

Genomic DNA extraction of *Tenebrio molitor* using the Omni Bead Ruptor 4 bead mill homogenizer.

Introduction

Many species of beetles are known world-wide to be pests causing damage to stored food products and crops. One of the most common studied species of beetles is the *Tenebrio molitor*, also known as the meal worm beetle. *T. molitor*'s larvae are a common food source to a variety of captive animals, like reptiles, and a possible protein source for humans. *T. molitor* is used in a variety of scientific applications. It is one of the easiest species of beetles to study due to its size and ease of handling. Due to similar biological processes of other organisms in the *Tenebrio* genera, *T. molitor* is a model organism for studying population control and food safety. Typically, insect DNA extraction requires a chemical digestion that can be time consuming due to a long incubation lysis period which can take overnight. Another extraction method that is commonly used is dry grinding with a mortar and pestle, but limits processing to one sample at a time. Bead-mill homogenizers, like the Omni Bead Ruptor 4, allow fast and efficient disruption of multiple samples to extract analytes like nucleic acids or proteins. High-speed shaking of the sample in a tube with small beads allows processing in less amount of time than traditional methods.

In this study, we demonstrate an extraction method for DNA from *T. molitor* using the Omni Bead Ruptor 4 bead mill homogenizer. Extraction efficiency and analyte integrity were evaluated.

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)
- 2 mL Hard Tissue Homogenizing Mix (Cat #19-628)

DNA extraction and separation

T. molitor beetles were obtained from Niles Biological Inc. in Sacramento, Ca. Beetles were cut to obtain about 30 mg of tissue. Samples were transferred to a 2 mL reinforced tube including six 2.8 mm ceramic beads (Cat #19-628). 350 µL of lysis buffer containing CTAB buffer was added to each tube. Samples were disrupted at various speeds and times (Table 1) on the Omni Bead Ruptor 4 bead mill homogenizer. 25 µL of Proteinase K was added to each tube, vortexed briefly, and placed in a water bath of 60 °C for 1 hour. DNA was then extracted using a commercially available insect DNA extraction kit following manufacturer’s instructions. The final elution volume was 100 µL for each sample. 2 µL of each elution was used to determine DNA concentration on the Nanodrop spectrophotometer (Thermo Fisher Scientific).

About 850 ng of total DNA from each sample was mixed in a 1:1 (v/v) ratio with TBE/Urea sample buffer and separated by electrophoresis on a 1% Agarose gel at 140V for about 50 minutes or until the samples travelled 3/4th of the way down the gel. The gel was stained with ethidium-bromide in DDH₂O for 20 min and then visualized on the Gel-Doc EZ system (Bio-Rad).

Table 1: Sample size and Omni Bead Ruptor 4 settings

Sample size	Speed	Time
27 mg	3	2 x 30 sec (10 sec dwell)
34 mg	3	45 sec
24 mg	4	30 sec

Results

In this study, we determined the Omni Bead Ruptor 4’s ability to process *T. molitor* in order to extract its DNA. The isolation of DNA is the first step in understanding insect genetic and biochemical mechanisms for reproduction, mating or insecticide resistance.

Beetles have a strong exoskeleton made of chitin, thus small pieces of the exoskeleton could be seen in solution. This did not affect extraction efficiency because they appeared uniform in size. DNA concentration was determined through spectrophotometry and ranged from 289 ng/µL to 565.2 ng/µL as seen in Table 2. The amount of genomic DNA recovered was independent of tissue size. Electrophoresis analysis showed that genomic DNA recovered was of high quality with little DNA shearing. Though samples were processed at different parameters 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 size (mg)	Avg. DNA concentration (ng/µL)
27	407.9
34	289.0
24	565.2

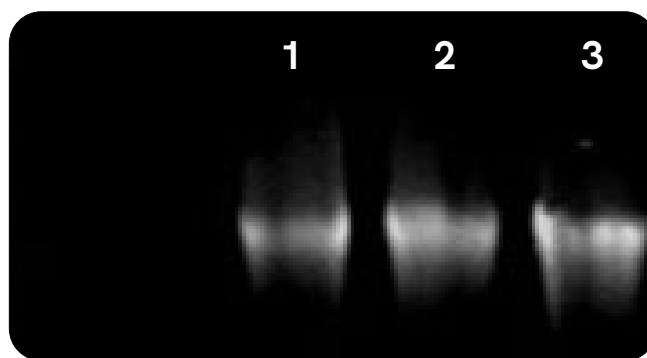


Figure 1 : Electrophoresis analysis of *T. molitor*

Lane 1: 27 mg sample,

Lane 2: 34 mg sample,

Lane 3: 24 mg sample

Conclusion

The Omni Bead Ruptor 4 bead mill homogenizer is capable of homogenizing *T. molitor* in up to 1 minute. Due to the nature of beetles' exoskeleton, different times and speeds on the Omni Bead Ruptor 4 bead mill homogenizer were demonstrated to obtain uniform results. DNA extracted was achieved at speeds 3 and 4; no detectable shearing was observed. At speed 5 there is excess shearing (data not shown), indicative of poor molecular integrity.



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