APPLICATION NOTE

# DNA extraction from *Turbatrix aceti* using the Omni Bead Ruptor 4 bead mill homogenizer.

## 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 aging[1]. *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 workflow from *T. aceti* using the Omni Bead Ruptor 4 bead mill homogenizer for sample preparation of nematode lysates. Extraction efficiency and analyte integrity were evaluated downstream.

**Omni Bead Ruptor 4 bead mill homogenizer**


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

## Materials and methods

### Equipment

- Omni Bead Ruptor 4 bead mill homogenizer (Cat # 25-010)
- Tough Micro-organism Lysing Mix 0.5 mm Glass (2 mL) (Cat # 19-622)

### 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 were 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 commercially available DNA lysis 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 Omni Bead Ruptor 4 bead mill homogenizer 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. After incubation, commercially available DNA extraction kit protocol was followed. DNA was eluted in 50 µL elution buffer. DNA concentration was determined on the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). Approximately 104 ng of DNA and 5 µL of TBE/Urea sample buffer (Bio-Rad) 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 (Bio-Rad).

Table 1: Sample size and Omni Bead Ruptor 4 bead mill homogenizer settings

| Sample | Speed | Time |
|---|---|---|
| 1 | 5 | 20 sec |
| 2 | 5 | 30 sec |

## Results

In this study, we showcased the capability of the Omni Bead Ruptor 4 bead mill homogenizer in preparing adequate homogenates from *Turbatrix aceti* suitable for downstream DNA extraction. 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 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 commercially available Tris EDTA buffer solution 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 integrity, with little DNA shearing. Though samples were processed at different times on the Omni Bead Ruptor 4 bead mill homogenizer, 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 |

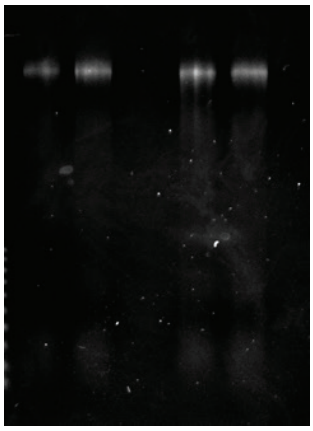

Figure 1: Electrophoresis analysis of *T. aceti.*
Lanes 1 and 2: Sample 1, Lanes 4 and 5: Sample 2

## Conclusion

The Omni Bead Ruptor 4 bead mill homogenizer was able to homogenize *T. aceti* in less than 30 seconds. Different homogenization times were demonstrated to showcase effect of homogenization time on downstream DNA concentration. High-integrity DNA was successfully recovered using a commercially available yeast DNA extraction kit.

## References

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

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