Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Impact of disruption methods on high molecular weight DNA extracted from archived frozen tissues

Kaiyu Qian

qky1009@whu.edu.cn

Wuhan University https://orcid.org/0000-0003-4153-1265

Research Article

Keywords: HMW DNA, quality, quantity, TissueLyser homogenization, PBS, ATL buffer

Posted Date: November 3rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11218928/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: The authors declare no competing interests.

Abstract

Background

Tissue samples can provide high molecular weight (HMW) DNA for advanced genomic applications, such as long-read sequencing. Archived frozen tissues stored in biobanks or tissue repositories could retain DNA quality. However, the impact of different tissue disruption methods on DNA yield, purity and integrity remains incompletely characterized.

Methods

The impact of three disruption methods on DNA was initially compared using rabbit liver tissues: liquid nitrogen (LN) coarse smashing, LN fine grinding, and TissueLyser homogenization. Key parameters, including lysis time, homogenization buffer, agitation frequency, and running duration, were subsequently optimized to maximize DNA yield or preserve fragment integrity. Different disruption methods were further evaluated using archived frozen human liver, kidney, bladder, and pancreas tissues to assess their applicability across different tissue types.

Results

All methods yielded A260/280 ratios of ~ 1.8. LN fine grinding resulted in the lowest DNA yield, DNA integrity number (DIN) and A260/230. LN coarse smashing yielded optimal integrity (DIN > 8, peak size ~ 50 kb) but moderate yield, and prolonged lysis did not improve DNA recovery. The TissueLyser group with nuclease-free water had the highest yield and purity (A260/230 > 2.0) but the smallest fragment size (~ 25 kb). Buffer selection markedly affected the outcomes of TissueLyser homogenization. PBS achieved high integrity (DIN ~ 9, peak size ~ 60 kb), whereas ATL buffer (lysis solution of DNA extraction kit) produced smaller DNA fragments (~ 22 kb) but significantly increased the yield (1245.93 ± 168.37 ng/mg). Validation in human tissues demonstrated that the optimized TissueLyser protocol consistently outperformed the LN disruption methods across all four tissue types.

Conclusions

Tissue disruption should balance DNA recovery and integrity. TissueLyser homogenization offers a standardized and efficient approach for HMW DNA extraction. The buffer and parameters should be guided by the specific requirements of downstream applications.

INTRODUCTION

DNA extracted from human tissues is a core resource in biomedical research, precision medical diagnosis, targeted therapy development and cutting-edge interdisciplinary exploration [1–3]. Large-scale genomic initiatives, such as the Mutographs project and the Genotype-Tissue Expression (GTEx) project, rely on the systematic collection and long-term cryopreservation of large numbers of human tissue specimens, as well as subsequent extraction of high-quality DNA [4–6]. High-quality DNA not only serves as a prerequisite for diverse applications with specific requirements but also directly affects the depth, breadth, and reliability of the research [7]. In particular, third-generation sequencing technologies, including long-read sequencing, require high-molecular-weight (HMW) DNA with sufficient quantity and integrity to achieve optimal sequencing performance [8–10].

DNA quality is commonly assessed in terms of purity and integrity. The purity of DNA is evaluated through the ratios of A260/280 (indicating protein contamination) and A260/230 (indicating polysaccharides, organic solvent or salt contamination) [11, 12]. The integrity of DNA is assessed by the DNA integrity number (DIN) and DNA fragment size. The DIN value ranges from 1 (highly degraded) to 10 (highly intact) [13–15], and DIN ≥ 8 is considered high quality [16]. DNA

fragmentation makes DNA less suitable for certain applications that require HMW DNA and have specific, strict standards for the length of DNA [13, 17, 18]. HMW DNA is typically defined as fragments larger than 10 kb [17]. The importance of DNA molecular weight is particularly evident in long-read sequencing. For example, the mean insert size recommended for PacBio HiFi whole-genome sequencing libraries is 10—25 kb [19]. Despite this relatively smaller library size, PacBio recommends starting with genomic DNA larger than 40 kb on average, to ensure a high-quality input and a more narrowly focused library size distribution after shearing [19]. Oxford Nanopore Technologies is able to sequence very long DNA samples (> 50 kb) [20, 21]. However, obtaining DNA of the desired molecular weight often requires specialized extraction protocols followed by controlled fragmentation or shearing, which can increase procedural complexity, time, and cost [21–24]. Any mechanical shearing, nuclease degradation, or chemical damage during sample pretreatment or DNA extraction procedures can result in DNA fragmentation [25]. Therefore, preserving DNA integrity during the processing of tissues is particularly important for generating HMW DNA suitable for advanced genomic applications.

The quality and quantity of DNA are influenced by the tissue type, initial state of the tissue sample, pretreatment method, and extraction procedure [26–29]. Prior to DNA extraction, it is essential to lyse tissue samples to release DNA [30]. The majority of commercial DNA extraction kits suggest that tissue disruption can reduce lysis time. Mechanical disruption methods, such as liquid nitrogen (LN) grinding via pestles and mortars, cutting tissues into pieces with scissors, and bead-based homogenization, are widely adopted to accelerate cell lysis in tissue DNA extraction [26, 30–32]. However, mechanical disruption can also introduce shear forces that may fragment genomic DNA [33]. The choice and optimization of tissue disruption method may be particularly important when HMW DNA is required.

Notably, bead-based homogenization is increasingly used for tissue disruption because it can homogenize extremely small tissues through the crushing action of beads against the tissue, combined with localized shear forces generated in the surrounding buffer [27]. The impact of this approach on DNA quality may depend on several operational parameters, including the composition of the liquid phase, agitation speed, and running duration [27, 31]. However, even in the instructions of well-established commercial kits, recommendations regarding the homogenization buffer are either absent or inconsistent across different manufacturers. Examples include the QIAwave DNA Blood & Tissue Kit (Qiagen, Hilden, Germany), PureLink® Genomic DNA Kit (Thermo Fisher Scientific, Waltham, MA, USA), TIANamp Genomic DNA Kit (Tiangen Biotech, Beijing, China), Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) and HiPure Tissue DNA Kit (Magen Biotechnology, Guangzhou, China). Despite the widespread use of these approaches, few studies have systematically evaluated how tissue disruption methods and their key operational parameters influence DNA yield, purity, and integrity, particularly in archived frozen tissues.

To address this gap, we first evaluated the impact of three disruption methods on the quality and quantity of DNA in archived frozen rabbit liver tissues. Subsequently, an attempt was made to optimize the LN coarse smashing method by extending the lysis time, and the effects of buffer solutions, agitation speed and running duration of the bead-based homogenization method on DNA quality were evaluated. Finally, four types of frozen human tissues, namely, liver, kidney, bladder, and pancreas, were used to validate the influence of different disruption methods on DNA quality.

MATERIALS AND METHODS

Tissue preparation

The rabbit liver tissues used in this study were specimens acquired from our prior research, which had been stored in vapor-phase LN since May 2025 [34]. These specimens were collected after the rabbit was anesthetized by intravenous injection of 3% pentobarbital sodium (30 mg/kg) into the ear vein and euthanized through the intravenous administration of an overdose of 10% pentobarbital sodium (100 mg/kg). Human tissues cryopreserved in vapor-phase LN for several months to four years were provided by the Department of Biological Repositories, Zhongnan Hospital of Wuhan University, a member of International Society for Biological and Environmental Repositories (ID:49623232). This study was approved by the Experimental Animal Welfare and Ethics Committee of Zhongnan Hospital, Wuhan University (Approval No. 2025109) and

the Medical Ethics Committee of Zhongnan Hospital of Wuhan University (Approval No. 2017038). All experiments were conducted with four biological replicates (n = 4).

Tissue disruption methods

An excessive tissue input, particularly for samples with rich DNA (e.g., liver, lung, or spleen), can reduce both DNA yield and purity. Therefore, the majority of commercial tissue DNA extraction kits (e.g., QIAwave DNA Blood & Tissue Kit, PureLink® Genomic DNA Kit, TIANamp Genomic DNA Kit, Wizard® Genomic DNA Purification Kit and HiPure Tissue DNA Kit) specify a maximum amount of starting material in their protocols. Therefore, in the present study, frozen rabbit liver tissues were first placed in a mortar precooled with LN and gently smashed into small pieces using a pestle. These pieces were then quickly weighed on an electronic balance (#HZT-A + 300, HUAZHI, USA) without thawing. Pieces weighing approximately 10 mg, as recommended by the Hipure Tissue DNA Mini Kit (#D312102, 2024, Magen, China) for liver tissue, were selected and randomly assigned to three disruption groups (Fig. 1).

For the LN coarse smashing group, 10 mg tissue pieces were cryogenically smashed 3–5 times in LN with a pestle to obtain smaller fragments of approximately 1–2 mm in diameter, yielding coarse tissue pieces. For the LN fine grinding group, 10 mg tissue pieces were first processed as described for coarse smashing and then further ground into a fine powder. For the TissueLyser group, 10 mg tissue pieces were placed into 2 mL RNase-/DNase-free sterile polypropylene centrifuge tubes (# MCT-200-C-S, Axygen, USA) and mechanically homogenized via a high throughput bead-based shaking TissueLyser (#Scientz-48, Ningbo SCIENTZ Biotechnology Ltd., China) under the following conditions: 250 μL of nuclease-free water (#BL510A, Biosharp, China), two different sized nuclease-free homogenization beads (4 mm and 2 mm diameter, respectively) [34], and a frequency of 50 Hz with continuous operation for 30 seconds. After homogenization, the samples were centrifuged at 12000 × g for 1 minute, after which the supernatant and beads were discarded. The disrupted tissues of the three groups were placed into 2 mL centrifuge tubes and supplemented with 230 μL ATL buffer and 20 μL proteinase K, followed by incubation in a 55°C water bath for 30 minutes. Both ATL buffer and proteinase K were provided by the Hipure Tissue DNA Mini Kit (#D312102, 2024, Magen, China).

Extended lysis time of coarse smashed tissues

Approximately 10 mg frozen rabbit liver tissue piece was cryogenically smashed 3–5 times in LN. After 230 μL ATL buffer and 20 μL proteinase K were added, the coarse smashed tissues were randomly allocated to three extended lysis time groups: 60, 90, and 120 minutes in a 55°C water bath.

Homogenization buffer

Frozen rabbit liver tissues were cryogenically smashed into 10 mg tissue pieces and transferred to two other buffer solutions containing two different sized nuclease-free homogenization beads (4 mm and 2 mm diameter, respectively): 230 μL ATL buffer with 20 μL proteinase K and 250 μL phosphate buffered saline (PBS, pH 7.4, #C10010500BT; Gibco, USA). The tissues were homogenized via a TissueLyser at a frequency of 50 Hz with continuous operation for 30 seconds. After homogenization, the samples in the PBS group were treated in the same way as those in the TissueLyser group with nuclease-free water. For the ATL group, the beads were removed with sterile tweezers. The treated tissues were incubated in a 55°C water bath for 30 minutes.

Homogenization parameters

Frozen rabbit liver tissues were cryogenically smashed into 10 mg pieces. The agitation frequency and running duration of homogenization were adjusted for the two buffer solutions. For the ATL groups, 10 mg tissue pieces were transferred to 230

μL ATL buffer combined with 20 μL proteinase K, followed by homogenization with continuous operation for 30 seconds under lower agitation frequencies of 20, 30, and 40 Hz. For the PBS groups, 10 mg tissue pieces were transferred to 250 μL PBS and homogenized at 50 Hz for longer running durations of 60 and 90 seconds.

Validation in frozen human tissues

Four types of frozen human tissues were cryogenically smashed into small pieces according to the instructions of the HiPure Tissue DNA Mini Kit: liver (~ 10 mg), kidney (~ 10 mg), bladder (~ 20 mg), and pancreas (~ 20 mg). The tissue pieces were randomly assigned to the LN coarse smashing group, the fine grinding group, and the TissueLyser group using PBS and ATL as the homogenization buffer (50 Hz, 30 seconds). The disrupted tissues were incubated in a 55°C water bath for 30 minutes.

DNA extraction and quality assessment

After the tissues were incubated in a 55°C water bath, DNA was extracted using the HiPure Tissue DNA Mini Kit. The primary steps were as follows: (1) 10 μL of RNase A was added, followed by incubation at room temperature for 20 minutes, (2) 250 μL of DL buffer was added, followed by incubation at 70°C for 10 minutes, (3) 250 μL of anhydrous ethanol was added and DNA purification was performed using a silica column, and (4) the extracted DNA was eluted with 50 μL of AE buffer.

The DNA concentration and purity (A260/280 and A260/230 ratios) were measured with a NanoDrop One spectrophotometer (Thermo Fisher Scientific Ltd.). The DNA yields were normalized to the tissue mass (ng DNA/mg tissue). DIN and fragment size (200 bp to > 60 kb) were determined using an Agilent 4200 TapeStation system with Genomic DNA ScreenTape (#5067–5365, Agilent Biotechnologies Ltd., USA). The peak size is recommended as the sizing metric [29]. For statistical analysis, peak sizes > 60 kb were assigned to a nominal value of 60 kb.

Statistical analysis

Statistical analyses were conducted via GraphPad Prism 8.0.1 (GraphPad Software, San Diego, CA). The Shapiro-Wilk normality test was performed to determine whether the data followed a normal distribution, and the homogeneity of variance was also examined. For the rabbit tissues, one-way ANOVA was used when normality and homogeneity of variance were satisfied. Otherwise, the Kruskal-Wallis U/H test was used. For the human tissues, repeated measures one-way ANOVA or the Friedman test was used. If the test result was statistically significant, a post hoc test for multiple comparisons was performed.

RESULTS

The effects of disruption methods on DNA quality of frozen rabbit liver

Three methods were used to disrupt the frozen rabbit liver: LN coarse smashing, LN fine grinding, and TissueLyser homogenization with nuclease-free water. The yield, purity, and integrity of the DNA were analyzed. As shown in Fig. 2A, the LN fine grinding group presented the lowest DNA yield, followed by the coarse smashing group, and the TissueLyser group presented the highest DNA yield (145.13 ± 16.65, 504.41 ± 140.48 and 862.58 ± 96.01 ng/mg). The A260/280 ratio of all the groups was approximately 1.8 (Table 1). The A260/230 ratio in the TissueLyser group was greater than 2.0, which was significantly greater than that in the LN fine grinding group (Table 1). The coarse smashing group showed significantly higher DIN values (8.75 ± 0.31, Fig. 2B) and larger DNA peak sizes (~ 50 kb, Fig. 2C) than the fine grinding and TissueLyser groups. The LN fine grinding group showed considerable variability in peak size (33.16 ± 11.10 kb, with the standard deviation exceeding 30% of the mean), and the TissueLyser group exhibited the lowest peak size (25.77 ± 3.20 kb).

Table 1
Evaluation of DNA extracted from frozen rabbit liver.

Disruption methods			Lysis time (min)	Yield (ng/mg)	DIN	Peak size (kb)	A260/280	A260/230
LN fine grinding			30	145.13 ± 16.65	7.35 ± 0.42	33.16 ± 11.10	1.80 ± 0.07	1.52 ± 0.15
LN coarse smashing			30	504.41 ± 140.48	8.75 ± 0.31	53.31 ± 3.52	1.85 ± 0.04	2.01 ± 0.30
			60	432.12 ± 91.32	8.88 ± 0.22	54.24 ± 2.05	1.84 ± 0.04	1.93 ± 0.13
			90	375.30 ± 47.47	8.30 ± 0.14	47.96 ± 3.38	1.85 ± 0.02	1.98 ± 0.19
			120	480.00 ± 39.20	8.15 ± 0.31	47.07 ± 8.39	1.86 ± 0.02	2.00 ± 0.06
TissueLyser homogenization (buffer, agitation frequency, and running duration)	NFW	50 Hz 30 s	30	862.58 ± 96.01	7.55 ± 0.10	25.77 ± 3.19	1.87 ± 0.01	2.14 ± 0.05
	ATL	50 Hz 30 s	30	1245.93 ± 168.37	8.33 ± 0.17	21.79 ± 0.61	1.88 ± 0.01	2.21 ± 0.10
	ATL	40 Hz 30 s	30	1300.27 ± 71.41	8.70 ± 0.08	30.48 ± 3.93	1.87 ± 0.02	2.15 ± 0.17
	ATL	30 Hz 30 s	30	880.04 ± 322.76	8.68 ± 0.19	53.73 ± 3.03	1.87 ± 0.01	2.07 ± 0.10
	ATL	20 Hz 30 s	30	733.22 ± 130.62	8.68 ± 0.13	58.30 ± 2.57	1.87 ± 0.02	2.02 ± 0.14
	PBS	50 Hz 30 s	30	687.12 ± 106.06	8.88 ± 0.05	57.44 ± 1.90	1.87 ± 0.02	2.09 ± 0.11
	PBS	50 Hz 60 s	30	858.56 ± 142.94	8.73 ± 0.33	59.34 ± 1.24	1.87 ± 0.02	2.19 ± 0.29
	PBS	50 Hz 90 s	30	735.08 ± 66.44	8.40 ± 0.14	58.73 ± 2.44	1.82 ± 0.05	1.87 ± 0.38

NFW/ATL/PBS represent different homogenization buffers when a TissueLyser is used.

LN, liquid nitrogen; **DIN**, DNA integrity number; **NFW**, nuclease-free water; **PBS**, phosphate buffered saline.

The impact of lysis time on the DNA quality of coarse smashed frozen rabbit liver

A 30-minute incubation for the coarse smashed tissue was insufficient to achieve complete lysis, as evidenced by the residual macroscopic tissue clumps. We therefore extended the incubation time. No significant differences in the DNA yield, purity, or peak size were observed across incubation times up to 120 minutes (Table 1, Figure S1A, S1C). However, DINs were significantly lower in the 90-minute and 120-minute groups than in the 30-minute and 60-minute groups (Figure S1B).

The effects of different homogenization parameters on the DNA quality

Compared with homogenization with nuclease-free water, homogenization with ATL buffer resulted in a significantly increased DNA yield, whereas PBS showed no significant difference in DNA yield (Fig. 3A). DNA purity was comparable among the three groups, as evidenced by both the A260/280 and A260/230 ratios (Table 1). The PBS group presented significantly greater DINs than the nuclease-free water group (8.88 ± 0.05 versus 7.55 ± 0.10). The DINs of the ATL group (8.33 ± 0.17) tended to be greater than those of the nuclease-free water group, although the difference was not statistically significant (Fig. 3B). The PBS group exhibited a significantly larger DNA peak size than the ATL and nuclease-free water groups (57.44 ± 1.90 kb versus 21.79 ± 0.61 kb and 25.77 ± 3.19 kb, Fig. 3C).

In light of the highest DNA yield but the lowest DNA peak size observed in the ATL group, we subsequently decreased the agitation frequency. DNA purity remained consistent under all conditions (Table 1). For DNA integrity, homogenization at 20–40 Hz produced significantly higher DINs than homogenization at 50 Hz (Fig. 3E). Compared with higher frequencies (40 Hz and 50 Hz), lower homogenization frequencies (20 Hz and 30 Hz) resulted in larger peak sizes but lower DNA yields (Fig. 3D, 3F). The DNA peak size was lowest at 50 Hz.

Considering that the PBS group presented the greatest DINs and the largest DNA peak size but a lower DNA yield, we enhanced the disruption intensity by extending the running duration. No differences were observed in the DNA yield, purity, or DNA peak size (Table 1, Fig. 3G, 3I). Nevertheless, more intensive treatments (50 Hz, 90 seconds) resulted in lower DINs, however, all values still remained above 8 (Fig. 3H).

The effect of disruption methods on the DNA quality of frozen human tissues

In the validation experiment on four types of frozen human tissues, the average DNA yield was the highest for the ATL TissueLyser group (liver, 2021.48 ± 497.97 ng/mg; kidney, 1219.45 ± 317.98 ng/mg; bladder, 2123.04 ± 1558.40 ng/mg; and pancreas, 2214.50 ± 483.88 ng/mg) and the lowest for the LN fine grinding group (Fig. 4A–4D). For the liver tissues, the LN fine grinding group showed the lowest DINs (Fig. 4E). For the other three types of tissues, no significant differences in DINs were observed (Fig. 4F–4H). Notably, apart from a single kidney tissue that exhibited DINs < 8 under four disruption methods, the PBS TissueLyser group of all other tissues presented DINs > 8 (Table 2). Furthermore, the PBS TissueLyser group of all tissues generated HMW DNA with a peak size > 50 kb (Fig. 4I–4L). The ATL TissueLyser group generated relatively lower peak sizes (liver, 21.40 ± 3.27 kb; kidney, 34.03 ± 7.55 kb; bladder, 44.05 ± 11.59 kb; and pancreas, 34.99 ± 9.70 kb, Table 2), with the exception of only two bladder tissues that exhibited a peak size > 50 kb. The LN fine grinding group of pancreas tissues and the coarse smashing group of bladder tissues presented a DNA peak size > 50 kb, whereas these two disruption methods showed marked fluctuations in the other three tissue types. In terms of DNA purity, significant differences were observed in the bladder and liver tissues among the four disruption methods, with the TissueLyser groups exhibiting superior purity (Table 2). No significant differences were found in kidney and pancreas tissues.

Table 2
Evaluation of DNA extracted from four types of frozen human tissues.

Tissue type	Disruption methods	DNA yield (ng/mg)		DIN		DNA peak size (kb)		A260/280		A260/230	
		Mean ± SD	*p* value	Mean ± SD	*p* value	Mean ± SD	*p* value	Mean ± SD	*p* value	Mean ± SD	*p* value
Liver	*LN fine grinding*	250.45 ± 37.13	**< 0.001**	7.60 ± 0.74	**0.027**	36.19 ± 11.61	**0.004**	1.83 ± 0.03	**0.007**	1.57 ± 0.39	**0.026**
	LN coarse smashing	614.41 ± 234.23		8.43 ± 0.86		55.30 ± 7.63		1.75 ± 0.07		1.29 ± 0.36	
	TissueLyser (PBS)	961.04 ± 413.01		8.83 ± 0.54		58.76 ± 1.67		1.83 ± 0.03		1.84 ± 0.21	
	TissueLyser (ATL)	2021.48 ± 497.97		8.10 ± 0.16		21.40 ± 3.27		1.84 ± 0.02		1.94 ± 0.18	
Kidney	*LN fine grinding*	379.89 ± 135.75	**0.004**	8.08 ± 0.33	0.196	51.11 ± 1.92	**0.002**	1.86 ± 0.05	0.151	1.83 ± 0.36	0.347
	LN coarse smashing	607.03 ± 118.27		8.25 ± 0.79		51.84 ± 8.05		1.87 ± 0.01		2.03 ± 0.12	
	TissueLyser (PBS)	978.21 ± 492.77		8.50 ± 0.69		56.03 ± 2.70		1.88 ± 0.01		2.16 ± 0.11	
	TissueLyser (ATL)	1219.45 ± 317.98		8.35 ± 0.51		34.03 ± 7.55		1.82 ± 0.05		1.90 ± 0.40	
Bladder	*LN fine grinding*	157.85 ± 68.14	**0.019**	8.50 ± 0.52	0.821	51.47 ± 7.76	**0.035**	1.80 ± 0.04	**0.004**	1.67 ± 0.28	**0.001**
	LN coarse smashing	443.66 ± 278.85		8.70 ± 0.53		56.24 ± 1.36		1.78 ± 0.04		1.42 ± 0.33	
	TissueLyser (PBS)	629.51 ± 125.97		8.75 ± 0.39		55.98 ± 2.77		1.84 ± 0.04		1.97 ± 0.26	
	TissueLyser (ATL)	2123.04 ± 1558.40		8.55 ± 0.35		44.05 ± 11.59		1.87 ± 0.01		2.29 ± 0.11	
Pancreas	*LN fine grinding*	577.51 ± 106.23	**0.002**	8.20 ± 0.45	0.058	58.59 ± 1.04	**0.012**	1.89 ± 0.03	0.181	2.09 ± 0.18	0.087
	LN coarse smashing	615.21 ± 213.10		8.30 ± 0.22		55.72 ± 4.99		1.87 ± 0.01		2.17 ± 0.08	
	TissueLyser (PBS)	1301.49 ± 299.61		9.00 ± 0.29		59.27 ± 1.47		1.88 ± 0.02		2.29 ± 0.09	
	TissueLyser (ATL)	2214.50 ± 483.88		8.65 ± 0.35		34.99 ± 9.70		1.86 ± 0.02		2.30 ± 0.07	

LN, liquid nitrogen; **DIN**, DNA integrity number; **PBS**, phosphate buffered saline.

DISCUSSION

Tissue samples procured from various organs contain a large amount of highly diverse information (such as DNA, RNA, and proteins) [31], which is highly valuable for biomedical research. As a crucial preprocessing step in biomedical research, tissue disruption is intended to break down the cell and nuclear membrane via physical, chemical, or enzymatic means to release intracellular components for subsequent molecular analysis [31]. The quality of this process directly influences the accuracy and reproducibility of subsequent operations, especially for those requiring batch processing. Therefore, selecting the most appropriate disruption method according to sample type, target analyte stability, and experimental throughput is essential to maximize the utilization of archived frozen tissues preserved in biobanks [34]. In the present study, we systematically compared tissue disruption methods to guide the extraction of high-quality DNA from archived frozen tissue samples.

Our results indicated that LN fine grinding resulted in the lowest DNA yield and A260/230 ratio. This finding aligns with our previous findings regarding tissue RNA, in which ground tissue powder adhering to a mortar and pestle significantly reduced the nucleic acid yield [34]. Compared with coarse smashing method, LN fine grinding resulted in lower DINs and DNA peak sizes. The mechanical forces involved in the grinding process may cause physical shearing of DNA molecules, leading to DNA fragmentation [33]. Angthong P *et al*. similarly reported that additional LN homogenization of adipose tissue caused lower DINs [35]. Furthermore, LN grinding is a technically demanding, complex and high-risk procedure that is prone to introducing pollutants, which may affect the efficiency of subsequent nucleic acid purification [12, 17].

LN coarse smashing is a relatively mild disruption method compared to LN fine grinding. The DNA integrity of the coarse smashing group was commendable, and the purity was within the acceptable ranges. However, this group yielded a moderate quantity of DNA among the three disruption methods, which correlated with the observation that undissolved tissue fragments remained after 30 minutes of incubation in a water bath. This suggested incomplete lysis, potentially compromising DNA yield [30]. It is crucial to adjust the lysis time according to the tissue state [36]. While previous investigations have reported that prolonged lysis (e.g., overnight) increases yields without decreasing quality [36, 37] or has no substantial influence [38, 39], our results contrast with these findings. Extending the lysis time up to 120 minutes, which completely dissolved the 10 mg tissue pieces, failed to increase DNA yield and instead resulted in DNA smearing and a trend toward lower fragment peak sizes at 90 and 120 minutes. We hypothesize that this discrepancy may arise because the amount of DNA within the exposed cellular structure is prone to reduction during chemical lysis, which exacerbates DNA degradation [40]. Considering the extraction efficiency and the hypothesis that there might be an improvement in yield but a decrease in DNA integrity, no additional groups with longer lysis times were conducted.

Bead-beating homogenization has been proven to be a simple, effective, and labor-saving mechanical disruption method that increases the DNA yield [41, 42]. In this study, bead beating homogenization via a TissueLyser with nuclease-free water yielded high purity DNA, with a maximum quantity of 862.6 ± 96.0 ng/mg. Nevertheless, the TissueLyser group exhibited lower DINs with DNA peak sizes of ~ 25 kb. Similarly, Hermans N *et al*. reported that the implementation of a bead beating step led to an approximately 60-fold increase in DNA yield but resulted in DNA fragmentation [43]. We hypothesized that the observed fragmentation might be caused by either inappropriate buffer or the excessive intensity of homogenization. Consequently, we introduced two other buffers (PBS and ATL buffer) for comparison with nuclease-free water under the same homogenization parameters (50 Hz for 30 s). All buffers produced high purity DNA. Notably, the ATL buffer significantly increased the mean DNA yield to 1245.93 ± 168.37 ng/mg without mitigating DNA fragmentation. Despite slightly higher DIN values (> 8), the DNA peak size remained approximately 22 kb. These results suggest that ATL buffer can facilitate cell lysis and is highly efficient in releasing DNA, but the released DNA may remain susceptible to physical shearing caused by mechanical homogenization. Thus, the trade-off between yield and integrity needs to be further tuned.

In contrast, PBS exhibited a slightly lower DNA yield but markedly improved DNA integrity, resulting in DINs near 9 and peak sizes approaching 60 kb. This difference indicated that the buffer environment itself may substantially influence the extent of DNA fragmentation. Simpson RJ. suggested that the choice of homogenization buffer depends on the nature of the extract required and a buffer of moderate ionic strength at neutral pH is generally used [44]. Presumably, the isotonic characteristic of PBS more effectively maintains the osmotic equilibrium of the dispersed tissue cells during

homogenization. This enables tissue fragments to be dissociated into cell clumps without impairing the integrity of the plasma membrane, thereby preventing DNA exposure and mechanical shearing.

Beyond buffer selection, optimization of homogenization parameters may further improve the quality of the target outcomes [43, 45]. Given that ATL and PBS exhibited distinct advantages over nuclease-free water, with ATL favoring DNA recovery and PBS better preserving DNA integrity, we subsequently examined the effects of agitation frequency and homogenization duration for these two buffers. In the ATL buffer, decreasing the agitation frequency to 20 Hz or 30 Hz notably diminished DNA yield but maintained DNA purity and improved DNA integrity, with HMW DNA > 50 kb. This phenomenon may be attributed to the fact that lower agitation frequencies impose milder mechanical shearing forces on the released DNA, thereby protecting long DNA strands from fragmentation. However, the reduced yield likely reflects incomplete tissue disruption, as small tissue remnants were observed. In PBS, apart from the slightly lower DINs after 90 seconds of homogenization, extending the running duration of PBS at a frequency of 50 Hz had no effect on the DNA yield, purity or peak size. Extending the running duration of PBS homogenization did not increase the DNA yield, likely because the tissue disruption had already reached an effective level at 50 Hz for 30 seconds, as evidenced by the absence of visible tissue remnants.

The findings from the four types of human tissues were generally consistent with those from rabbit liver tissue. TissueLyser homogenization demonstrated relatively stable overall performance. Among them, the PBS groups resulted in a moderate yield, yet the largest DNA peak size across the four types of human tissues. The ATL buffer yielded the highest amount of DNA but the lowest DNA peak sizes, which varied across different types of tissue. This may be attributed to tissue heterogeneity. For example, the DNA peak size of liver tissue was approximately 20 kb, which is consistent with the results for rabbit liver tissue. Liver tissue is readily disrupted, but HMW DNA recovery from it has been reported to be challenging, potentially owing to its high endogenous enzymatic activity [26]. Bladder tissue is more tough and more challenging to disrupt. Siuta J *et al*. also reported substantial differences in DNA yields among human liver, kidney, and pancreas tissues [26]. The TissueLyser groups in our study presented higher DNA yields. Both the LN grinding and minced groups presented marked fluctuations in the DIN and DNA size of the liver tissue. In contrast, the TissueLyser groups produced more stable results, suggesting that bead-based homogenization was more suitable for liver tissue. Nevertheless, with respect to the other three tissue types, the disruption methods had a minor influence on the DIN. Taken together, cross-tissue validation suggests that the effect of tissue disruption methods on DNA quality may vary with tissue characteristic.

Several limitations should be considered. First, the optimization experiments were primarily performed using rabbit liver tissue, and only four types of human tissues were used for validation. Second, the number of biological replicates was limited. Considering the impact of tissue heterogeneity and individual variations, the findings may not be directly generalizable to other tissue types, and larger cohorts would be conducive to establishing more stable and reliable results, thereby enabling the specific selection of appropriate disruption conditions for each type of tissue. Third, the performance of the extracted DNA in downstream long-read sequencing was not directly assessed.

CONCLUSION

In conclusion, this study demonstrates that tissue disruption conditions substantially influence both DNA recovery and molecular integrity during the extraction of HMW DNA from archived frozen tissues. Compared with the LN fine grinding and coarse smashing, the bead-based homogenization tends to be more efficient. It consistently yields DNA with stable quality and quantity, which is crucial for the accuracy of downstream analyses. Moreover, it facilitates the batch processing of tissue samples, substantially improving efficiency. Specifically, the choice of homogenization buffer is critical. PBS can generate high-quality HMW DNA with a DIN greater than 8 and a peak size > 50 kb, regardless of the sample type, agitation frequency, and running duration. Conversely, the quality of DNA extracted from tissues treated with ATL buffer is more prone to being influenced by these factors. ATL buffer produces moderate size of HMW DNA and provides a considerable DNA yield higher than that of PBS. These findings may facilitate the preparation of high quality DNA for downstream genomic applications requiring HMW DNA.

Abbreviations

LN: liquid nitrogen;

DIN: DNA integrity number;

HMW DNA: high molecular weight DNA;

NFW: nuclease-free water;

ATL, lysis buffer from Hipure Tissue DNA Kit;

PBS: phosphate buffered saline.

Declarations

Ethics statement

The animal study protocol was approved by the Experimental Animal Welfare and Ethics Committee of Zhongnan Hospital, Wuhan University (Approval No. 2025109). The human study was conducted in accordance with the Declaration of Helsinki and approved by the Medical Ethics Committee of Zhongnan Hospital of Wuhan University (Approval No. 2017038). Informed consent was obtained from all participants involved in this study.

Conflicts of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

Shanshan Zhang, Wenjing Li, and Cong Zou contributed equally to this work. Kaiyu Qian, Shanshan Zhang, Cong Zou, Yi Zhang, and Zhangyan Zhou contributed to the concept and design of the work. Shanshan Zhang, Cong Zou, Yu Xiao, Hongwei Peng, Zongning Zhou, and Wenjing Li contributed to the acquisition, analysis, or interpretation of data. Shanshan Zhang, Cong Zou, Wenjing Li, Zongning Zhou, Zhangyan Zhou, Kaiyu Qian, Yi Zhang, and Yu Xiao drafted the work and revised it. Wenjing Li and Yu Xiao contributed to the visualization; Kaiyu Qian, Yi Zhang, and Zhangyan Zhou contributed to resources, project administration, funding acquisition, and supervision. All the authors read and approved the final manuscript.

Acknowledgments

The authors thank Dr. Yuruo Chen for assistance with figure editing. We gratefully acknowledge and thank the participants for their help in the experiment and support from the funds. The human tissues were obtained from the Human Genetic Resources Preservation Center of Hubei Province (Department of Biological Repositories, Zhongnan Hospital of Wuhan University), China, a member of the International Society for Biological and Environmental Repositories (ID:49623232). This

study was funded by the Hubei Provincial Science and Technology Plan Project (2025CFC012), and the Open Research Fund of Hubei Key Laboratory of Urological Diseases (MNXTJB202414).

Data availability statement

All data generated or analyzed during this study are included in this manuscript and the supplementary materials.

References

1. Nurk S, Koren S, Rhie A et al (2022) The complete sequence of a human genome. Science 376:44–53. 10.1126/science.abj6987
2. Sedlazeck FJ, Rescheneder P, Smolka M et al (2018) Accurate detection of complex structural variations using single-molecule sequencing. Nat Methods 15:461–468. 10.1038/s41592-018-0001-7
3. Esmaeilpour D, Ghomi M, Zare EN et al (2025) Recent advances in DNA nanotechnology for cancer detection and therapy: A review. Int J Biol Macromol 307:142136. 10.1016/j.ijbiomac.2025.142136
4. Perdomo S, Abedi-Ardekani B, de Carvalho AC et al (2024) The Mutographs biorepository: A unique genomic resource to study cancer around the world. Cell Genom 4:100500. 10.1016/j.xgen.2024.100500
5. GTEx Consortium, Laboratory, Data Analysis &Coordinating Center (LDACC)—Analysis Working Group; Statistical Methods groups—Analysis Working Group (2017) Genetic effects on gene expression across human tissues. Nature 550:204–213. 10.1038/nature24277
6. Abedi-Ardekani B, Nikmanesh A, Sotoudeh M et al (2026) Centralized processing of frozen tumor tissues for global cancer genomics. Nat Protoc Published online August 17. 10.1038/s41596-026-01426-x
7. Lyander A et al (2024) NGS method for parallel processing of high quality, damaged or fragmented input material using target enrichment. PLoS ONE 19:e0304411. 10.1371/journal.pone.0304411
8. Devonshire AS et al (2025) Interlaboratory evaluation of high molecular weight DNA extraction methods for long-read sequencing and structural variant analysis. BMC Genomics 26:698. 10.1186/s12864-025-11792-7
9. Blom MPK (2021) Opportunities and challenges for high-quality biodiversity tissue archives in the age of long-read sequencing. Mol Ecol 30:5935–5948. 10.1111/mec.15909
10. Mayjonade B, Gouzy J, Donnadieu C, Pouilly N, Marande W, Callot C, Langlade N, Muños S (2016) Extraction of high-molecular-weight genomic DNA for long-read sequencing of single molecules. Biotechniques 61:203–205. 10.2144/000114460
11. Bo YY, Liang LD, Hua YJ et al (2021) High-purity DNA extraction from animal tissue using picking in the TRIzol-based method. Biotechniques 70:186–190. 10.2144/btn-2020-0142
12. Shi X, Hu Z, Gan B et al (2023) Multivariate evaluation of DNA quality differences in different preanalytical procedures in mouse livers. Biopreserv Biobank 21:378–387. 10.1089/bio.2022.0027
13. Burke D, Pinheiro L, Somerville Glover E et al (2022) Between laboratory reproducibility of DNA extraction from human blood and fresh frozen tissue. J Mol Diagn 24:1041–1049. 10.1016/j.jmoldx.2022.06.003
14. Lu X, Wei Y, Sun J et al (2023) A comparative study of three nucleic acid integrity assay systems. Biopreserv Biobank 21:624–630. 10.1089/bio.2022.0171
15. Padmanaban A (2015) DNA integrity number (DIN) for the assessment of genomic DNA samples in real-time quantitative PCR (qPCR) experiments, Agilent Technologies Application Note. ; publication number 5991-6368EN
16. Dabrowski P, Rasmus M, Jundzill A et al (2024) A comparison of five methods to maximize RNA and DNA isolation yield from adipose tissue. PeerJ 12:e17071. 10.7717/peerj.17071
17. Messner E, Becker L, DeSanctis ML et al (2025) Perish the thawed? EDTA reduces DNA degradation during extraction from frozen tissue. PLoS ONE 20:e0321872. 10.1371/journal.pone.0321872

18. Dahn HA, Mountcastle J, Balacco J et al (2022) Benchmarking ultrahigh molecular weight DNA preservation methods for long-read and long-range sequencing. Gigascience 11:giac068. 10.1093/gigascience/giac068
19. PREPARING DNA FOR PACBIO HIFI SEQUENCING — EXTRACTION AND QUALITY CONTROL, Accessed (2026) September 28. https://www.pacb.com/wp-content/uploads/Technical-Note-Preparing-DNA-for-PacBio-HiFi-Sequencing-Extraction-and-Quality-Control.pdf
20. Akaçin İ, Ersoy Ş, Doluca O et al (2022) Comparing the significance of the utilization of next generation and third generation sequencing technologies in microbial metagenomics. Microbiol Res 264:127154. 10.1016/j.micres.2022.127154
21. Koren S, Bao Z, Guarracino A et al (2024) Gapless assembly of complete human and plant chromosomes using only nanopore sequencing. Genome Res 34:1919–1930. 10.1101/gr.279334.124
22. Kalendar R, Ivanov KI, Samuilova O et al (2023) Isolation of high-molecular-weight DNA for long-read sequencing using a high-salt gel electroelution trap. Anal Chem 95:17818–17825. 10.1021/acs.analchem.3c03894
23. Agyabeng-Dadzie F, Beaudry MS, Deyanov A et al (2025) Evaluating the benefits and limits of multiple displacement amplification with whole-Genome Oxford Nanopore Sequencing. Mol Ecol Resour 25:e14094. 10.1111/1755-0998
24. Gong L, Wong CH, Idol J et al (2019) Ultralong Read Sequencing for Whole Genomic DNA Analysis. J Vis Exp 145:e58954. 10.3791/58954
25. Russo A, Mayjonade B, Frei D et al (2022) Low-input high-molecular-weight DNA extraction for long-read sequencing from plants of diverse families. Front Plant Sci 13:883897. 10.3389/fpls.2022.883897
26. Siuta J, Dobosz A, Kawecki J et al (2023) DNA content of various fluids and tissues of the human body. Genes (Basel) 15:17. 10.3390/genes15010017
27. Goldberg S (2021) Mechanical/physical methods of cell disruption and tissue homogenization. Methods Mol Biol 2261:563–585. 10.1007/978-1-0716-1186-9_36
28. Wang YS, Dai TM, Tian H et al (2019) Comparative analysis of eight DNA extraction methods for molecular research in mealybugs. PLoS ONE 14:e0226818. 10.1371/journal.pone.0226818
29. Zhang S, Cheng X, Yang G et al (2024) Evaluation of DNA extracted from residual blood clots after serological testing. Cell Biochem Funct 42:e4023. 10.1002/cbf.4023
30. Wei S, Levy B, Hoffman N et al (2020) A rapid and simple bead-bashing-based method for genomic DNA extraction from mammalian tissue. Biotechniques 68:240–244. 10.2144/btn-2019-0172
31. Hao Y, Horak J, Stijepic Z et al (2025) Comprehensive tissue homogenization and metabolite extraction for application in clinical metabolomics. Anal Chim Acta 1344:343728. 10.1016/j.aca.2025.343728
32. Pearson H, Stirling D (2003) DNA extraction from tissue. Methods Mol Biol 226:33–34. 10.1385/1-59259-384-4:33
33. Kussler AP, Pimentel AM, Alcoba DD et al (2014) Mechanical processing of hyperviscous semen specimens can negatively affect sperm DNA fragmentation. Int Urol Nephrol 46:737–742. 10.1007/s11255-013-0578-9
34. Zou C, Yu M, Peng H et al (2025) Optimizing RNA quality in cryopreserved tissues without preservatives: impact of preservatives, thawing methods and tissue aliquot sizes. BMC Biotechnol 25:120. 10.1186/s12896-025-01059-0
35. Angthong P, Uengwetwanit T, Pootakham W et al (2020) Optimization of high molecular weight DNA extraction methods in shrimp for a long-read sequencing platform. PeerJ 8:e10340. 10.7717/peerj.10340
36. Ondracek RP, Chen J, Marosy B et al (2022) The results and lessons from dual extraction of DNA and RNA from formalin-fixed paraffin-embedded breast tumor tissues for a large Cancer epidemiologic study. BMC Genomics 23:614. 10.1186/s12864-022-08837-6
37. Janecka A, Adamczyk A, Gasińska A (2015) Comparison of eight commercially available kits for DNA extraction from formalin-fixed paraffin-embedded tissues. Anal Biochem 476:8–10. 10.1016/j.ab.2015.01.019
38. Frazer Z, Yoo C, Sroya M et al (2020) Effect of different proteinase K digest protocols and deparaffinization methods on yield and integrity of DNA extracted from formalin-fixed, paraffin-embedded tissue. J Histochem Cytochem 68:171–184.

10.1369/0022155420906234
39. Hidalgo A, Melo A, Romero F et al (2018) DNA extraction in Echinococcus granulosus and Taenia spp. eggs in dogs stool samples applying thermal shock. Exp Parasitol 186:10–16. 10.1016/j.exppara.2018.01.016
40. Buttar BS, Nelson ML, Busboom JR et al (2013) Effect of heat treatment on viability of Taenia hydatigena eggs. Exp Parasitol 133:421–426. 10.1016/j.exppara.2013.01.004
41. Hieda Y, Choe H, Ike H et al (2024) Bead-beating assay during synovial fluid DNA extraction improves real-time PCR accuracy for periprosthetic joint infection. J Orthop Res 42:2123–2130. 10.1002/jor.25871
42. Katayama YA, Hayase S, Iwamoto R et al (2024) Simultaneous extraction and detection of DNA and RNA from viruses, prokaryotes, and eukaryotes in wastewater using a modified COPMAN. Sci Total Environ 907:167866. 10.1016/j.scitotenv.2023.167866
43. Hermans N, de Zwaan R, Mulder A et al (2024) Mycobacterium tuberculosis complex sample processing by mechanical lysis, an essential step for reliable whole genome sequencing. J Microbiol Methods 227:107053. 10.1016/j.mimet.2024.107053
44. Simpson RJ (2010) Homogenization of mammalian tissue. *Cold Spring Harb Protoc*. ; 7: pdb.prot5455. 10.1101/pdb.prot5455
45. Tarazona Carrillo K, Nam SL, de la Mata AP et al (2023) Optimization of fecal sample homogenization for untargeted metabolomics. Metabolomics 19:74. 10.1007/s11306-023-02036-4

Figures

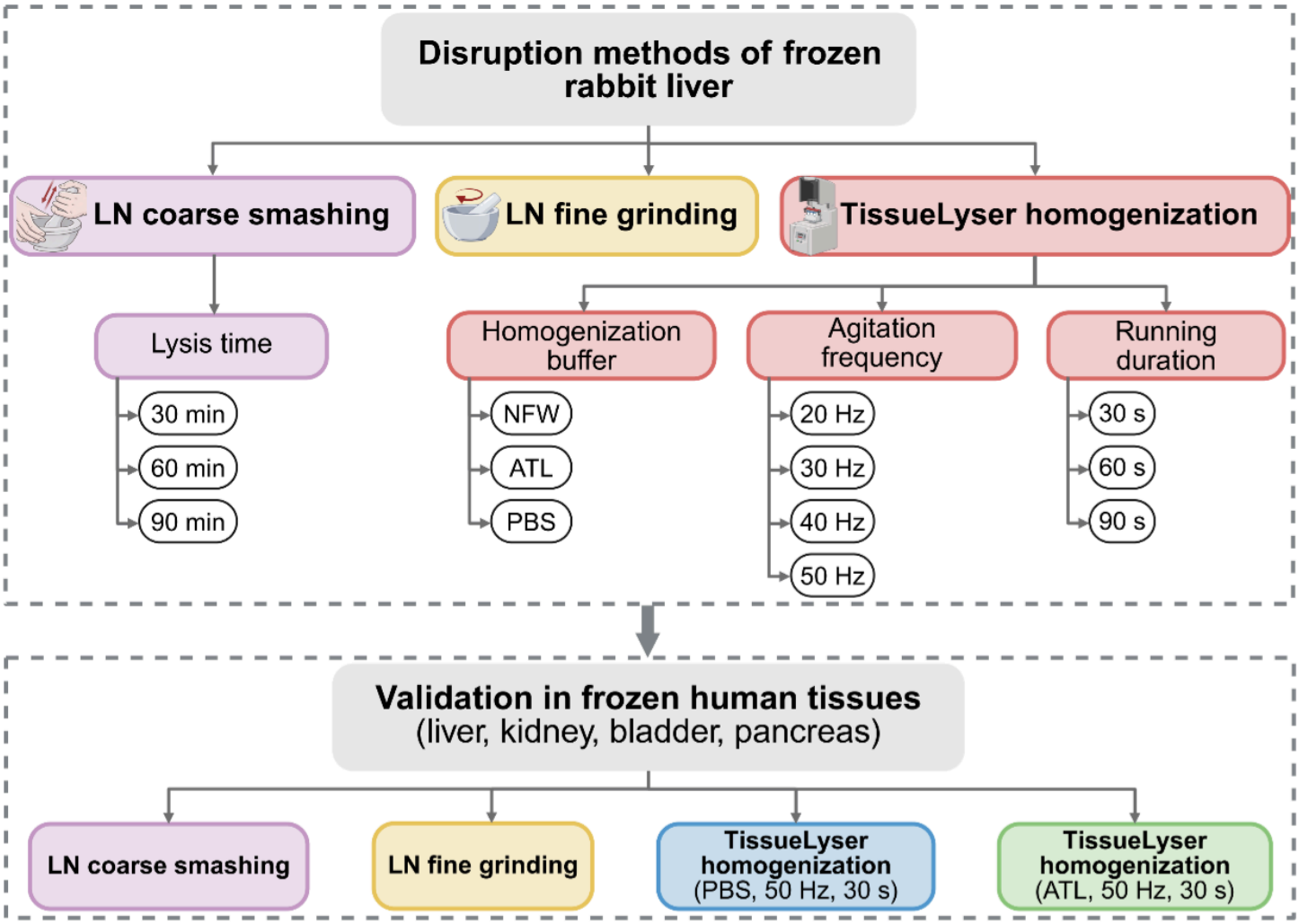


Figure 1

Overall schematic diagram.

LN, liquid nitrogen; NFW, nuclease-free water; PBS, phosphate buffered saline.

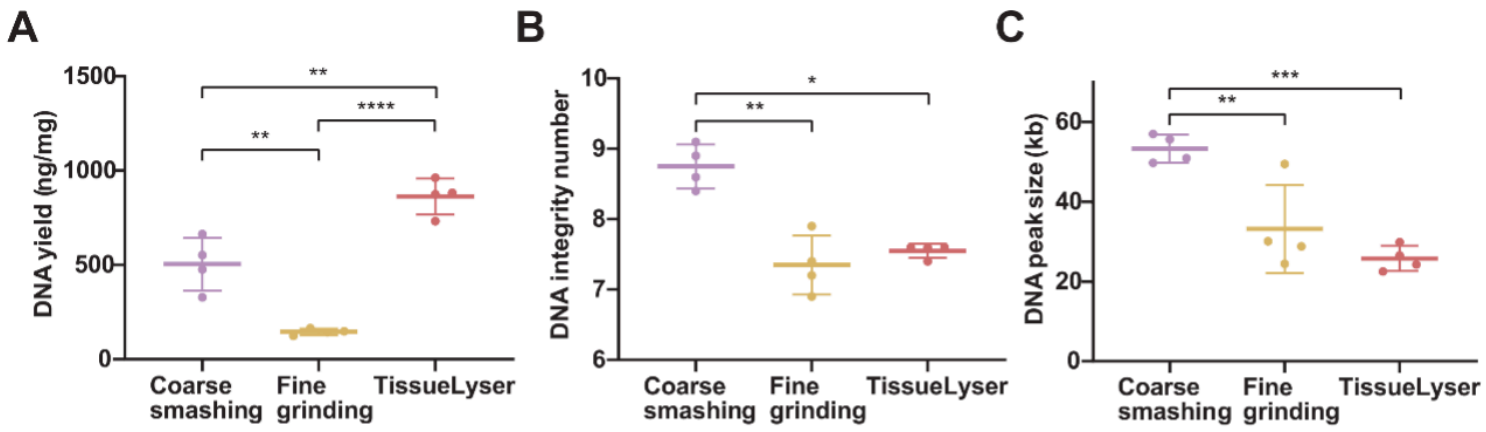


Figure 2

Evaluation of DNA in frozen rabbit liver subjected to different disruption methods.

(A) DNA yields, ng DNA/mg tissue. (B) DNA integrity number values. (C) DNA peak sizes. *, p values < 0.05; **, p values < 0.01; ***, p values < 0.001; ****, p values < 0.0001. Ordinary one-way ANOVA (A, C) and the Kruskal-Wallis test (B).

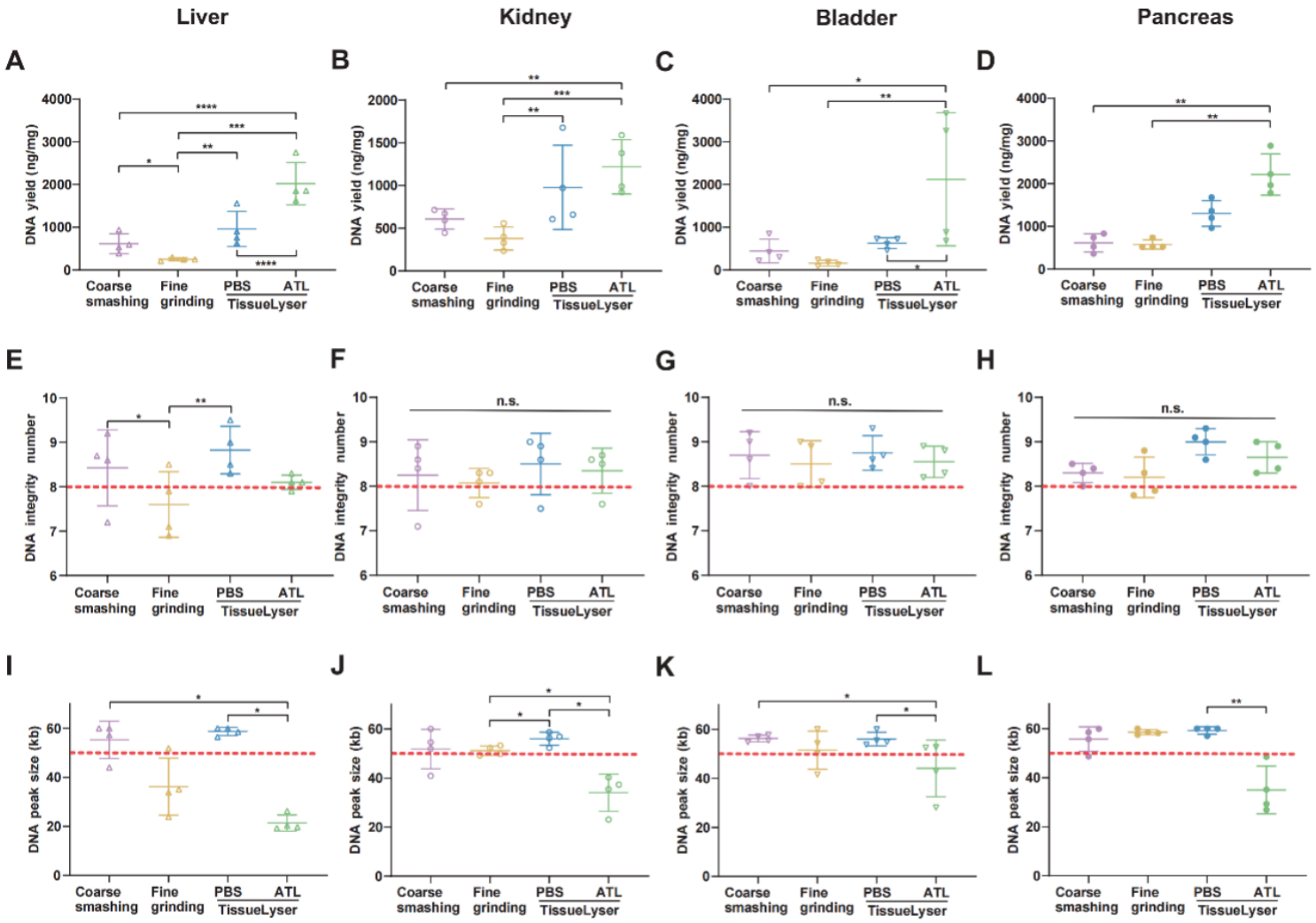


Figure 3

Evaluation of DNA in four types of frozen human tissues.

DNA yields of the liver (A), kidney (B), bladder (C), and pancreas (D), ng DNA/mg tissue. DNA integrity number values of the liver (E), kidney (F), bladder (G), and pancreas (H). DNA peak sizes of the liver (I), kidney (J), bladder (K), and pancreas (L). PBS, phosphate buffered saline. △, liver; ○, kidney; ▽, bladder; ●, pancreas. n.s., non-significant; *, p values < 0.05; **, p values < 0.01; ***, p values < 0.001; ****, p values < 0.0001. Repeated measures one-way ANOVA (A, B, C, E, F, G, H, J, K) and the Friedman test (D, I, L).

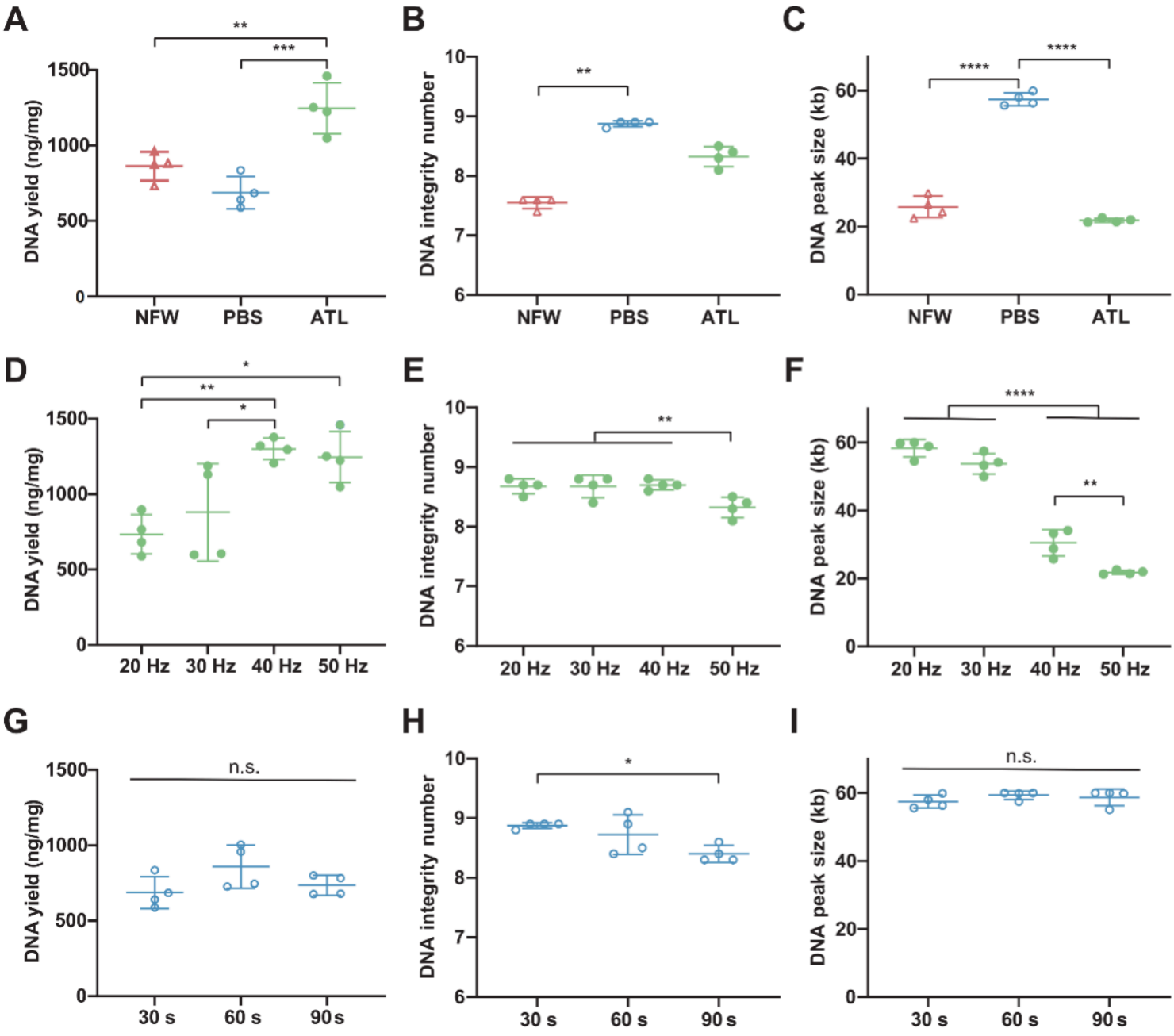


Figure 4

Evaluation of DNA in frozen rabbit liver under different homogenization parameters via a TissueLyser.

DNA yields (A), DNA integrity number values (B), and DNA peak sizes (C) of DNA under three homogenization buffers. DNA yields (D), DNA integrity number values (E), and DNA peak sizes (F) of DNA in the homogenization group with ATL buffer under four agitation frequencies. DNA yields (G), DNA integrity number values (H), and DNA peak sizes (I) of DNA in the homogenization group with PBS under three running durations. DNA yields, ng DNA/mg tissue. NFW, nuclease-free water; PBS, phosphate buffered saline. △, NFW; ○, PBS; ●, ATL. n.s., non-significant, *, p values < 0.05; **, p values < 0.01; ***, p values < 0.001; ****, p values < 0.0001. Ordinary one-way ANOVA (A, C, D, E, F, G) and the Kruskal-Wallis test (B, H, I).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Suppl.docx