APPLICATION NOTE

Nucleic acid extraction from *Rattus norvegicus* liver using the Omni Bead Ruptor Elite bead mill homogenizer for sample preparation.

Summary

Animal tissue is a widely used model for analysis of nucleic acids in downstream processes like PCR or next-generation sequencing (NGS) to allow for disease screening, gene identification and other relevant assays. Upstream of PCR or NGS, a nucleic acid extraction step is required which necessitates the input of lysed tissue cells. Traditional lysis methods involve enzymatic digestion, manual dissociation, or a combination of both, which can be time consuming and labor intensive processes for larger tissue samples.

The Omni Bead Ruptor Elite™ bead mill homogenizer offers scientists a sample preparation workflow allowing for homogenization of large tissue samples in a quick and efficient process, yielding a homogenate conducive to downstream nucleic acid isolation, purification, and analysis.

Herein, we evaluate the capability of the Omni Bead Ruptor Elite bead mill homogenizer in homogenization of *Rattus norvegicus* liver for downstream nucleic acid extraction and evaluation.

Authors

Gabriella Ryan, M.S.
Rodney J Nash, Ph.D.

Revvity, Inc.

Omni Bead Ruptor Elite bead mill homogenizer

Materials and methods

Equipment

- Omni Bead Ruptor Elite bead mill homogenizer (Cat # 19-042E)
- Omni Bead Ruptor Elite 30 mL tube carriage & finger plate (Cat # 19-376-HT)
- Hard Tissue Homogenizing Mix 2.8 mm Ceramic (30 mL) (Cat # 19-6358)

Procedure

Tissue DNA Extraction

For sample preparation, 10 g of liver tissue was added to a 30 mL Hard Tissue Homogenizing Mix tube (Cat #19-6358) along with 6 mL commercially available tissue lysis buffer. Samples were weighed out with a tolerance ± 10 mg. The liver samples were homogenized on the Omni Bead Ruptor Elite bead mill homogenizer at 4.5 m/s for 2 cycles of 20 seconds with a 10 second dwell (Table 1). After processing, 200 µL of homogenate was transferred to a 1.5 mL microcentrifuge tube (USA Scientific, Cat # 1615-5510) along with 25 µL of Proteinase K solution. Samples were incubated at 55 °C for 1 hour. During incubation, samples were vortexed for 10 seconds after 20 minutes and 40 minutes had elapsed. After incubation, 8 µL of RNase A (100 mg/mL) was added to each sample tube and allowed to incubate at room temperature (22 °C) for 2 minutes, according to instructions in commercially available tissue DNA extraction kit. After RNase A incubation, the remainder of the DNA extraction was carried out using a commercially available tissue DNA extraction kit per manufacturer's instructions.

After analysis of concentration and integrity, approximately 100 ng of DNA was separated on a 1.5 % agarose gel stained with ethidium bromide. DNA bands were visualized on a gel documentation system following standard procedures.

Tissue RNA Extraction

For sample preparation, 10 g of liver tissue was added to a 30 mL Hard Tissue Homogenizing Mix tube (Cat # 19-6358) along with 6 mL commercially available tissue lysis buffer. The liver samples were homogenized on the Omni Bead Ruptor Elite bead mill homogenizer at 4.5 m/s for 2 cycles of 20 seconds with a 10 second dwell (Table 1). After processing, 200 µL of homogenate was transferred to a 1.5 mL microcentrifuge tube (USA Scientific, Cat # 1615-5510). After sample transfer, the remainder of the RNA extraction was carried out using a commercially available tissue RNA extraction kit following manufacturer's instructions.

Samples were eluted with 100 µL of Nuclease-Free water. RNA concentration and integrity were determined by A_{260}/A_{280} spectrophotometry.

After analysis of concentration and integrity, eluted RNA was also analyzed using the 2100 Bioanalyzer® Instrument (Agilent, Cat # G2939B) with the RNA 600 Nano Kit (Agilent, Cat # 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 and gel electropherogram were obtained from the instrument readout upon protocol completion.

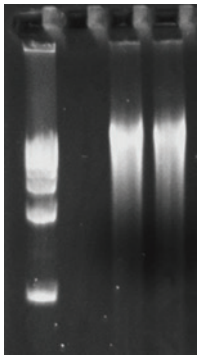
Table 1: Omni Bead Ruptor Elite bead mill homogenizer homogenization summary.

Sample type	Speed (m/s)	Time (sec)	Cycles	Dwell time (sec)
Liver	4.5 m/s	20 sec	2	10 sec

Results

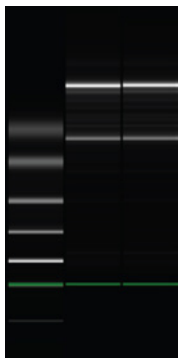
Homogenization of 10 g of rat liver tissue on the Omni Bead Ruptor Elite bead mill homogenizer yielded an average concentration of 24 ng/µL of DNA and 21 ng/µL of RNA (Table 2, Table 3). Eluted DNA had an average A_{260}/A_{280} ratio of 1.90 (Table 2). Eluted RNA had an average A_{260}/A_{280} ratio of 1.82 and an average RIN value of 8.6 (Table 3), indicating that the extracted nucleic acid is intact and free of contaminants.

An A_{260}/A_{280} ratio greater than 1.8 corresponds to >90% pure nucleic acid whilst a RIN value of >8.0 corresponds to majority intact RNA sample and indicates 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. Ultimately, after separation via gel electrophoresis, eluted nucleic acid demonstrated high molecular weight when compared to the molecular ruler (Figure 1, Figure 2).



Sample name	DNA concentration (ng/µL)	A_{260}/A_{280}
Liver DNA 1	25.6	1.89
Liver DNA 2	23.9	1.92
Liver DNA 3	23.8	1.91

Figure 1, Table 2: Gel electropherogram and spectrophotometric data obtained from tissue DNA eluate.



Sample name	RNA concentration (ng/µL)	A_{260}/A_{280}	RIN value
Liver RNA 1	21.4	1.79	8.6
Liver RNA 2	21.1	1.84	8.6
Liver RNA 3	21.5	1.83	8.7

Figure 2, Table 3: Gel electropherogram, spectrophotometric data, and RIN value data obtained from tissue RNA eluate.

Conclusions

The Omni Bead Ruptor Elite bead mill homogenizer and 30 mL Hard Tissue Homogenization tube provides a robust solution for front-end sample preparation of large tissue samples in less than 60 seconds and eliminates the need for manual homogenization techniques. The resulting homogenate is suitable for extraction and purification of nucleic acids that are intact and suitable for further analysis.

1552214 (202104)