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Assessment of DNA Concentration and Degradation from Extracted Bumble Bee Thorax Muscle 2023

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Assessment of DNA Concentration and Degradation from Extracted Bumble Bee Thorax Muscle 2023

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Objectives

- Determine the correlation between concentration measured by NanoDrop vs. Qubit methods.
- Determine presence of RNA in extracted wild bee DNA samples and whether it contributes to the difference of Nanodrop and Qubit measurement.
- Treat DNA samples with RNase to improve extraction outcome.
- Compare the quality, quantity, purity, & overall concentration of the samples extracted from bees collected in 2022 compared to 2023.

Introduction

DNA is the foundation of an organism's ability to develop, survive, and reproduce. High genomic DNA is imperative for many scientific studies and applications such as Biotechnology, Genetics, & Pharmaceuticals. The separation of genomic DNA once purified from other cellular components (RNA, protein) can be sequenced to study entire genomes, develop antibodies, and limit genetic mutations.

Although a very stable molecule, DNA is susceptible to degradation under normal cellular conditions to various factors. Hence it is essential to properly extract genetic material from bee specimens, to better understand their population genetics.

DNA often needs to be stored for various lengths of time. To produce reliable and reproducible results, stability without contamination needs to be ensured to prevent degradation. In this study the goal is to show the stability & difference in quality of DNA purified from wild bees using the QIAGEN DNeasy® Blood & Tissue kit.

Methods

➤ **DNA Extraction:** DNA was extracted and purified from 48 wild bees using the QIAGEN DNeasy® Blood & Tissue kit Protocol: Purification of Total DNA from Animal Blood or Cells (Spin-Column Protocol).

➤ **DNA Analysis & Quantification:** DNA samples were quantified using agarose gel electrophoresis analysis. Indicating the presence of high-quality DNA where no smears appear. PCR amplification was performed on extracted samples.

➤ **DNA Yield:** DNA yield was analyzed by the measurement of absorbance in a Nanodrop spectrophotometer machine. Absorbance readings are performed at 260nm (A260) where DNA absorbs the greatest amount of light. In addition, a Qubit 2.0 Fluorometer was also used to measure the concentration of DNA samples. This measures DNA concentrations based on fluorescence of molecular probe dyes to provide an absorbance reading.

➤ **RNase treatment:** In order to test the presence of RNA in the DNA extractions and to examine the difference between the Nanodrop and Qubit measurement. We selectively treated four DNA extractions using a standard RNase protocol. The treated samples were measured using Nanodrop again to evaluate the change of DNA concentration. A RNase cocktail was used to treat the DNA samples, followed by an alcohol (chloroform & ethyl) wash. Treated samples were then eluted using the QIAGEN DNeasy® Blood & Tissue kit day 2 extraction protocol.



Results

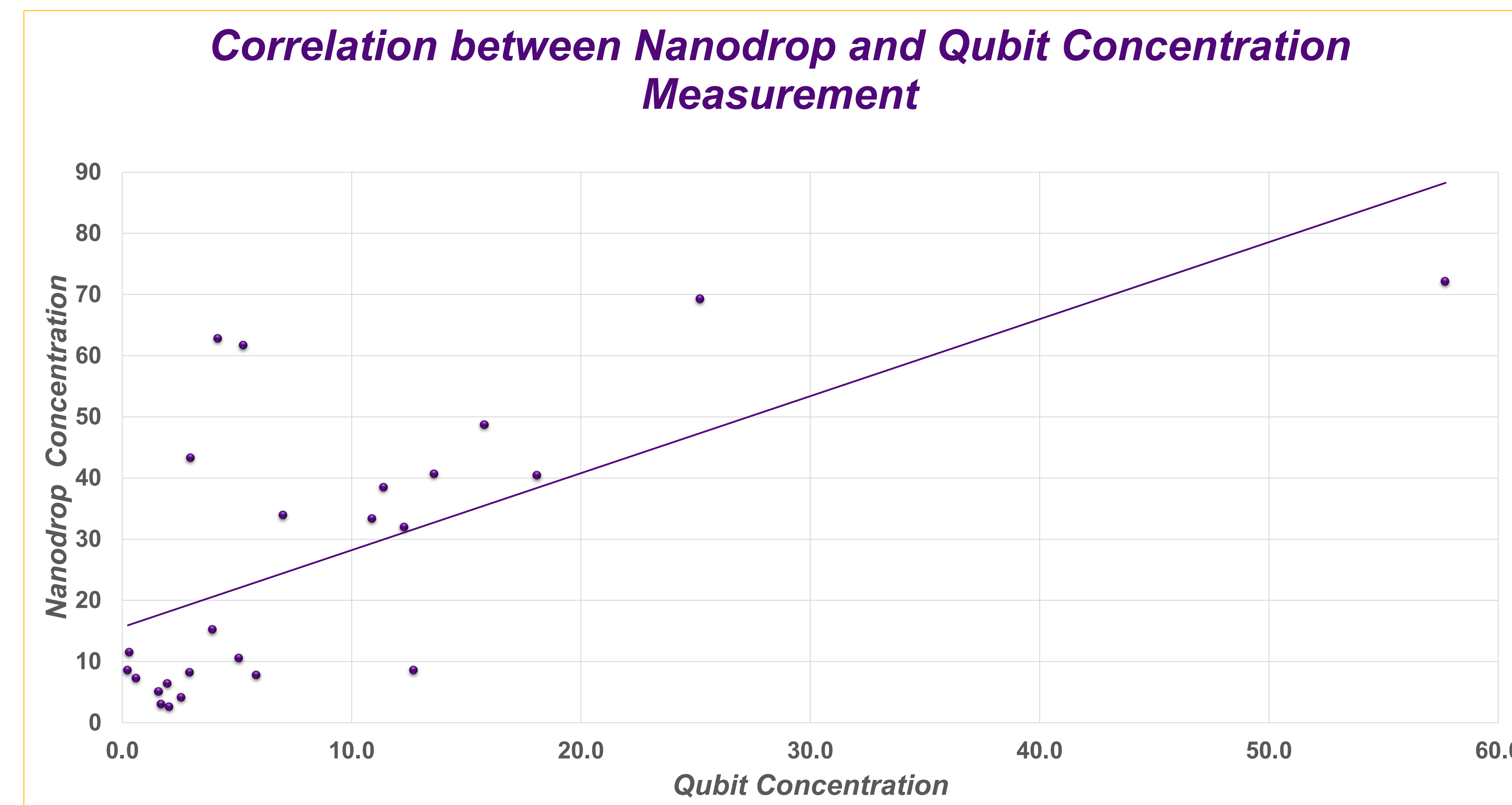


Figure 1. Linear Regression line plot showing a significant positive linear correlation between Nanodrop and Qubit purity concentration readings in nanogram/microliter. (p-value=0.000365, R²=0.656533339)

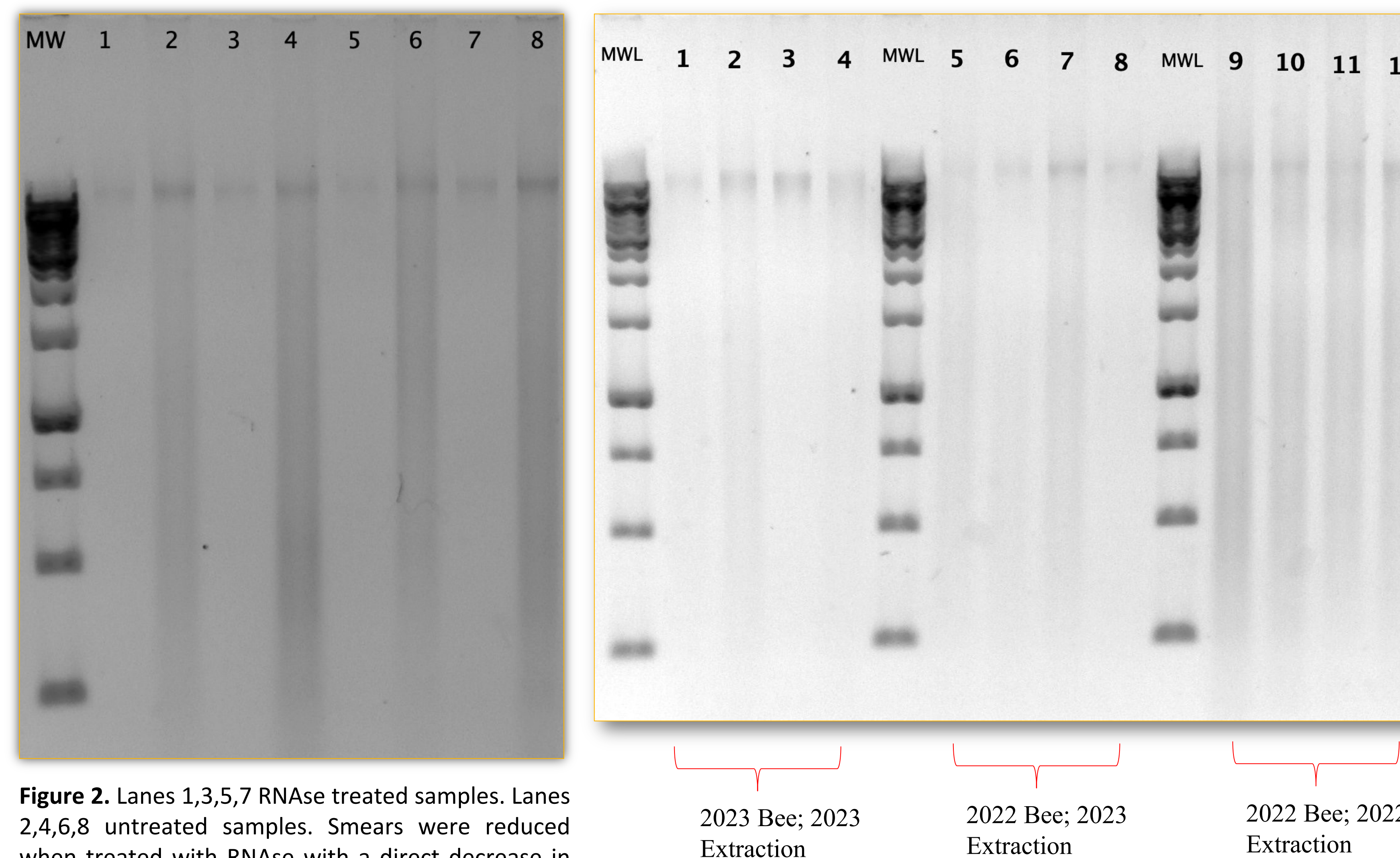


Figure 2. Lanes 1,3,5,7 RNase treated samples. Lanes 2,4,6,8 untreated samples. Smears were reduced when treated with RNase with a direct decrease in DNA concentration.

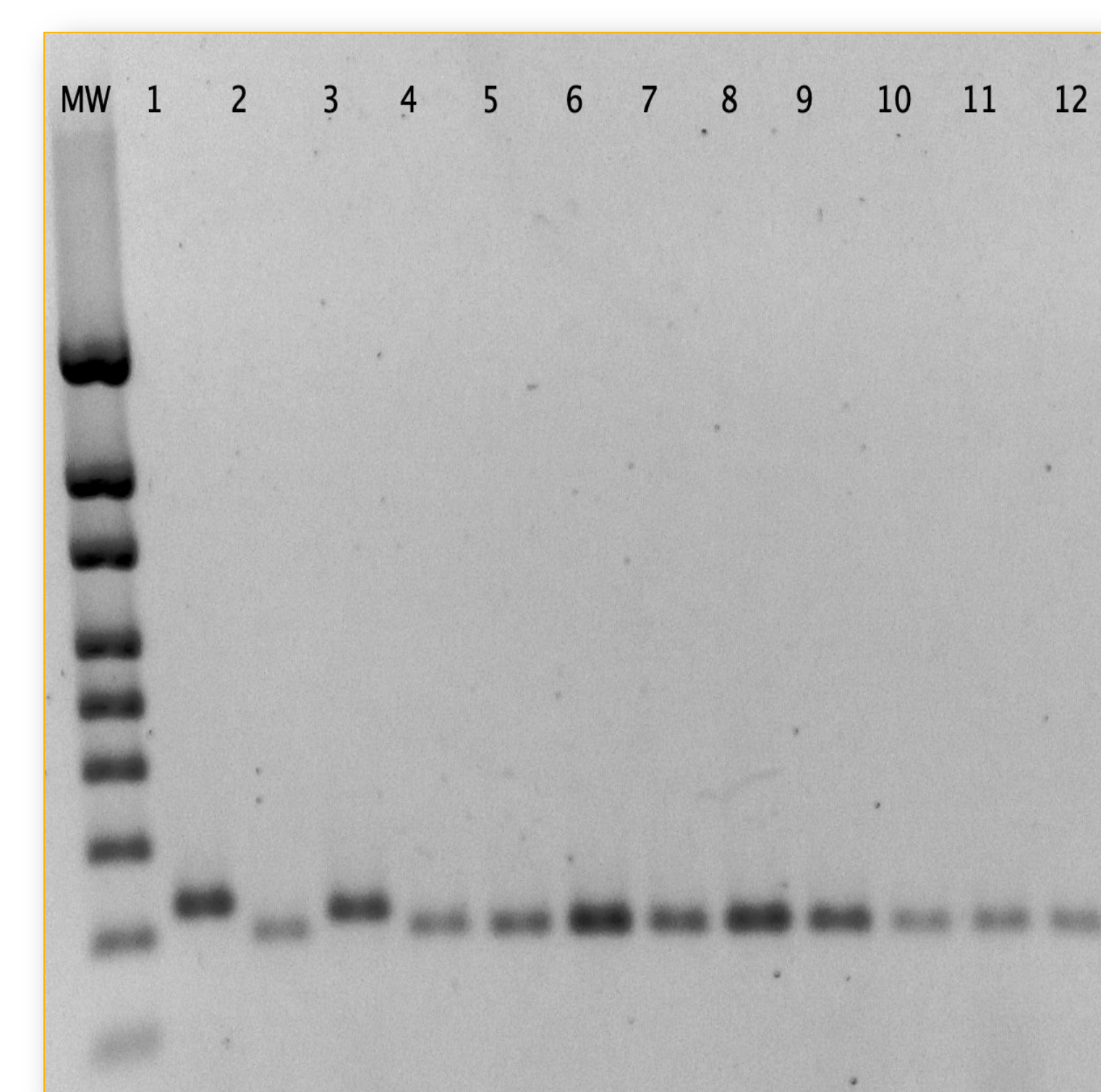


Figure 3. (1 kb DNA Ladder; Lanes 1,4,7,8,11,12 ♂ Male Bees) DNA from 2022 bees extracted in 2022 showed the highest level of degradation and possible RNA presence. DNA from 2022 bees but extracted in 2023 showed lower level of degradation. DNA from newly collected and newly extracted bees showed the highest concentration of high-molecular weight bands.

Figure 4. PCR amplification of genomic DNA. Lanes 1-8 are newly extracted bees captured from UNI campus. Lanes 10, 12 are Bees collected from sites in 2022 extracted summer 2023 treated with RNase to increase/purify protein concentration. Lanes 9, 11 are the same Bees left untreated without RNase.

Data

Lane Samples	Initial Nanodrop DNA Concentration (ng/ul)	Initial Qubit DNA Concentration (ng/ul)	RNA Treated Nanodrop Concentration (ng/ul)	RNA Treated Qubit Concentration (ng/ul)
Lane 1 & 2 AW2022CRP3 39	43.3	2.98	3.15	0.938
Lane 3 & 4 AW2022CRP3 40	62.8	4.18	2.95	0.96
Lane 5 & 6 AW2022CRP4 05	43	33.6	2.65	0.696
Lane 7 & 8 AW2022CRP4 08	60.8	10	2.45	1.19

Figure 5. Samples from DNA gel electrophoresis image (Figure 2.) showing a decrease in RNA contamination directly correlated with an overall decrease in DNA concentration, prior to and after RNase treatment.

Conclusions

- DNA purified with the QIAGEN DNeasy® Blood & Tissue kit proved to be stable after prolonged storage.
- However, DNA extracted in 2022 showed higher levels of degradation than 2023 specimens extracted in 2023. Indicating preserving DNA in animal muscle tissues is better than extracted and eluted DNA.
- Newly captured and extracted bees showed the least DNA degradation.
- RNA was detected in extracted DNA. Which largely contributes to much higher DNA concentration reading using NanoDrop than Qubit.
- Samples treated with RNase reduced RNA contamination; however also decreased overall DNA yield.
- RNase reduced the Nanodrop reading close to the Qubit reading, indicating the presence of RNA in extracted DNA samples.

References

- "HB-2061-003 HB Dny Blood Tissue 0720 WW." Scribd, www.scribd.com/document/517855501/HB-2061-003-HB-DNY-Blood-Tissue-0720-WW-6. Accessed 21 July 2023.
- Holloway, Beth A, et al. An Economical and Effective High-Throughput DNA Extraction Protocol for Molecular Marker Analysis in Honeybees.
- DNA Repair - Molecular Biology of the Cell - NCBI Bookshelf, www.ncbi.nlm.nih.gov/books/NBK26879/. Accessed 21 July 2023.
- Biolabs, New England. "High Molecular Weight DNA Extraction." NEB, www.neb.com/monarch/high-molecular-weight-dna-extraction. Accessed 21 July 2023.
- Aboul-Maaty, Nadia Aboul-Ftooh, and Hanaa Abdel-Sadek Oraby. "Extraction of High-Quality Genomic DNA from Different Plant Orders Applying a Modified CTAB-Based Method." Bulletin of the National Research Centre, vol. 43, no. 1, 2019, https://doi.org/10.1186/s42269-019-0066-1.
- "Cunanan, Wu J, et al. Stability of Genomic DNA at Various Storage Conditions .