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Nucleic Acid Extraction from *Mus musculus* Tissues Using the Bead Ruptor Elite and Stainless Steel Lysing/Grinding Tubes

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Background

The use of animal models in biomedical research often relies on fresh tissue samples for the evaluation and use of DNA and RNA in downstream molecular applications. The process of nucleic acid extraction from fresh tissues is implemented prior to molecular applications like qPCR, RT-PCR and Next Gen Sequencing in these studies. Extracted nucleic acids can be utilized in identifying genes of interest relating to disease pathology, pharmaceutical and genomic modification analysis. For those reasons, it is crucial that high quality DNA and RNA is obtained during the extraction process to ensure the efficacy and accuracy of experiments and results.

To retrieve nucleic acid from fresh tissue samples, the samples must be disrupted through a process such as mechanical homogenization. Through mechanical homogenization the sample anatomy is disrupted and many of the cells are lysed to expose metabolites and nucleic acids contained within the cells. This lysis step can be difficult depending on tissue consistency and methodology used. Commonly used lysis methods including enzymatic digestion or manual homogenization can be time consuming and inadequate processes.

Implementing the Omni Bead Ruptor Elite for the homogenization and lysis steps provides an effective approach to disrupt tissue samples, while decreasing total processing time. Additionally, Omni Stainless Steel Lysing/Grinding Tubes and Grinding Cylinder provides durability to withstand cryogenic sample preparation along with homogenization of your toughest samples.

Herein, we evaluate the capability of Omni Stainless Steel Lysing/Grinding Tube, along with the Bead Ruptor Elite, to homogenize tissue samples for isolation of pure and intact nucleic acid.

Materials and Methods

Materials

- Bead Ruptor Elite (PN 19-040E)
- 2 mL Tube Carriage Kit (PN 19-010-310)
- Stainless Steel Lysing/Grinding Tube with Grinding Cylinder (PN 19-6001)
- Omni Tissue DNA Kit (PN 26-007)
- Omni Tissue RNA Kit (PN 26-010)

Bead Ruptor Elite

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Methods

Tissue DNA Extaction

30 mg of liver tissue was added to a Stainless Steel Lysing/Grinding Tube with Grinding Cylinder (Omni Cat. No. 19-6001). After closing the tube containing tissue sample, the entire tube was submerged in LN_2 for 15 seconds. The tube was then immediately processed on the Bead Ruptor Elite (Omni Cat. No. 19-040E) for 20 seconds at 3.9 m/s (Table 1). Powdered homogenate was then transferred to a 1.5 mL microcentrifuge tube containing 200 µl of DNA Lysis Buffer (DLB) (Omni Tissue DNA Kit, Cat No. 26-007) Tissue DNA extraction was then carried out per kit instructions (Omni Cat. No. 26-007).

Following DNA extraction, eluted DNA concentration and integrity were determined by A_{260}/A_{280} spectrophotometry. After spectrophotometric analysis, approximately 100 ng of eluted DNA was electrophoresed on a 2.0% agarose gel in Tris-Borate-EDTA (TBE). After ethidium bromide staining, DNA bands were visualized on a gel documentation system following standard procedures.

Tissue RNA Extraction

30 mg of liver tissue was added to a Stainless Steel Lysing/Grinding Tube with Grinding Cylinder (Omni Cat. No. 19-6001). After closing the tube containing tissue sample, the entire tube was submerged in LN_2 for 15 seconds. The tube was then immediately processed on the Bead Ruptor Elite for 20 seconds at 3.9 m/s (Table 1). Powdered homogenate was transferred to a 1.5 mL microcentrifuge tube containing 700 µl pre-chilled RNA Lysis Buffer (RLB) (Omni Tissue RNA Kit, Cat No. 26-010). RLB was prepared with β-mercaptoethanol (βME), per kit instructions. Tissue RNA extraction was then carried out per kit instructions (Omni Cat. No. 26-010).

Following RNA extraction, eluted RNA concentration and integrity were determined by A_{260}/A_{280} spectrophotometry. After spectrophotometric analysis, eluted RNA was further analyzed using the 2100 Bioanalyzer Instrument (Agilent, Cat No. G2939B) with the Agilent RNA 6000 Nano Kit (Agilent, Cat No. 5067-1511) using the Eukaryote Total RNA Nano Assay. Standard kit protocol was followed when preparing samples for analysis on the Bioanalyzer. RNA Integrity Number (RIN) and gel electropherogram were obtained from the instrument readout upon protocol completion.

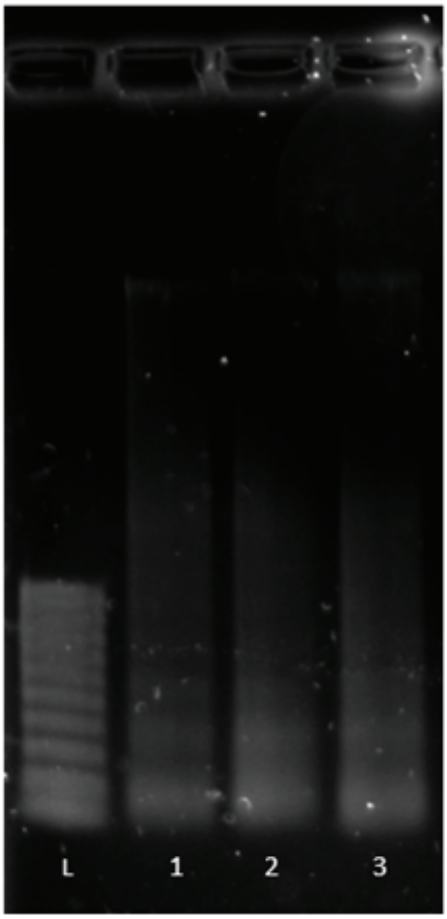
Results

Homogenization of 30 mg of liver tissue on the Bead Ruptor Elite using the Stainless Steel Lysing/Grinding tube with Grinding Cylinder, yielded an average concentration of 156 ng/µl of DNA and 423 ng/µl of RNA (Table 2, Table 3). DNA had an average A_{260}/A_{280} ratio of 1.9, whilst RNA had an average A_{260}/A_{280} ratio of 2.07, with an average RIN value of 8.4 (Table 2, Table 3).

Note that a A_{260}/A_{280} ratio of 2.0 is considered pure nucleic acid whilst a RIN value of >8 corresponds to majority intact RNA sample and indicate suitability for transcriptome analysis. Furthermore, RIN values are assigned based on an algorithm that measures the ratio of 18S to 28S ribosomal subunits and predicts RNA intactness or degradation, accordingly. Finally, after separation by gel electrophoresis, the eluted nucleic acid was shown to be high molecular weight and exhibited minimal signs of degradation (Figure 1, Figure 2).

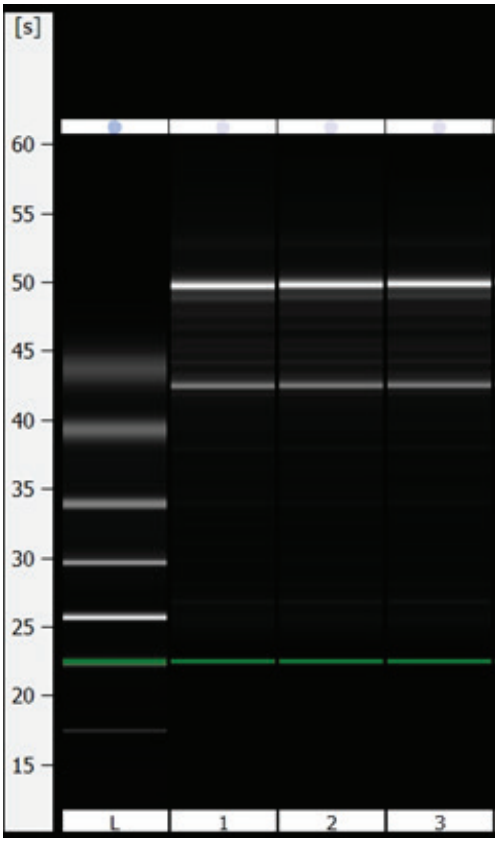
Sample Type	Sample Weight (mg)	Speed (m/s)	Time (sec)	Cycles	Dwell Time (sec)
Tissue	30	3.9	20	1	N/A

Table 1: Bead Ruptor Elite Sample Homogenization Summary



Sample	DNA Concentration (ng/µl)	A_{260}/A_{280}
Liver 1	128.7	1.9
Liver 2	188.2	1.9
Liver 3	162.0	1.8

Figure 1, Table 2. Gel Electrophoresis & Spectrophotometric data obtained from Tissue DNA eluate showing concentration and A_{260}/A_{280} values.



Sample	DNA Concentration (ng/µl)	A_{260}/A_{280}	RIN Value
Liver 1	429.3	2.06	8.2
Liver 2	429.7	2.07	8.6
Liver 3	410.8	2.09	8.5

Figure 2, Table 3. Gel Electrophoresis & Spectrophotometric data obtained from Tissue RNA eluate showing concentration and A_{260}/A_{280} values.

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Conclusions

The data presented show that the Stainless Steel Lysing/Grinding Tube with Grinding Cylinder is successful in generating a complete homogenate that is suitable for downstream analysis. After nucleic acid purification, A_{260}/A_{280} spectrophotometric analysis & gel visualization show that high concentrations of pure nucleic acid were obtained.

In addition to tissue sample preparation, the Stainless Steel Lysing/Grinding tube provides users with a durable alternative for use with hard and fibrous tissue samples and can withstand cryogenic sample preparation with LN_2 before processing on the Bead Ruptor Elite.

Ordering Information

Contact Your Local OMNI International Representative for:

- Bead Ruptor Elite (PN 19-040E)
- 2 mL Tube Carriage Kit (PN 19-010-310)
- Stainless Steel Lysing/Grinding Tube with Grinding Cylinder (PN 19-6001)
- Omni Tissue DNA Kit (PN 26-007)
- Omni Tissue RNA Kit (PN 26-010)

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