrated among the larger quantity of RNA and the RNA stabilizes itself.

RESULTS

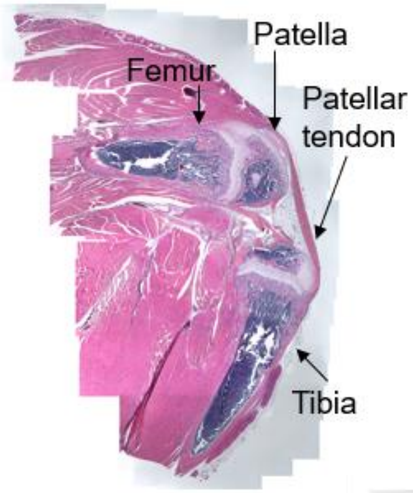


Fig. 6. Representative image of knee joint section. Knee joint section of a patellar tendon extending from the patella to the tibia. Knees were isolated from one-month-old mouse, decalcified, embedded in paraffin wax, and sectioned at 6.0 µm. Sectioning perpendicular to the tendon yielded the best sections.

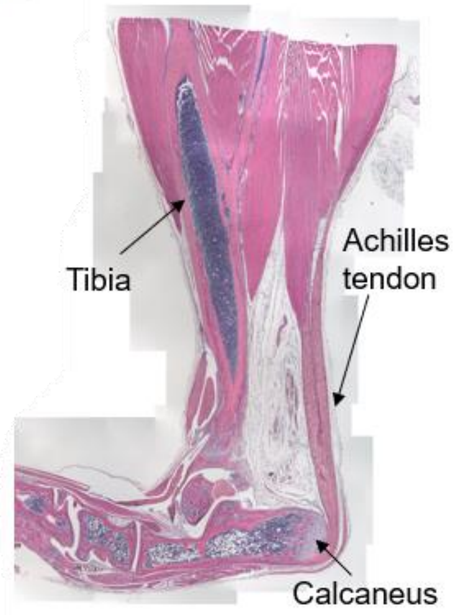


Fig. 7. Representative image of ankle joint section. Ankle joint section of an Achilles tendon extending from gastrocnemius muscle to calcaneus. Joint isolated from one-month-old mouse, decalcified, embedded in paraffin wax, and sectioned at 6.0 µm. Sectioning perpendicular to the tendon yielded the best sections.

FUTURE DIRECTION

- The RNA isolation methods have been improved, but there is still room for refinement, especially for smaller tissues like the Achilles and patellar tendons
- One option for further improvement is the stabilization of the low RNA quantities using carrier RNAs such as yeast tRNA or poly(A) RNA
- The final RNA isolation and histological techniques optimized here will will be used to characterize genetic mouse models of tendon disease

ACKNOWLEDGEMENTS

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