APPLICATION NOTE

Nucleic acid recovery from multiple tissues with scalable throughput utilizing the KingFisher Flex and Omni Bead Ruptor Elite bead mill homogenizer.

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Summary

Nucleic acid extractions from biological samples are pivotal for downstream molecular applications, driving advancements in genomics, transcriptomics, and biomolecular research. Often, these nucleic acid extractions are performed with saliva, cellular culture, transport media, or other bodily fluids which provide easy to acquire nucleic acids. However, many researchers utilize solid tissue samples for their fundamental research which require additional handling steps such as homogenization to rapidly release intracellular nucleic acids for extraction. Manual homogenization protocols, however, introduce significant limitations—high variability, prolonged hands-on time, and a lack of scalability, compromising both throughput and data integrity. To address these issues, researchers and technicians must deploy semi-automated or automated solutions into their laboratories to allow for high quality data to be produced[1].

The Omni Bead Ruptor Elite™ bead mill homogenizer, integrated with the KingFisher™ Flex, offers an automated, high-throughput solution that addresses these challenges with tissue samples. By enabling efficient and reproducible cellular lysis and magnetic bead based nucleic acid extraction from diverse tissue types, including soft tissues like brain and liver and more fibrous matrices like skin and tumors, this system dramatically reduces manual intervention while maintaining high nucleic acid yield and integrity. This application note presents an efficient protocol for extracting nucleic acids from *Mus musculus* tissues—including liver, kidney, heart, skin, skeletal muscle, and lung.

Omni Bead Ruptor Elite bead mill homogenizer

Capable of processing up to 100 mg of tissue, this scalable workflow ensures robust performance across varying sample types, delivering nucleic acids that are immediately ready for downstream analysis. Pairing the Omni Bead Ruptor Elite bead mill homogenizer and the KingFisher™ Flex using this optimized protocol enhances laboratory efficiency, offering a reliable, reproducible, and automated approach for researchers working with complex tissue extractions.

Materials & methods

Equipment

- Omni Bead Ruptor Elite bead mill homogenizer (Revvity, Cat # 19-042E)
- Bead Ruptor Elite bead mill homogenizer 2 mL Tube Carriage (Revvity, Cat # 19-373)
- Hard Tissue Homogenizing Mix 2.8 mm Ceramic (2 mL Reinforced Tubes) (Revvity, Cat # 19-628)

Procedure

Sample preparation

All *Mus musculus* organ samples were extracted and immediately stored intact in a -80°C freezer until the day of nucleic acid extraction. Hair from abdomen skin tissue was removed using a facial razor before being transferred to cold storage. Due to the limited availability, only 10 mg of lung tissue samples were processed. *Gallus domesticus* muscle tissue was obtained from a local grocery store and sectioned into smaller pieces. The samples were stored in the -20°C freezer prior to the day of testing.

Mus musculus liver, kidney, abdominal skin, skeletal muscle, heart, and lung samples were sectioned into 10 mg and 100 mg portions (±1%) and placed into individual 2 mL reinforced tubes containing 2.8 mm ceramic beads (Revvity, Cat # 19-628). Each tube was then filled with 400 µL of MagMAX™ stool lysis buffer (ThermoFisher, Cat # A58154) and 20 µL of proteinase K (Revvity, Cat # CMG-723). Samples were homogenized using the Omni Bead Ruptor Elite bead mill homogenizer (Revvity, Cat # 19-042E) utilizing the settings specified in Table 1. Following homogenization, the tubes were centrifuged at 14,000 x g for 2 minutes. Supernatant from each homogenization tube was utilized in the below nucleic acid extraction steps.

Additionally, *Gallus domesticus* skeletal muscle samples were sectioned into 10, 30 and 100 mg (±1%) portions and placed into individual 2 mL reinforced tubes containing 2.8 mm ceramic beads (Revvity, Cat # 19-628). The tubes were processed identically to *Mus musculus* muscle samples and were utilized in identical nucleic acid extractions. Data created from these samples were utilized to evaluate the efficiency of nucleic acid recovery across multiple sizes of tissue.

Nucleic acid extraction

All plates were prepared following the instructions provided in the MagMAX™ Prime Viral/Pathogen Nucleic Acid Isolation Kit (ThermoFisher, Cat # A58145) basic workflow using the user-filled method. An additional Wash III Plate was prepared in the same manner as the Wash II Plate containing 500 µL of 80% Ethanol. A 200 µL aliquot of the supernatant from each homogenization tube was transferred into individual wells on the Sample Plate. The Prime FLX script for the KingFisher™ Flex instrument was downloaded, and the protocol was adjusted to include the Wash III Plate (as shown in Table 2). The Elution Plate contained an elution volume of 60 µL per well. The prepared plates were loaded onto the instrument following prompts from the software. After the nucleic acid extraction was completed, the elution plate was removed. A 1 µL aliquot from each sample was analysed on a NanoDrop spectrophotometer to measure nucleic acid concentration. All remaining nucleic acid was stored at -20°C following spectrophotometric analysis.

Table 1: Homogenization parameters for all tissue samples.

Sample type	Homogenization parameters			
	Speed (m/s)	Time (s)	Cycles	Dwell time (s)
Liver	5	60	1	-
Kidney	5	60	1	-
Skin	6	60	2	60
Skeletal muscle	5	60	1	-
Heart	5	60	1	-
Lung	5	60	1	-

Table 2: Detailed protocol layout for nucleic acid extraction on KingFisher™ Flex instrument.

Plate ID	Plate type	Reagent	Volume per well	Customized script
Wash I plate	KingFisher™ 96 Deep-Well Plate	Wash solution	500 µL	
Wash II plate		80% Ethanol solution	500 µL	
Wash III plate		80% Ethanol solution	500 µL	
Elution plate		Elution buffer	60 µL	
Tip comb plate	Place a KingFisher™ 96 tip comb for deep-well magnets in a KingFisher™ 96 microplate			

Data processing

All data collected from NanoDrop spectrophotometry were uploaded to GraphPad Prism (software version 10.2.3). Replicate reads of nucleic acid concentration were averaged and outliers were identified using the analysis feature in GraphPad Prism (ROUT, Q=1%). Mean and standard deviation was calculated using row statistics feature in GraphPad. All graphical figures were plotted using GraphPad Prism.

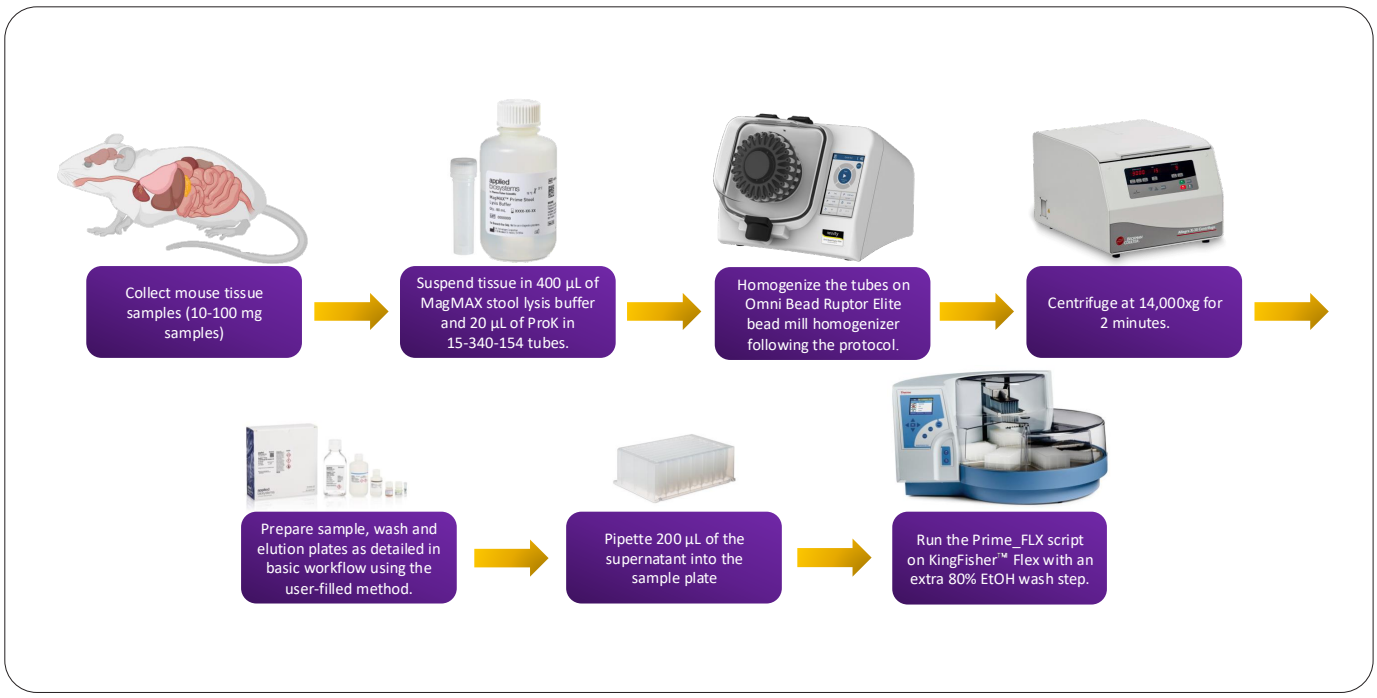

Figure 1: Overview of the steps for extracting Nucleic Acid from tissue samples.

Results

Nucleic acid extractions from *Mus musculus* tissues (liver, kidney, heart, skin, and skeletal muscle) were executed at two tissue masses: 10 mg and 100 mg. As illustrated in Figure 2a, nucleic acid concentrations increased correspondingly with tissue mass. The data demonstrates a clear linear correlation between sample starting mass and nucleic acid concentration across different tissue types, as seen in Figures 4 and 5 showing the compatibility of the workflow with variable sample masses. In Figure 5, *Gallus domesticus* skeletal muscle samples exhibit a strong correlation (R^2 = 0.9667) between sample mass and nucleic acid concentration, indicating that recovery of nucleic acid will linearly return concentrations as input sample weight increases or decreases prior to homogenization. Similarly, Figure 4 shows that the observed nucleic acid yields for 100 mg heart, skin, and skeletal muscle samples closely corresponded to values predicted by scaling the 10 mg yields by a factor of 10. Liver and kidney samples, however, showed significant deviations, with reduced nucleic acid concentrations at higher sample sizes. This reduction is likely associated with extraction limitations at concentrations exceeding 4000 ng/µL, possibly due to magnetic bead saturation, as marked by the shaded regions in the graphs.

Quality control assessments, including 260/280 and 260/230 absorbance ratios (Figure 3), were within acceptable ranges for all samples, with the exception of one outlier. This indicates high nucleic acid integrity and consistent extraction performance across replicates. The ratios provided by this extraction meet minimum criteria for compatibility with PCR, qPCR, and NGS[2].

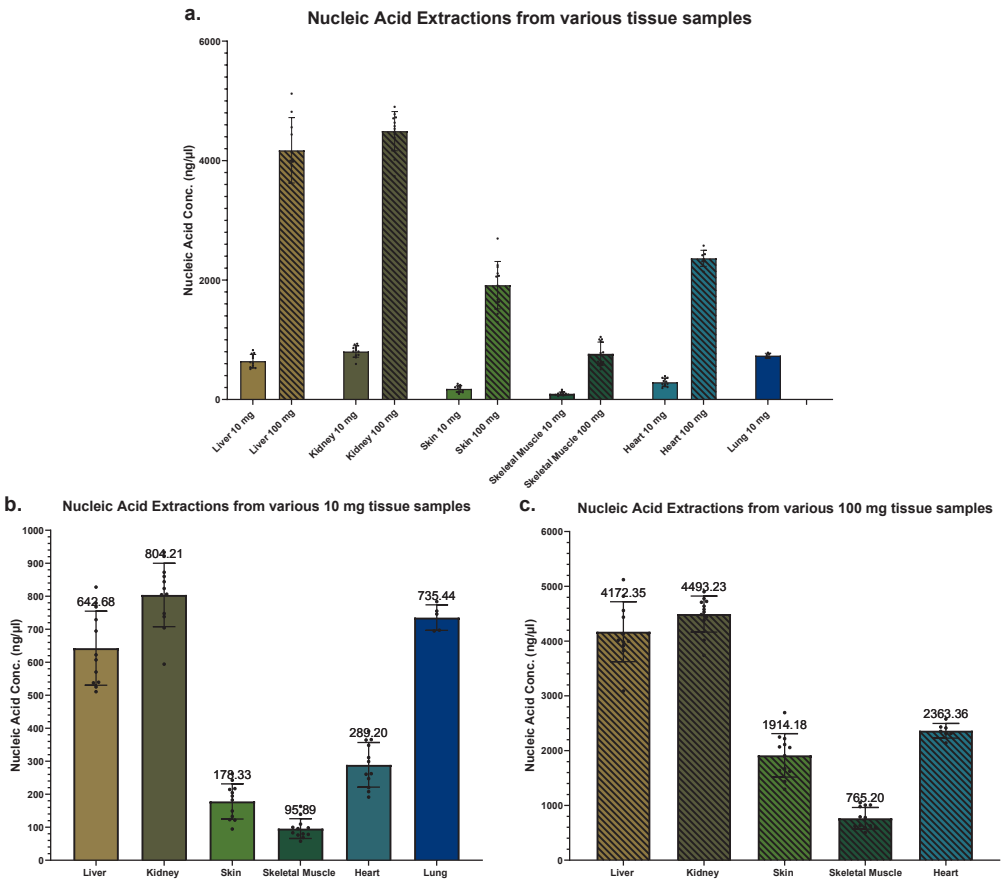

Figure 2: Nucleic Acid Extractions from *Mus musculus* tissues with error bars represent standard deviations across replicates. **(a.)** Comparative nucleic acid yields for 10 mg versus 100 mg tissue samples. Yields scale proportionally with tissue mass, with notably higher extractions in 100 mg liver and kidney samples. **(b.)** Detailed view of nucleic acid extractions from 10 mg tissue samples, with mean concentrations displayed at the top of each bar. **(c.)** Detailed view of nucleic acid extractions from 100 mg tissue samples, with mean concentrations indicated at the top of each bar.

Table 3: Quantitative and qualitative analysis of nucleic acid extractions from various mouse tissue types.

Sample type	Sample size (mg)	Average of Nucleic Acid Conc.	St. Deviation of Nucleic Acid Conc.	Quality metrics	
				Average of 260/280	Average of 260/230
Liver (n=12)	10	668.03	112.14	2.02	1.91
Liver (n=12)	100	3892.31	547.22	2.03	2.04
Kidney (n=12)	10	804.21	96.09	2.00	2.29
Kidney (n=12)	100	4493.23	328.56	2.01	2.29
Skin (n=12)	10	169.75	53.25	1.95	1.99
Skin (n=12)	100	1709.49	396.63	1.97	2.20
Skeletal muscle (n=12)	10	95.89	30.03	1.99	1.78
Skeletal muscle (n=12)	100	799.66	197.00	2.03	2.19
Heart (n=12)	10	277.29	67.49	2.04	2.14
Heart (n=7)	100	2363.36	134.35	2.01	2.29
Lung (n=5)	10	735.44	38.64	1.93	2.33

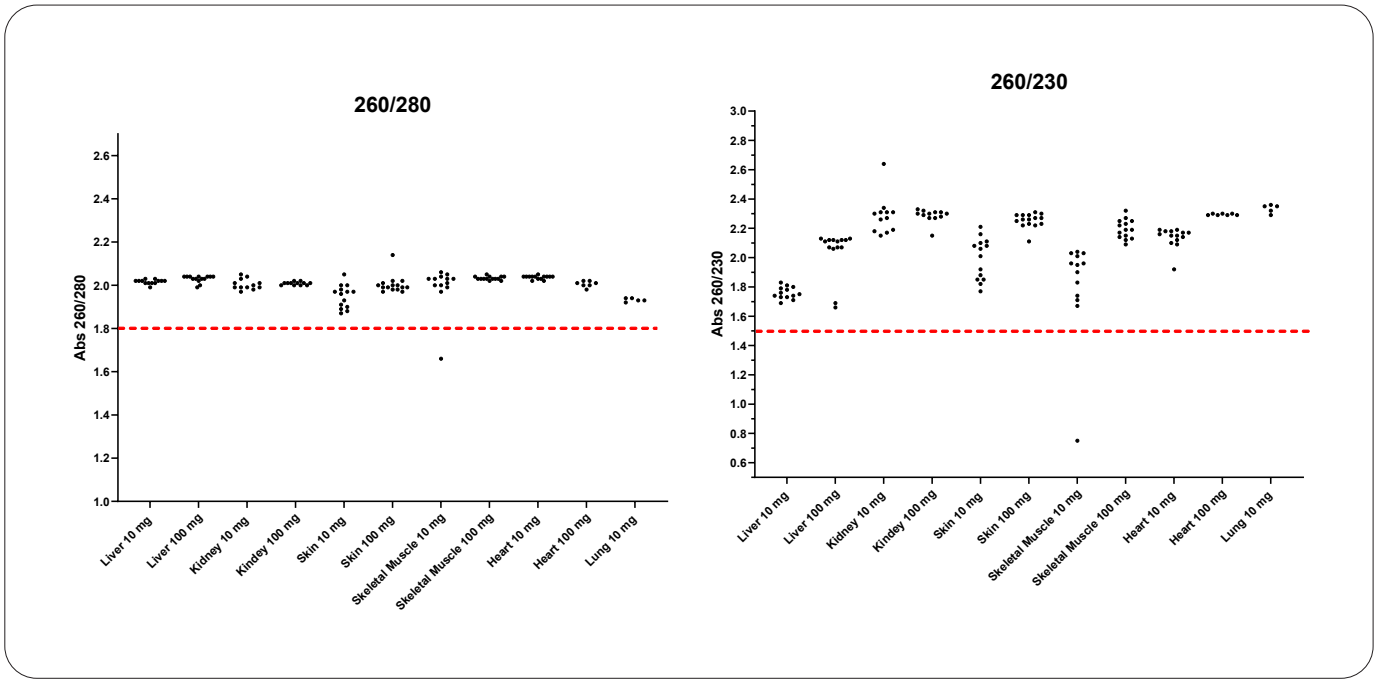

Figure 3: The 260/280 and 260/230 absorbance ratio plotted for each replicate. The red dotted line represents the minimum accepted value for both ratios. (A_{260}/A_{280}=1.8, A_{260}/A_{230}=1.5).

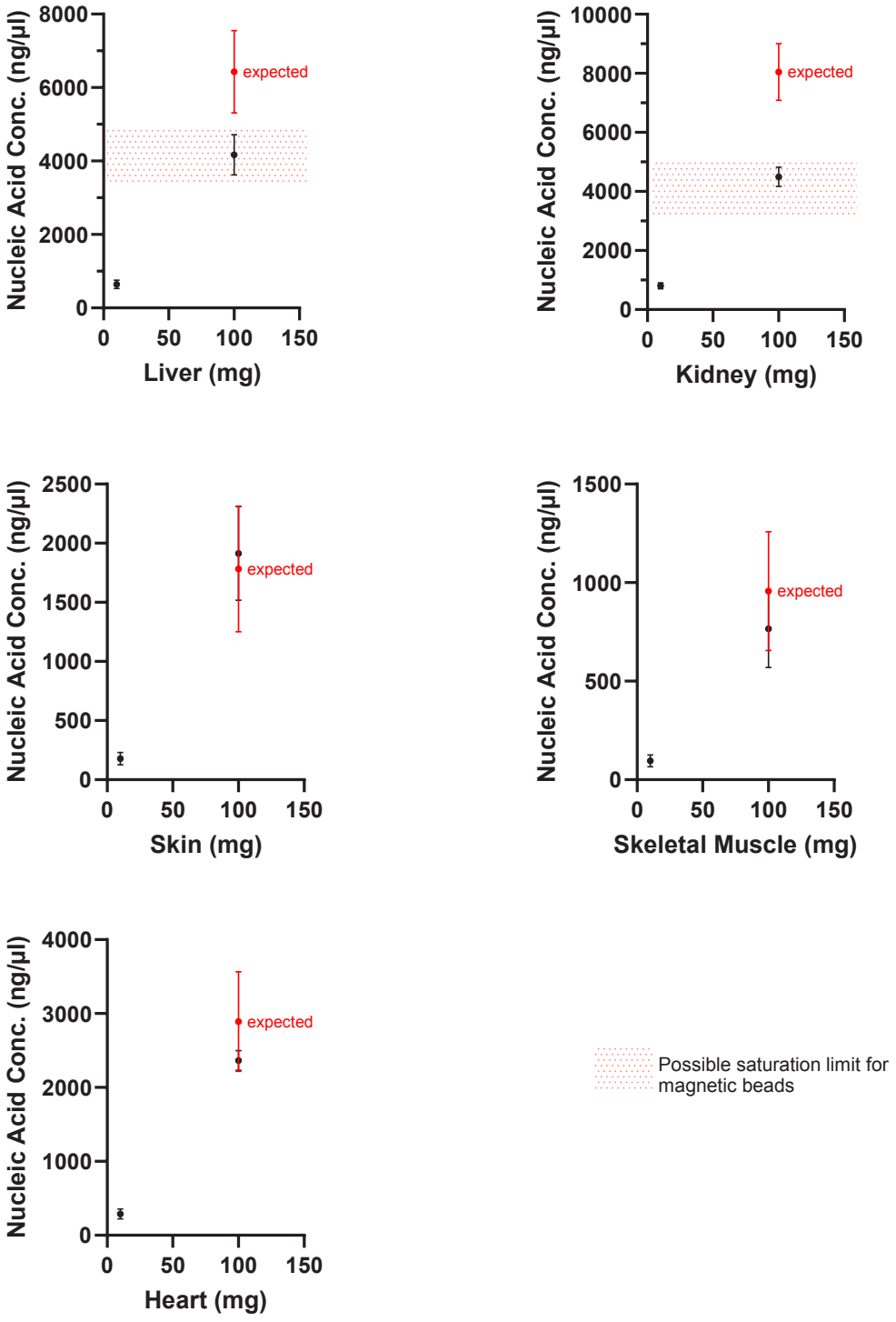

Figure 4: Nucleic acid concentration (ng/µl) extracted from various tissues (liver, kidney, heart, skin, and skeletal muscle) as a function of tissue mass (mg). Each graph shows the expected nucleic acid yield based on tissue type and mass (red dot with error bars) calculated by multiplying nucleic acid concentration values at 10 mg samples by 10, assuming a linear increase in concentration with tissue mass, and the actual measured concentration (black data points). The shaded area represents the potential saturation limit for magnetic beads in the extraction process.

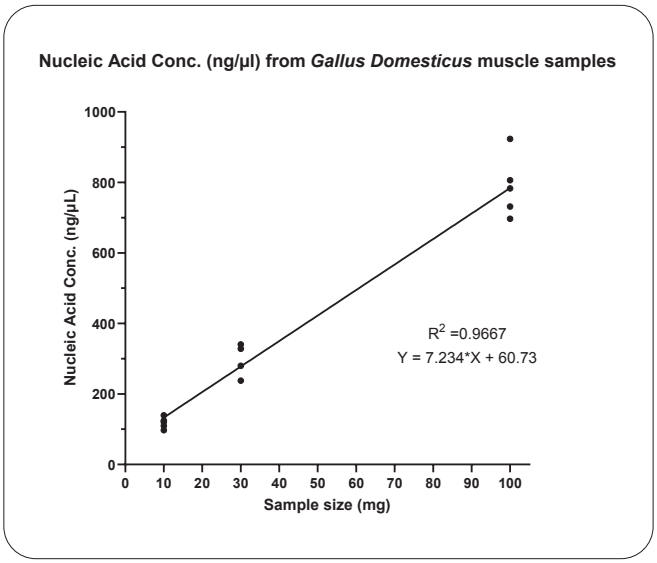

Figure 5: Correlation between sample mass and nucleic acid concentration from *Gallus domesticus* muscle samples.

Conclusions

The nucleic acid extraction protocol utilizing the Omni Bead Ruptor Elite bead mill homogenizer in conjunction with the KingFisher™ Flex proves to be an effective and scalable solution for various tissue types, including liver, kidney, heart, skin, skeletal muscle and lung. While the concentrations observed for liver and kidney samples were lower than expected, likely due to oversaturation of the magnetic beads, they remained sufficiently high quality and quantity for multiple downstream applications, such as PCR, qPCR, and NGS.

The yields for heart, skin, and skeletal muscle aligned closely with predicted values, further emphasizing the reliability of the method for tissue masses up to 100 mg. Furthermore, extractions using 10, 30 and 100 mg skeletal muscle samples created linear nucleic acid concentrations. This displays the reliability of the workflow to provide predictable results until the available DNA saturates the magnetic beads. Increasing the magnetic bead volume can help augment this saturation point when processing larger masses of nucleic acid-rich samples. Despite some variability, all tissues demonstrated acceptable quality control metrics, including 260/280 and 260/230 absorbance ratios, indicating robust nucleic acid integrity. This sample preparation and nucleic acid extraction process not only supports effective analysis but also advances molecular biology research by enabling reliable and scalable nucleic acid yields from a range of tissue types all within the same workflow.

References

1. Han, Soon M, and Arnold Munro. "Transfer from manual to Automated Sample Preparation: A Case Study." *Journal of Pharmaceutical and Biomedical Analysis,* vol. 20, no. 5, Sept. 1999, pp. 785–790, https://doi.org/10.1016/s0731-7085(99)00078-3.

2. Matlock, Brian. *Assessment of Nucleic Acid Purity,* Thermo Fisher Scientific, assets.thermofisher.com/TFS-Assets/CAD/Product-Bulletins/TN52646-E-0215M-NucleicAcid.pdf. Accessed 25 Sept. 2024.