APPLICATION NOTE

Purification and evaluation of total RNA from porcine skin on the Omni Bead Ruptor Elite bead mill homogenizer.

Introduction

RNA, specifically mRNA, is the intermediary between DNA and protein. By understanding the levels of RNA found in cells, scientists can better comprehend gene expression and regulation. Therefore, the extraction of high-quality RNA is the first and most vital step in performing many molecular techniques found in molecular biology, genetics, biochemistry and microbiological applications. While DNA can survive for extended periods of time and is relatively stable, RNA is typically short-lived and extremely temperature sensitive. Due to RNA's disposition to degenerate, extracting RNA from tissues requires a method that will isolate purified RNA while also minimizing degradation (1).

Hard samples such as skin and bone present a significant challenge for the extraction of RNA. Common methods require freezing the tissue in liquid nitrogen or dry ice and then pulverizing with a mortar and pestle. While freezing gives the researcher more control over their disruption conditions, it is typically a very involved and time-consuming process (2). Bead mill homogenizers such as the Omni Bead Ruptor Elite™ bead mill homogenizer can quickly and efficiently disrupt tough samples for the extraction of RNA. By selecting the most efficient bead media, high-quality RNA can be extracted for various downstream analyses.

Herein, we evaluate the potential for the extraction of RNA from porcine (*Sus scrofa*) skin on the Omni Bead Ruptor Elite bead mill homogenizer. The extraction efficiency and analyte integrity were evaluated via RT-PCR.

For research use only. Not for use in diagnostic procedures.

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 7 mL Tube Carriage (Cat # 19-374)
- 7 mL Reinforced Tubes with Screw Caps (Cat # 19-651)
- 6.5 mm Ceramic Bulk Beads (Cat # 19-682)

RNA extraction and separation

A 10 mm^2 section of porcine skin was excised from picnic shoulder obtained from a local grocer. The section was then added to a 7 mL screw cap tube with a single 6.5 mm ceramic bead. 2.5 mL of ice-cold Trizol® was added and processed on the Omni Bead Ruptor Elite bead mill homogenizer for two 30 second cycles at 5.5 m/s. The sample was placed in an ice water bath for 1 minute between each cycle.

1 mL of the lysate was removed, placed in an ice-cold 1.5 mL microcentrifuge tube and was centrifuged at 12,000 x g for 10 minutes at 4 °C. The supernatant was placed in an ice-cold 1.5 mL microcentrifuge tube and incubated in an ice water bath for 5 minutes. 200 µL of ice-cold chloroform was added; vigorously shaken by hand and incubated on ice for 2 minutes. The sample was centrifuged at 12,000 x g for 15 minutes at 4 °C and the upper aqueous layer was placed in an ice-cold microcentrifuge tube.

500 µL of ice-cold isopropyl alcohol was added to the solution; gently inverted several times and placed on ice for 10 minutes. The sample was centrifuged at 12,000 x g for 10 minutes at 4 °C. The supernatant was removed and the pellet was washed with 1 mL of 100 % ethanol. The suspended pellet was centrifuged at 9,000 x g for 5 minutes at 4 °C; the supernatant was removed and the pellet was air-dried at room temperature for 15 minutes. The RNA pellet was gently suspended in 40 µL of DEPC water by pipetting. 1 µL of purified RNA was quantified on a NanoDrop Spectrophotometer (Thermo Fisher Scientific) to determine RNA yields and A_{260}/A_{280} ratios.

The size and quality of the isolated RNA was determined by agarose gel electrophoresis. Approximately 600 ng of RNA was mixed with 2x RNA Loading Dye (New England BioLabs Cat#B0362S) and heated at 70 °C for 10 minutes with subsequent cooling in an ice bath for 2 minutes. The sample was separated in duplicate on a 1.2 % TBE agarose gel at 140 V and stained with ethidium bromide (Bio-Rad Cat#161-0433). The gel was washed with DD H_2O and visualized on a GelDoc EZ System (Bio-Rad).

RT-PCR assay and fragment analysis

Specific primers were chosen to amplify a region of the 18S (*Sus scrofa*) ribosome using published sequences (Table 1).

Table 1. PCR Primers

Name	Sequence (5′-3′)	Reference
18S-F	GGCCTCACTAAACCATCCAA	3
8S-R	TAGAGGGACAAGTGGCGTTC	3

One step amplification was performed in duplicate using the SuperScript III One-Step RT-PCR System with Platinum Taq DNA polymerase (Invitrogen), 0.5 µM of each primer and 1 ng of purified RNA. Amplification was carried out using the T100 Thermal Cycler by Bio-Rad per the settings in table 2.

Table 2. PCR Program

	Temperature	Time
cDNA synthesis	55 °C	30 minutes
Pre-denaturation	94 °C	2 minutes
35 Cycles	94 °C	30 seconds
	58 °C	1 minute
	72 °C	30 seconds
Final extension	72 °C	2 minutes

PCR products were analyzed on a 2 % agarose gel and stained using ethidium bromide. Fragments were visualized and analyzed on Bio-Rad's Gel Doc EZ system.

Results

In this study, we investigated the ability of the Omni Bead Ruptor Elite bead mill homogenizer to process porcine skin for the extraction of high-quality RNA for RT-PCR. Excessive heat can be a concern when processing tough samples with small bead media. The energy released due to the frequent impacts of multiple small beads can increase intratube temperatures dramatically. By utilizing a single large bead, there are less impacts, but each impact imparts more force causing complete dissociation. In this particular assay, porcine skin was processed with a 6.5 mm ceramic bead. Total RNA was extracted and the 18S rRNA gene was detected via end-point RT-PCR.

RNA quantification and separation

After extraction, the purified RNA yields were quantified by spectrophotometry. The average RNA yield was 604 ng/µL. The absorbance A_{260}/A_{280} ratio was 1.9 indicating that there was no significant amount of contamination. The RNA was then separated and visualized by gel electrophoresis. Based on the gel analysis, the extracted RNA was of good quality with a sharp and clear 28S and 18S rRNA band. The image indicated that there was no high-molecular weight shearing indicating that the RNA is intact and ready for further RT-PCR analysis.

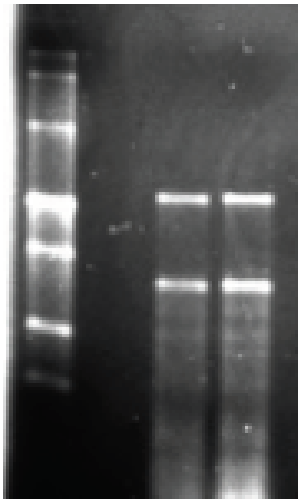

Figure 1. Purified RNA.
Lane 1: RNA Ladder, Lane 3-4: Porcine RNA

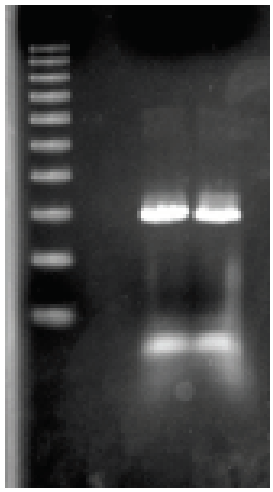

Figure 2. 18S rRNA Detection.
Lane 1: 100 bp Ladder, Lane 3-4: Porcine 18S fragment

RT-PCR detection

To assess the quality of the RNA, RT-PCR was performed to amplify a specific region from the 18S rRNA gene. The fragments were analyzed on a 2 % agarose gel. The fragment size from the amplicon is shown to be 295 bp. As indicated, the skin sample contained a band at the expected base pair length.

Conclusion

Bead mill homogenizers such as the Omni Bead Ruptor Elite bead mill homogenizer are capable of disrupting skin tissue for RNA extraction with subsequent PCR analysis. The Omni Bead Ruptor Elite bead mill homogenizer is excellent at processing skin samples for the extraction of total RNA in excess of 600 ng/µL. By utilizing a 6.5 mm ceramic bead the RNA was shown to be of excellent quality for RT-PCR analysis. The 6.5 mm ceramic bead allows for tough samples to be gently processed without the generation of excessive heat assuring high yields and analyte integrity.

References

1. Grigoryev, Y. (2013, March 19). Isolation of total RNA, including microRNA, from cells and tissues [Web log post]. Retrieved from: http://bitesizebio.com/13529/isolation-of-total-rna-including-microrna-from-cells-and-tissues/

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3. Méndez, V., Avelar, E., Morales, A., Cervantes, M., Araiza, A., & González, D. (2011). Methodology A rapid protocol for purification of total RNA for tissues collected from pigs at a slaughterhouse. Genetics and Molecular Research Genet. Mol. Res., 10(4), 3251-3255. doi:10.4238/2011.december.22.3

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