Nucleic acid extraction

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

Authors

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Introduction

Nucleic acid extractions from biological samples are pivotal for downstream molecular applications, helping drive advancements in genomics, transcriptomics, and biomolecular research. Often, these extractions are performed with saliva, cellular culture, transport media, or other bodily fluids that provide easy-to-acquire nucleic acids. However, many researchers utilize solid tissue samples for their fundamental research. Tissue samples 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 help address these issues, researchers and technicians must deploy semiautomated 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 Thermo Scientific™ KingFisher™ Flex Purification System, offers an automated, high-throughput solution that helps address these challenges with tissue samples. By enabling efficient and reproducible cellular lysis and nucleic acid extraction from diverse tissue types, including soft tissues like brain and liver and more fibrous matrices like skin and tumors, this integrated 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 mouse tissues—including liver, kidney, skin, skeletal muscle, heart, and lung. Capable of processing up to 100 mg of tissue, this scalable workflow helps ensure robust performance across varying sample types, delivering nucleic acids that are immediately ready for downstream analysis. Pairing the Omni Bead Ruptor Elite homogenizer and the KingFisher Flex system using this optimized protocol enhances laboratory efficiency, offering a reliable, reproducible, and automated approach for researchers working with complex tissue extractions.

Materials and methods

Equipment

- Bead Ruptor Elite bead mill homogenizer (Revvity, Cat. No. 19-042E) and Fisherbrand™ Bead Mill 24 Homogenizer (Fisher Scientific, Cat. No. 15-340-163)
- Revvity™ Hard Tissue Homogenizing Mix 2.8 mm Ceramic (2 mL Reinforced Tubes) (Revvity, Cat. No. 19-628) and Fisherbrand™ Pre-Filled Bead Mill Tubes (Fisher Scientific, Cat. No. 15-340-154)
- Omni™ Bead Ruptor Elite™ 2 mL Tube Carriage (Revvity, Cat. No. 19-373)
- Applied Biosystems™ MagMAX™ Prime Viral/Pathogen NA Isolation Kit (Cat. No. A58145)
- Applied Biosystems™ MagMAX™ Prime Stool Lysis Buffer (Cat. No. A58154)
- KingFisher Flex Purification System, KingFisher with 96 Deep-Well Head (Cat. No. 5400630)

Sample preparation

All mouse organ samples were excised from the donor organism and immediately stored intact in a –80°C freezer until the day of nucleic acid extraction. Hair from abdominal skin tissue was removed using a facial razor before the sample was transferred to cold storage. Due to the limited availability, only 10 mg of lung tissue samples were processed. Chicken muscle tissue was obtained from a local grocery store and sectioned into smaller pieces. The samples were stored in a –20°C freezer until the day of nucleic acid extraction.

Mouse liver, kidney, abdominal skin, skeletal muscle, heart, and lung samples were sectioned into 10 mg and 100 mg (±1%) portions and placed into Hard Tissue Homogenizing Mix 2.8 mm Ceramic (2 mL Reinforced Tubes) (Figure 1), and 400 µL of MagMAX Prime Stool Lysis Buffer and 20 µL of proteinase K were then placed in each tube. Samples were homogenized using the Bead Ruptor Elite homogenizer with the settings specified in Table 1. Following homogenization, the tubes were centrifuged at 14,000 x *g* for 2 min. Supernatant from each homogenization tube was utilized in the following nucleic acid extraction steps.

Additionally, chicken skeletal muscle samples were sectioned into 10, 30, and 100 mg (±1%) portions and placed into 2 mL reinforced tubes from the Hard Tissue Homogenizing Mix. The tubes were processed identically to those containing the mouse muscle samples and were utilized in identical nucleic acid extractions. Data from these extractions were analyzed to evaluate the efficiency of nucleic acid recovery across multiple sizes of tissue samples.

Figure 1. Overview of steps for extracting nucleic acid from tissue samples.

Table 1. Homogenization parameters for mouse tissue samples.

Sample type	Parameters			
	Speed (m/sec)	Time (sec)	No. cycles	Dwell time (sec)
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	–

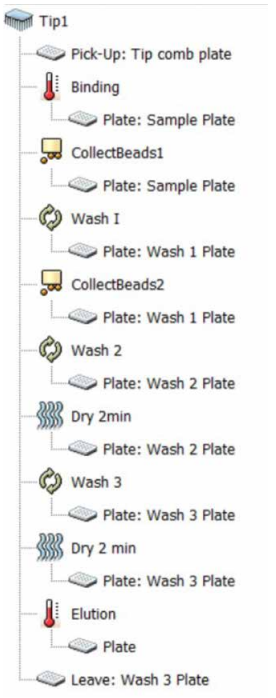
Nucleic acid extraction

All plates were prepared following the instructions provided in the MagMAX Prime Viral/Pathogen NA Isolation Kit basic workflow using the user-filled method. An additional plate for Wash III 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 of buffer per well. The prepared plates were loaded onto the KingFisher Flex 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 analyzed on a Thermo Scientific™ NanoDrop™ 2000 Spectrophotometer to measure nucleic acid concentration. All remaining nucleic acid was stored at –20°C following spectrophotometric analysis.

Data processing

All data collected from the NanoDrop spectrophotometer 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 of the software (ROUT method, Q = 1%). Mean and standard deviation were calculated using the row statistics feature. All graphical figures were plotted using GraphPad Prism Software.

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

Plate ID	Plate type	Reagent	Volume per well	Customized script
Sample Plate	KingFisher 96 Deep-Well Plate	Binding bead mix + sample	275 µL + 200 µL	
Wash I 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			

Results

Nucleic acid extractions from mouse tissues (liver, kidney, skin, skeletal muscle, and heart) were executed on two tissue masses: 10 mg and 100 mg. Nucleic acid extractions from lung tissues were executed from 10 mg samples. As illustrated in Figure 2A, nucleic acid concentrations increased with tissue mass.

Quality control assessments, including A_{260}/A_{280} and A_{260}/A_{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 absorbance ratios resulting from this extraction meet minimum criteria for compatibility with PCR, qPCR, and next-generation sequencing (NGS) [2].

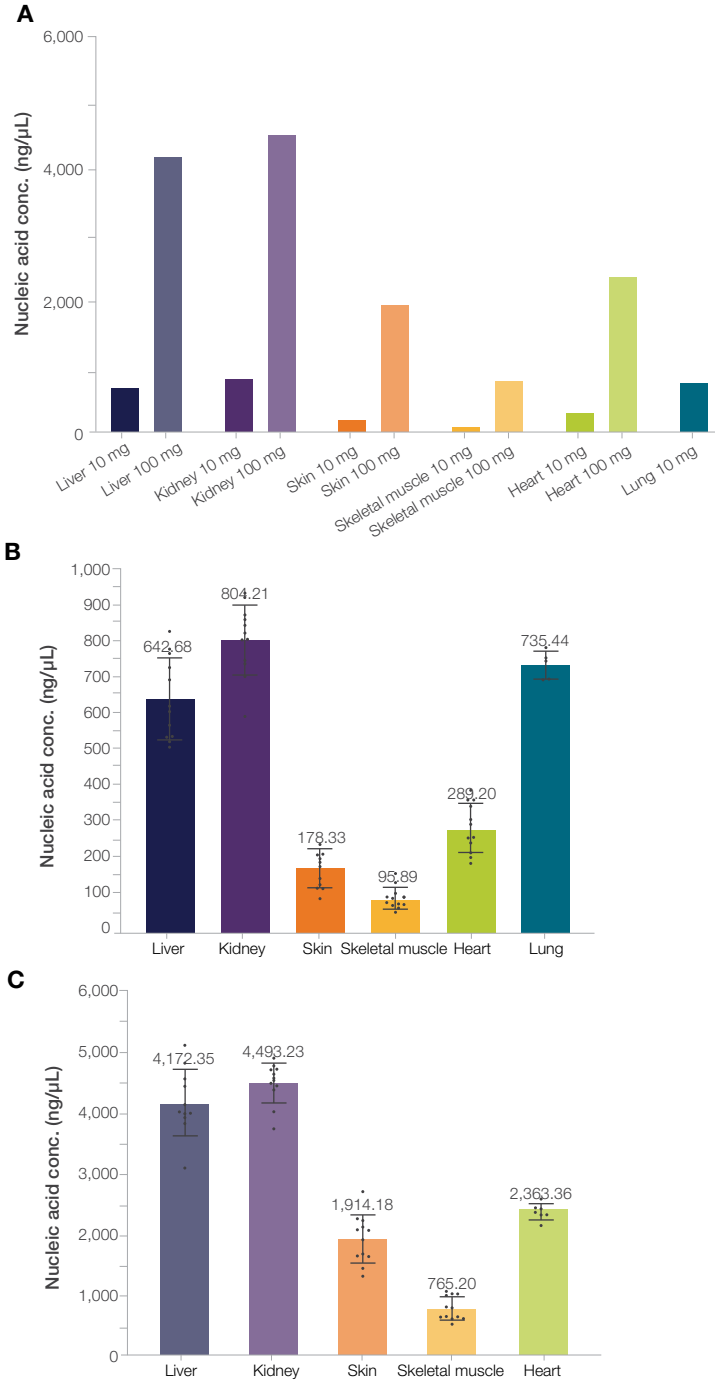


Figure 2. Nucleic acid extractions from mouse tissues. Error bars represent standard deviations across replicates. **(A)** Comparative nucleic acid yields for 10 mg and 100 mg tissue samples. Yields scale proportionally with tissue mass, with notably higher amounts extracted from 100 mg liver and kidney 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 displayed at the top of each bar.

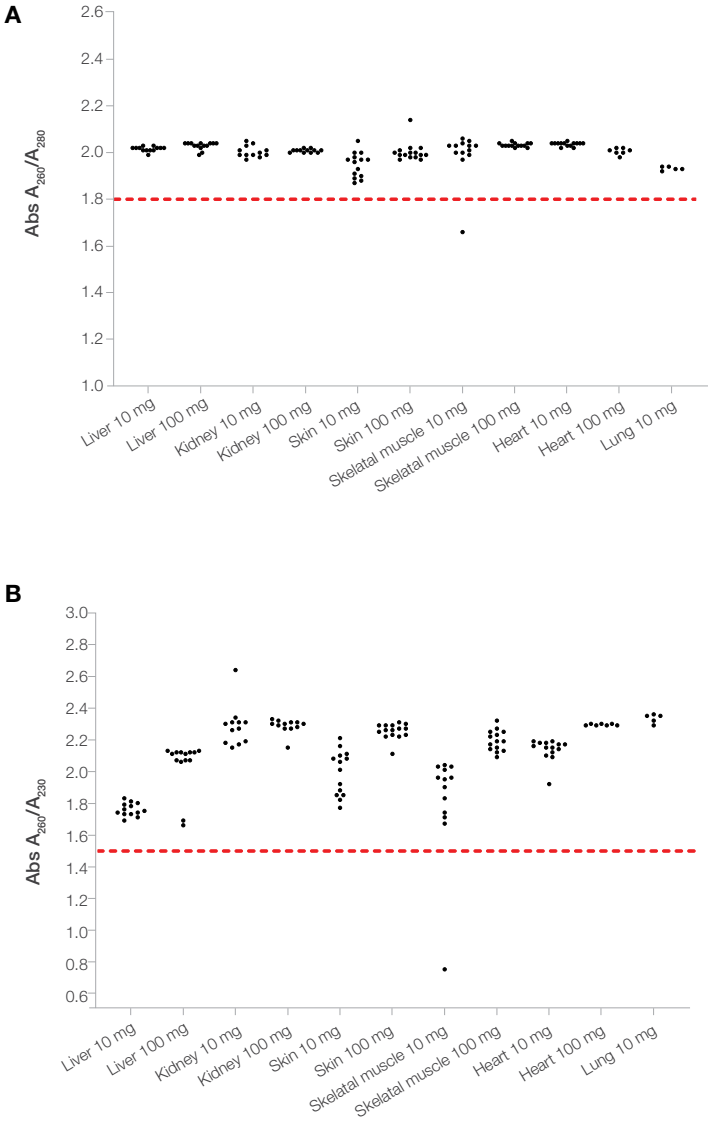


Figure 3. (A) A_{260}/A_{280} and (B) A_{260}/A_{230} plotted for each replicate. The red dotted line represents the minimum accepted value for each ratio: 1.8 for A_{260}/A_{280} and 1.5 for A_{260}/A_{230}.

Table 3 provides the results of quantitative and qualitative analyses of nucleic acid extractions from various mouse tissue types. The data demonstrate a clear linear correlation between starting sample 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, chicken 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 that correspond with input sample weight prior to homogenization. Similarly, Figure 4 shows that the observed nucleic acid yields for 100 mg skin, skeletal muscle, and heart samples closely corresponded to values predicted by scaling the 10 mg yields by a factor of 10. Liver and kidney samples, however, deviated significantly from predicted values, with reduced nucleic acid concentrations at higher sample sizes. This reduction is likely associated with extraction limitations at concentrations exceeding 4,000 ng/µL, possibly due to saturation of the magnetic beads.

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. (ng/µL)	Standard deviation of nucleic acid conc. (ng/µL)	Quality metrics	
				Average A_{260}/A_{280}	Average A_{260}/A_{230}
Liver (n = 12)	10	642.7	112.1	2.02	1.76
Liver (n = 12)	100	4,172.4	547.2	2.03	2.04
Kidney (n = 12)	10	804.2	96.1	2.00	2.29
Kidney (n = 12)	100	4,493.2	328.6	2.01	2.29
Skin (n = 12)	10	178.3	53.3	1.95	1.99
Skin (n = 12)	100	1,914.2	396.6	2.00	2.25
Skeletal muscle (n = 12)	10	95.9	30.0	1.99	1.81
Skeletal muscle (n = 12)	100	765.2	197.0	2.03	2.19
Heart (n = 12)	10	289.2	67.5	2.04	2.14
Heart (n = 7)	100	2,363.4	134.4	2.01	2.29
Lung (n = 5)	10	735.4	38.6	1.93	2.33

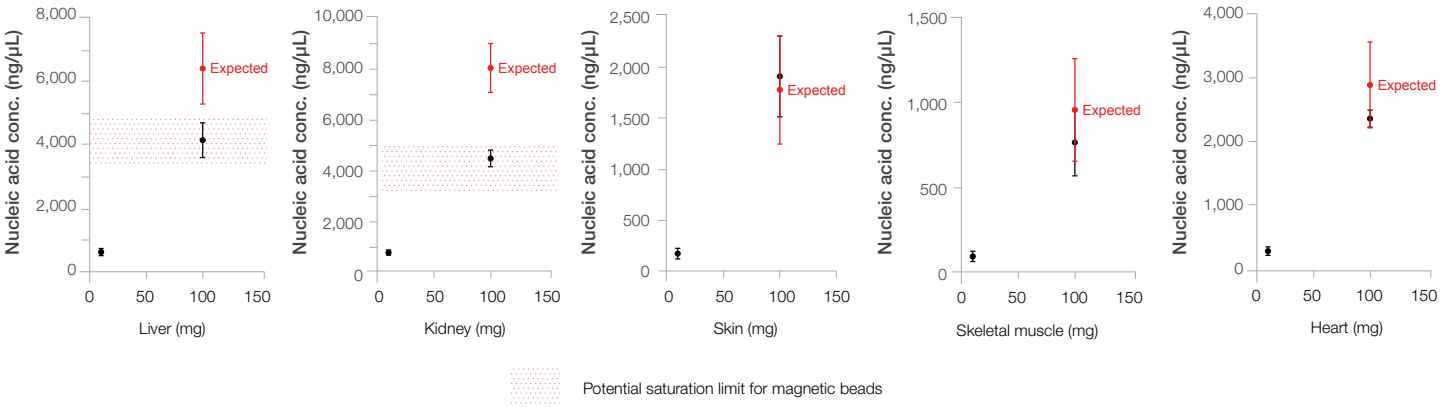


Figure 4. Concentration (ng/µL) of nucleic acid extracted from various mouse tissues (liver, kidney, skin, skeletal muscle, and heart) as a function of tissue mass (mg). Each graph shows the expected nucleic acid yield based on tissue type and mass (red dots with error bars) calculated by multiplying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

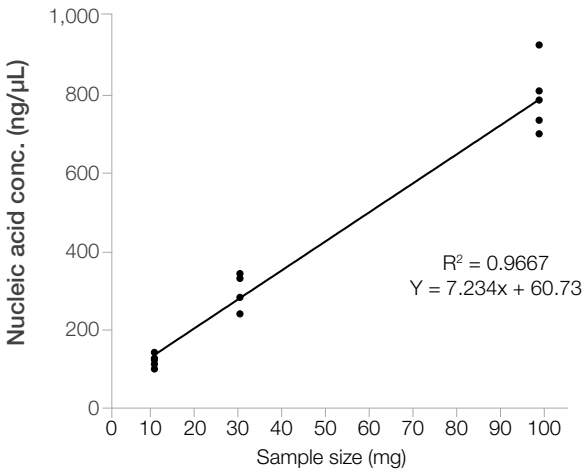


Figure 5. Correlation between sample mass and nucleic acid concentration from chicken muscle samples.

The yields for skin, skeletal muscle, and heart 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 samples yielded 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 A_{260}/A_{280} and A_{260}/A_{230}, indicating robust nucleic acid purity. 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.

Conclusions

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

References

1. Han SM, Munro A (1999) Transfer from manual to automated sample preparation: a case study. *J Pharm Biomed Anal* 20(5):785–790. doi.org/10.1016/s0731-7085(99)00078-3.
2. Matlock B (2015) 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.

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