APPLICATION NOTE #201506

Genomic DNA Extraction of Tenebrio molitor using the Bead Ruptor 4

Shari Garrett, Omni International, Inc.

Introduction

Many species of beetles are known worldwide 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 foodsource 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 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 Bead Ruptor 4. Extraction efficiency and analyte integrity were evaluated.

Materials & Methods

Equipment

- **Bead Ruptor 4** (Cat #25-010)
- **2 mL Hard Tissue Homogenizing Mix** (Cat #19-628)
- **Omega E.Z.N.A Insect DNA Kit** (Cat #D60926-01)
- **Sigma - Antifoam** Y-**30 Emulsion** (Cat #A5758)

DNA Extraction and Separation

T.molitor beetles were obtained fromNiles Biological Inc. in Sacremento, Ca.Beetles were cut to obtain about 30 mg oftissue. Samples were transferred to a 2 mLreinforced tube including six 2.8 mm ceramic beads (Cat #19-628). 350 µL of CTL from theOmega E.Z.N.A Insect DNA Kit was addedto each tube along with 15 µL of anti-foam.Samples were disrupted at various speedsand times (Table 1) on the Bead Ruptor 4.25 µL of Proteinase K (Omega-Biotek) wasadded to each tube, vortexed briefly, andplaced in a water bath of 60oC for 1 hour.The homogenate was transferred to a clean1.5 mL tube and the Omega E.Z.N.A InsectDNA Kit protocol was followed henceforth.The final elution volume was 100 µL foreach sample. 2 µL of each elution was usedto determine DNA concentration on theNanodrop spectrophotometer (ThermoFisher).

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 3/4's 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).

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

Table 1 Sample size and Bead Ruptor 4 Settings.

Results

In this study, we determined the 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 Bead Ruptor 4, there was minimal lane to lane variation (Figure 1).

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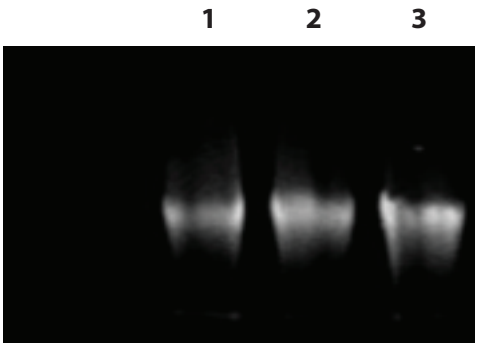
Sample Size	Avg. DNA Concentration
27mg	407.9 ng/µL
34mg	407.9 ng/µL
24mg	565.2 ng/µL

Table 2 Average DNA Concentrations of Each Sample



Lane 1: 27mg sample,
Lane 2: 34mg sample,
Lane 3: 24mg sample

Figure 1 : Electrophoresis Analysis of *T. molitor*

Conclusion

The Bead Ruptor 4 is capable of homogenizing *T. molitor* in up to 1 minute. Due to the nature of beetles' exoskeleton, different times and speeds on the Bead Ruptor 4 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 indicative of poor molecular integrity.

Bead Ruptor 4: 25-010

OMNI International
The Homogenizer Company®

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