APPLICATION NOTE

Mosquito DNA Extraction and High Throughput Gene Targeting on Bead Ruptor 96 Bead Mill Homogenizer

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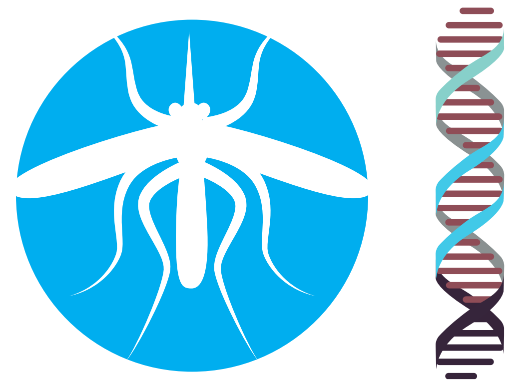
Introduction

Mosquitos, specifically *Aedes aegypti*, are known to act as a host for vector borne diseases such as Yellow fever, Dengue fever, and Zika (1). Most of these diseases are associated with tropical climates, but *Aedes aegypti* are found throughout the Southeast United States, with a likely range from Virginia to Texas (2). To monitor the spread of vector borne diseases, researchers typically sample mosquitos from the field and dissociate the organism to release the internal pathogens to quantify pathogen load. Researchers require a fast and repeatable sample preparation technique to rapidly dissociate a large number of mosquitos while maintaining a high degree of vector and pathogen lysis. Herein, we demonstrate dissociation of *Aedes aegypti* using the Bead Ruptor 96 bead mill homogenizer for the purification of DNA and analysis by endpoint PCR.

Materials and Methods

Equipment

- **Bead Ruptor 96 (Cat# 27-0001)**
- **Bead Ruptor 96 Microtube Holder (Cat# 27-106)**
- **1.5 mL Pre-filled Tube with 2.4 mm Metal Beads (Cat# 19-610)**
- **2 mL 96 Deep Well Plate (Cat# 27-500)**
- **Deep Well Plate Sealing Mat (Cat# 27-510)**



Multiple mosquito DNA extraction in 1.5 ml tubes

Frozen *Aedes aegypti* were obtained from Benzon Research and stored at -20°C. For DNA extraction, 50 mg of thawed mosquitos were added to a 1.5 ml microcentrifuge tube pre-filled with 3 x 2.4 mm stainless steel beads. 350 µl CTL buffer (Omega Biotek D0926-01) was added and the sample was homogenized on the Bead Ruptor 96 at 30 hertz for 3 minutes. Post homogenization, DNA was purified using the Omega Biotek Insect E.Z.N.A Kit, per the manufacturer's instructions. Eluted DNA was stored at -20°C. DNA was quantified and analyzed on a Bioanalyzer and subjected to end point PCR using an 18S rRNA and a *Aedes* serine protease gene, Serpin 5b.

Bead Ruptor 96 (Cat# 27-0001)

Single mosquito DNA extraction in 96 well plates

A single mosquito was thawed and placed a 2 mL 96 deep well plate containing 3 X 2.4 mm stainless steel beads. 1 mL of 0.3 M sucrose, 0.3 M NaCl and 60 mM of Tris-HCl, pH 7.4 was added and the plate was sealed with a silicon mat. The sample was then homogenized on the Bead Ruptor 96, at 24 Hz, for 5 minutes. After processing, the 96 well plate was heated to 95°C in a water bath for 10 minutes. The sample was centrifuged at 4000 rpm for 5 minutes to pellet cell debris and placed on ice for 5 minutes. By processing in a high concentration of sucrose, following centrifugation, two phases were visible. The upper phase contained DNA while the cellular debris was portioned in the lower phase. One µL of the upper phase was removed and analyzed by end point PCR using a 18S rRNA and a *Aedes* serine protease gene, Serpin 5b.

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DNA Quantification

Eluted DNA from the 50 mg *Aedes* extraction was quantified, in triplicate, on a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific). Concentrations and purity levels are shown in Table 1. 1 µl of DNA was further analyzed, in triplicate, on an Agilent 2100 Bioanalyzer in a 7500 DNA chip (Figure 1)

Endpoint PCR

Endpoint PCR was performed on DNA from the 50 mg *Aedes* and single mosquito extraction. Eluted DNA was diluted to 1 pg/µL and 1 µL was added to 19 µL MolTaq PCR reactions. Two primers were used in this study, an 18S ribosomal subunit gene and an abundant *Aedes aegypti* serine protease gene, Serpin 5b shown in Table 2. The positive 18S control was 1 pg of purified *S. cerevisiae* DNA. The thermal cycler was set to cycle 30 times between 95°C for 30 seconds, 50.8°C for 30 seconds, 72°C for 30 seconds and 95°C for 30 seconds. The resulting amplicons were visualized on a 2% agarose gel. All amplicons were diluted 1:1 with TBE-urea and electrophoresis was performed for 1 hour at 140 V. After electrophoresis, amplicons were visualized by staining with 0.05% ethidium bromide for 20 minutes following by imaging on a BioRad Gel Doc EZ system.

Results

Field sampling of mosquitos followed by sample dissociation and DNA purification is a routine processing for surveying the spread of vector borne diseases. As the sample sizes are relatively small and the number of samples is high, bead beating is an ideal method for high-throughput sample disaggregation. The Bead Ruptor 96, when operated with two 96 well plates, allows up to 192 samples to be processed simultaneously in SBS format that is compatible with liquid handling systems. Here we demonstrate a method of DNA extraction using two different approaches. The first used multiple mosquitos (50 mg) combined with silica spin column-based DNA extraction methods while the second approach demonstrated the extraction of PCR suitable DNA from a single mosquito.

Table 1 and figure 1 show the DNA yields and integrity of DNA obtained from silica spin column purification following dissociation of 50 mg of mosquitos. Not surprisingly the 260/280 ratio was observed as 2.04 indicating the possible co-purification of RNA. The Bioanalyzer analysis indicated that most of the purified DNA was in the range of 3000-6000 base pairs.

Data

Sample	Average Nucleic Acid conc. (ng/ul)	Average Abs 260	Average Abs 280	Average 260/280
Aedes aegypti	1302.3	26.047	12.741	2.04

Table 1: DNA quantification and purity analysis by spectrophotometry using DNA purified from 50 mg of *Aedes aegypti*.

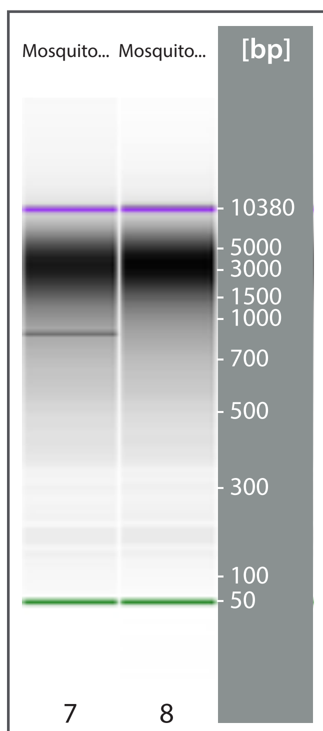

Figure 1: Bioanalyzer generated electropherogram resulting from the analysis of purified DNA obtained from 50 mg of *Aedes aegypti*.

Gene Target	Primer Sequence
Serpin 5B-Fw	TCA CCT TCC CAT TGG TGT GT
Serpin 5B-Rv	TGG AAT GAG ACC AGT GTG AGT
Universal 18S	AAC CTG GTT GAT CCT GCC AGT
Universal 18S	GGC ACC AGA CTT GCC CTC

Table 2: Primers ordered from IDT for each gene target.

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The purified DNA from both the 50 mg and single mosquito extraction was further analyzed by endpoint PCR targeting the 18S rRNA and a *Aedes* serine protease gene, Serpin 5b. For the 50 mg mosquito extraction, abundant amplicons were observed for both the 18S and Serpin 5b PCR. For the single mosquito DNA purification the 18S amplicon was detected while the Serpin targeted PCR did not yield a detectable result. This is presumably due to the lower concentration of DNA obtained from the single organism.

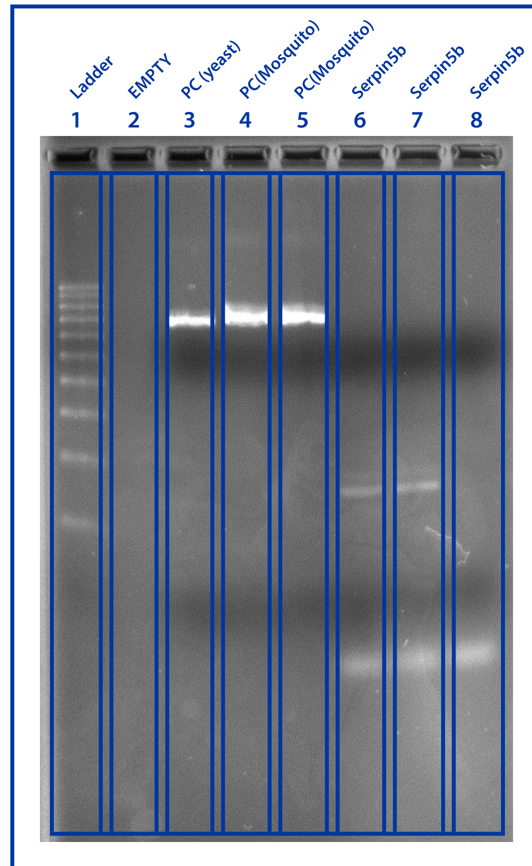

Figure 2: PCR products targeting 18S and serpin 5b from the 50 mg mosquito extraction. Extracted DNA was capable of being amplified by both species-specific primers (Serpin 5b) and eukaryotic specific primers (18s ribosomal subunit). PC indicates positive control.

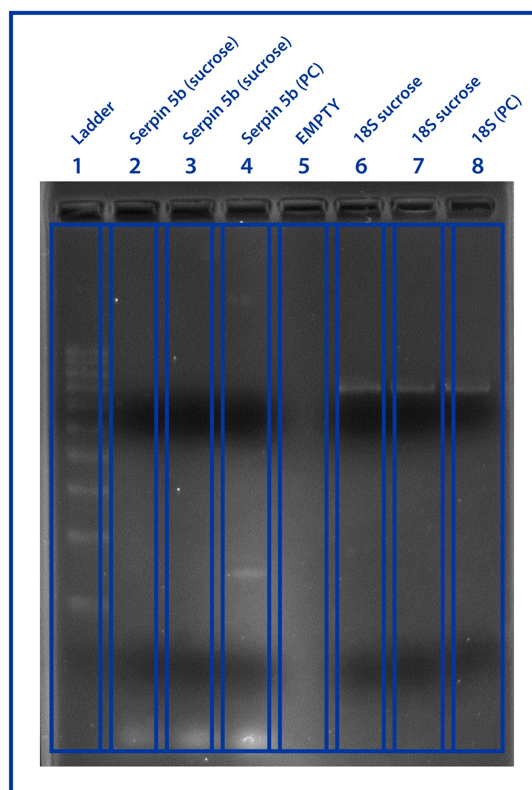

Figure 3: PCR products targeting 18S and serpin 5b from the single mosquito extraction. Extracted DNA was capable of being amplified for the eukaryotic specific primers (18s ribosomal subunit). PC indicates positive control.

References

1. Mousson, L., Dauga, C., Garrigues, T., Schaffner, F., Vazeille, M., & Failloux, A. (2005). Phylogeography of Aedes (Stegomyia) aegypti (L.) and Aedes (Stegomyia) albopictus (Skuse) (Diptera: Culicidae) based on mitochondrial DNA variations. Genetical Research, 86(1), 1-11.

2. Lars Eisen, Chester G. Moore; Aedes (Stegomyia) aegypti in the Continental United States: A Vector at the Cool Margin of Its Geographic Range, Journal of Medical Entomology, Volume 50, Issue 3, 1 May 2013, Pages 467–478,

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