APPLICATION NOTE #20173

DNA Extraction from Turbatrix aceti using the Bead Ruptor 4 and the Omni Nucelic Acid Purification Kit

Brandon Easparro, Omni International, Inc.

Introduction

Nematodes are roundworms that are numerous in species. With such a variety, they have nearly adapted to every kind of environment ranging from aqueous to soil environments and polar to tropical regions. More than half of nematode species are parasitic causing such conditions as trichinosis in humans, heart worm disease in domestic pets, and a variety of crop losses. Although there is such an abundance of parasitic nematodes, some are non-parasitic and are actually used as model organisms. One in particular, Turbatrix aceti, or the vinegar eel, has been used in DNA damage studies to understand the cause of aging1. T. aceti is one of the easiest species of nematodes to maintain since they can be grown in unpasteurized vinegar. T. aceti feeds off mother of vinegar, which is a microbial culture consisting of cellulose and acetic acid bacteria that forms in fermenting alcoholic liquids. To better understand the molecular processes that occur in the vinegar eel, an efficient DNA extraction method is important.

Herein, we demonstrate a DNA extraction protocol using the Omni Yeast DNA Purification Kit and the Bead Ruptor 4 bead mill homogenizer. Extraction efficiency and analyte integrity were evaluated.

Materials and Methods

Equipment

- Bead Ruptor 4 (Cat #: 25-010)
- Omni Yeast DNA Purification Kit (Cat #: 26-009B)

Bead Ruptor 4

Acquisition and Growth of T. aceti

Vinegar eels were obtained from Niles Biological Inc. (30 mL) and transferred to a stock of pasteurized apple cider vinegar that was unopened for 5 days prior to transfer. Eels were allowed to grow for two weeks prior to DNA extraction.

DNA Extraction and Separation

3 mL of culture was transferred to two 15 mL tubes and centrifuged at 10,000 x g for 10 minutes. Both samples' supernatant was removed and pellets were resuspended in 3 mL of TE buffer (10 mM Tris, 0.1 mM EDTA). Samples were centrifuged again at 10,000 x g for 10 minutes, twice. After centrifugation, pellets were resuspended in 220 µL of DLB buffer. Contents were transferred to 2 mL bead tubes containing 0.5 mm glass beads. 10 µL of Antifoam was added and the samples were disrupted on the Bead Ruptor 4 per settings seen in table 1. 25 µL of Protease solution was added to both samples. Samples were incubated at 55°C for one hour. Procedure was carried henceforth according to manufactures' protocol. DNA was eluted in 50 µL of EB buffer. DNA concentration was determined on the NanoDrop 2000 spectrophotometer (Thermo Fisher). Approximately 104 ng of DNA and 5 µL of TBE/Urea sample buffer (Biorad) were loaded onto a 1% agarose gel. DNA was separated by electrophoresis at 140 V for about 50 minutes or until the samples travelled 3/4's of the way down the gel. The gel was stained with ethidium-bromide and then visualized on the Gel-Doc EZ system (Biorad).

Sample	Speed	Time
1	5	20 sec
2	5	30 sec

Table 1. Sample size and Bead Ruptor 4 Settings

OMNI International
The Homogenizer Company®

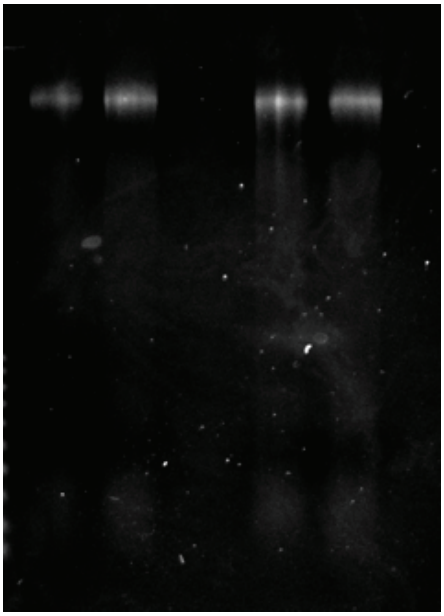
Results

In this study, we determined the efficiency of the Omni DNA purification kit and Bead Ruptor 4 to extract DNA from Turbatrix aceti. It is imperative to spin down the vinegar eels fast and long enough to ensure all eels are toward the bottom of the tube. Vinegar eels tend to swim and retain to the surface of the vinegar they are in. It is also imperative to remove as much of the vinegar as possible after spinning as the acetic acid can affect enzymes in the extraction process. Resuspending the nematode pellet with TE buffer dilutes any residual acetic acid that would otherwise effect the extraction process downstream. DNA concentration was determined through spectrophotometry and ranged from 6.98 ng/µL to 7.40 ng/µL as seen in table 2. Electrophoretic analysis showed bands of genomic DNA recovered of high quality with little DNA shearing. Though samples were processed at different times on the Bead Ruptor 4, there was minimal lane to lane variation (Figure 1).

Table 2: Average DNA Concentrations of Each Sample

Sample	Avg. DNA Concentration
1	7.40 ng/ µL
2	6.98 ng/ µL

Figure 1: Electrophoresis Analysis of T. aceti
Lanes 1 and 2: Sample 1, Lanes 3 and 4: Sample 2



Conclusion

The Bead Ruptor 4 was able to homogenize T. aceti in less than 30 seconds. Different times were demonstrated on the Bead Ruptor to obtain uniform results. High quality DNA was successfully recovered using the Omni Yeast DNA Purification Kit with some variation to the manufactures' protocol.

References

Gershon, D. (1970, April). Studies on aging in nematodes. Experimental Gerontology, 5(1), 7-12. doi:10.1016/0531-5565(70)90023-9

OMNI International
The Homogenizer Company®

935-C Cobb Place Blvd. NW
Kennesaw, GA 30144
800.776.4431 • 770.421.0058
www.omni-inc.com

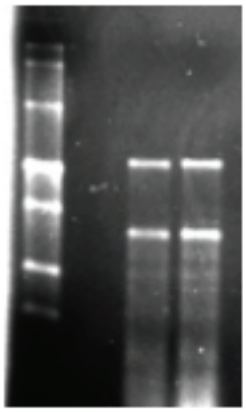


Figure 1. Purified RNA.
Lane 1: RNA Ladder, Lane 3-4: Porcine RNA

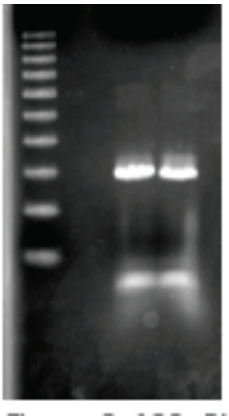


Figure 2: 18S rRNA Detection.
Lane 1: 100 bp Ladder, Lane 3-4: Porcine 18S fragment

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 Bead Ruptor 24 Elite are capable of disrupting skin tissue for RNA extraction with subsequent PCR analysis. The Bead Ruptor 24 Elite 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/

2. The Basics: RNA Isolation. (n.d), Retrieved from: https://www.thermofisher.com/us/en/home/references/ambion-tech-support/rna-isolation/general-articles/the-basicsrna-isolation.html

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

OMNI International
The Homogenizer Company®

935-C Cobb Place Blvd. NW
Kennesaw, GA 30144
800.776.4431 • 770.421.0058
www.omni-inc.com