APPLICATION NOTE #201801

High throughput RNA extraction from *Mus musculus* tissues using the Omni Tissue RNA kit and the Bead Ruptor 12

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Introduction

The extraction and isolation of RNA is an integral part of downstream analyses such as RT-PCR, RT-qPCR, Northern blotting, and cDNA library construction. The importance of using pure, intact RNA for these processes is well documented and a critical part of downstream analysis success. It is well known that RNA is sensitive to degradation due to mechanical shear, temperature, storage conditions and freeze-thawing. Furthermore, RNA is highly susceptible to RNAse degradation following release of nucleases during the tissue disaggregation process. Thus, proper sample handling during the homogenization process is crucial when performing an RNA based assay.

The Omni Tissue RNA Purification Kit supports rapid RNA purification with reproducible RNA yields and integrity. Extraction is based on bead based tissue disruption followed by RNA purification on silica spin columns. Herein, we demonstrate the performance of the Omni Tissue RNA Purification Kit for RNA purification from multiple murine tissues following disaggregation on the Bead Ruptor 12 Bead Mill Homogenizer. RNA integrity and yield was compared to tissues dissociated through cryomilling in a mortar & pestle under liquid nitrogen.

Materials and Methods

Equipment

- **Bead Ruptor 12** (Cat# 19-050A)
- **Omni Tissue RNA Purification Kit (**Cat# 26-010B)
- **Ambion DNase I** (Cat# AM2222, Thermo Fisher)
- **Agilent RNA 6000 Nano Kit** (Cat# 655097, Agilent)

Bead Ruptor 12

Tissue RNA Purification Mini Kit

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RNA Extraction

Murine tissues (kidney, liver, heart, and lung) were freshly harvested from CO2 euthanized BALB/c mice. 25 mg of each tissue was placed in a prechilled reinforced 2 mL tube containing 6 x 2.8 mm ceramic beads and 500 µL of prechilled RLB buffer containing 2-mercaptoethanol. Samples were disaggregated on the Bead Ruptor 12 at 2.9 m/s for 20 seconds. As a control, 25 mg of each tissue was cryomilling in prechilled mortar & pestle under liquid nitrogen. The powdered tissue was then transferred to a prechilled 1.5 mL microcentrifuge tube including 500 µL of prechilled RLB containing 2-mercaptoethanol. The Omni Tissue RNA Purification Kit protocol was followed henceforth with the following exceptions: all steps were performed on ice, all centrifugation steps were performed at 4°C, an optional on-column DNase treatment step was performed using a total of 12 Units, at room temperature for 15 minutes. RNA was eluted in 100 µL DEPC water.

RNA Quantification and Integrity Analysis

1 µL of purified RNA was analyzed, in triplicate, on a Agilent 2100 Bioanalyzer in a RNA 6000 Nano kit chip, as per the manufactures protocol. Gel images, electrophoretograms and RNA integrity numbers (RIN's) were visualized and analyzed on the 2100 Bioanalyzer Expert software (Agilent).

Results

Herein, we evaluated tissue RNA extraction on the Bead Ruptor 12 combined with RNA purification using the Omni Tissue RNA Purification Kit to extract high quality RNA as compared to a traditional cryomilling method. Table 1 shows the average RNA yield for each tissue type processed on the Bead Ruptor with heart tissue exhibiting the lowest yields at 105.7 ng/µL and liver yielding RNA at concentrations of 733.3 ng/µL. The bead milling approach produced RNA yields in excess of 2X compared to the cryomilling method for liver, heart and lung tissues. RNA integrity numbers (RIN) were comparable for both the bead mill and cryomilling methods. Based on gel analysis, the RNA was of good quality with prominent 28S and 18S bands (Figures 1-2). There was no high molecular weight shearing visible in the electrophoretograms indicating the RNA was intact and ready for further downstream analyses (Figures 1-2).

Tissue	Average Yield	Average RN
Kidney (mortar & pestle)	192.3 ng/µL	7.7
Liver (mortar & pestle)	167.3 ng/µL	7.5
Heart (mortar & pestle)	26.7 ng/µL	7.6
Lung (mortar & pestle)	44.7 ng/µL	7.2
Kidney (Bead Ruptor)	186 ng/µL	8.6
Liver (Bead Ruptor)	733.3 ng/µL	7.1
Heart (Bead Ruptor)	105.7 ng/µL	8.9
Lung (Bead Ruptor)	106 ng/µL	7.9

Conclusion

The Bead Ruptor was able to extract high quality RNA that was efficiently purified through the Omni Tissue RNA Kit. RNA yields were higher with the bead milling approach and RNA integrity was maintained at acceptable levels when compared to cryomilling.

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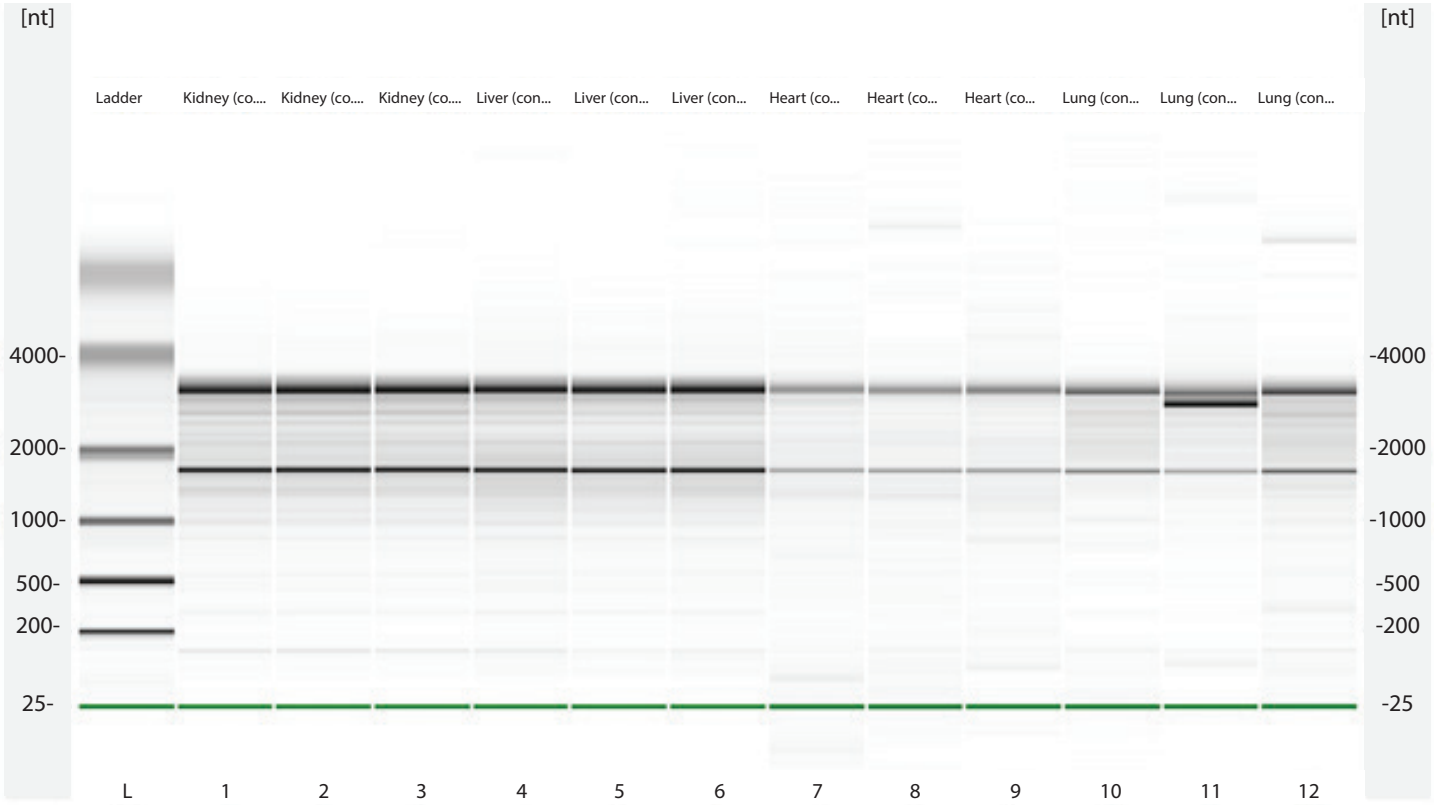
Figure 1 : RNA extracted by cryomilling on a liquid nitrogen cooled mortar pestle and purified using the Omni Tissue RNA Purification Kit. RNA analyzed on the Agilent 2100 Bioanalyzer.



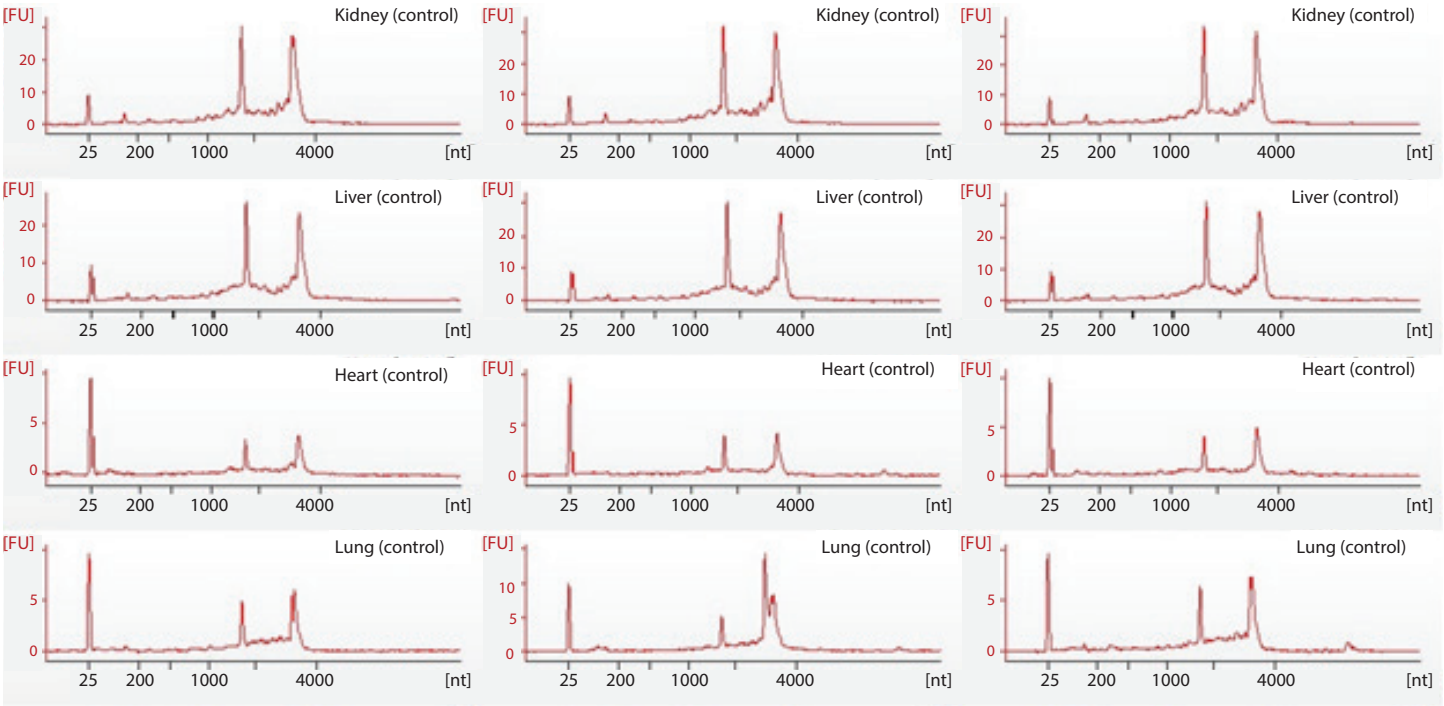
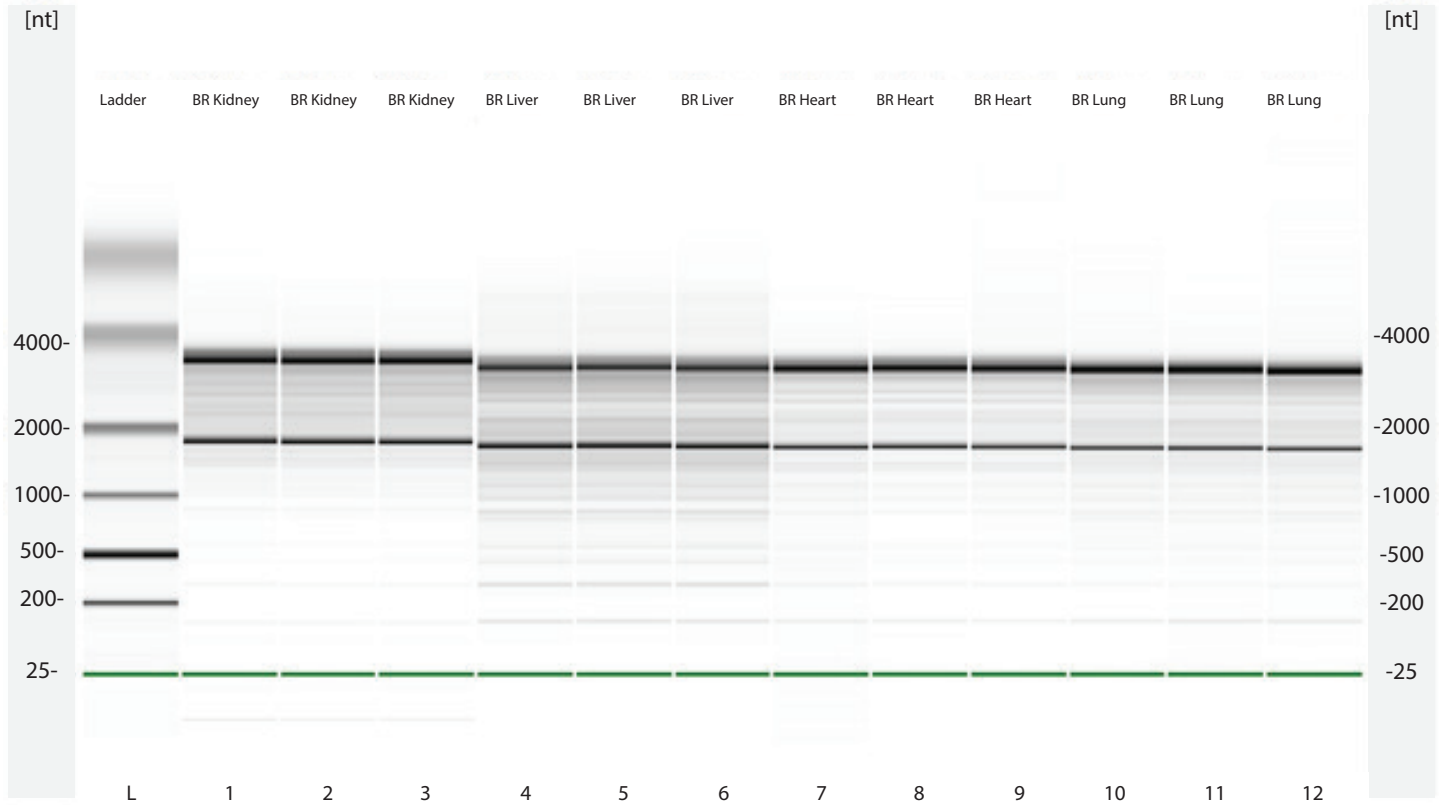
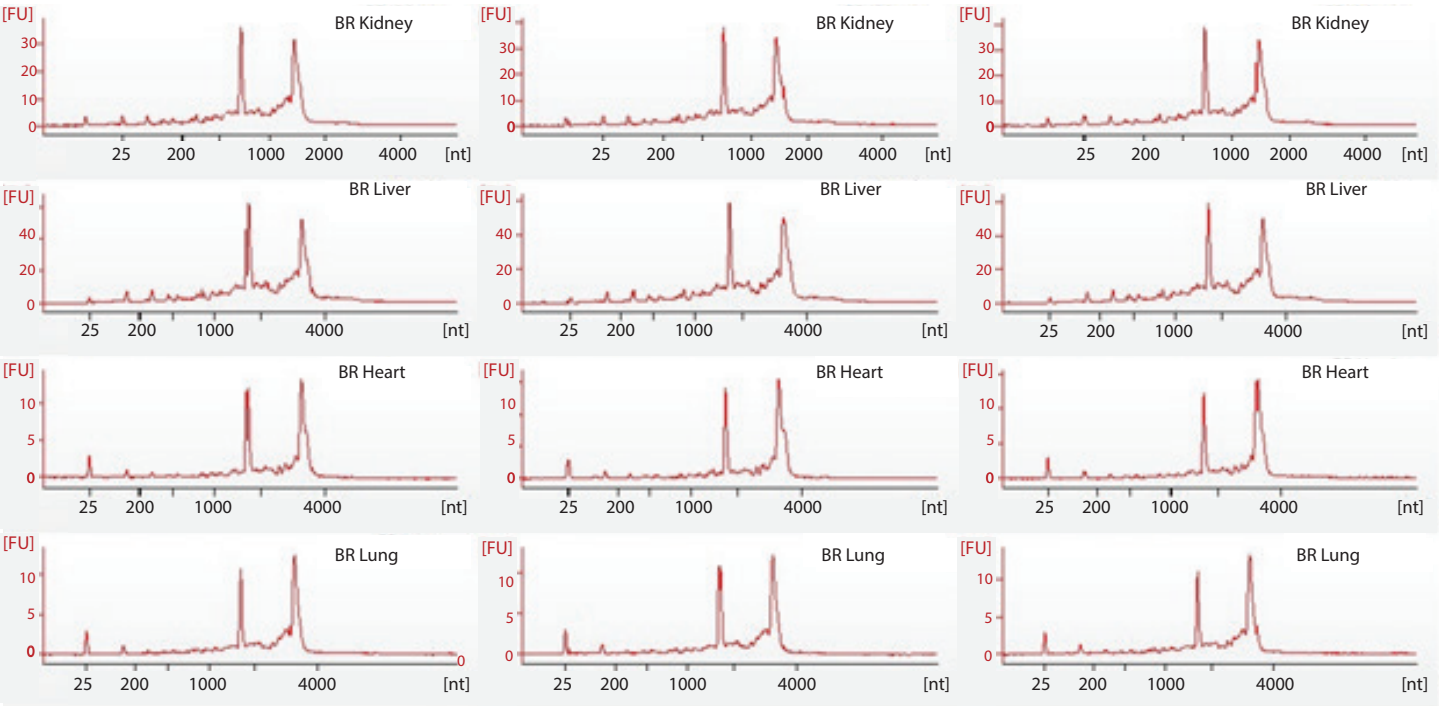


Figure 2 : RNA extracted by bead milling on the Bead Ruptor 12 and purified using the Omni Tissue RNA Purification Kit. RNA analyzed on the Agilent 2100 Bioanalyzer.





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