APPLICATION NOTE

# Mosquito DNA extraction and high-throughput gene targeting on the Omni Bead Ruptor 96 bead mill homogenizer.


## Summary

Mosquitoes, specifically *Aedes aegypti*, are known to act as a host for vector borne diseases such as yellow fever, Dengue fever, and Zika (Mousson, 2005). 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 (Eisen, 2013). To monitor the spread of vector borne diseases, researchers typically sample mosquitoes from the field and dissociate the organism to release internal pathogens for quantification of pathogen load. Researchers require a fast and repeatable sample preparation technique to rapidly dissociate a large number of mosquitoes while maintaining a high degree of vector and pathogen lysis. Herein, we demonstrate dissociation of *Aedes aegypti* using the Omni Bead Ruptor 96 bead mill homogenizer for the purification of DNA and analysis by endpoint PCR.



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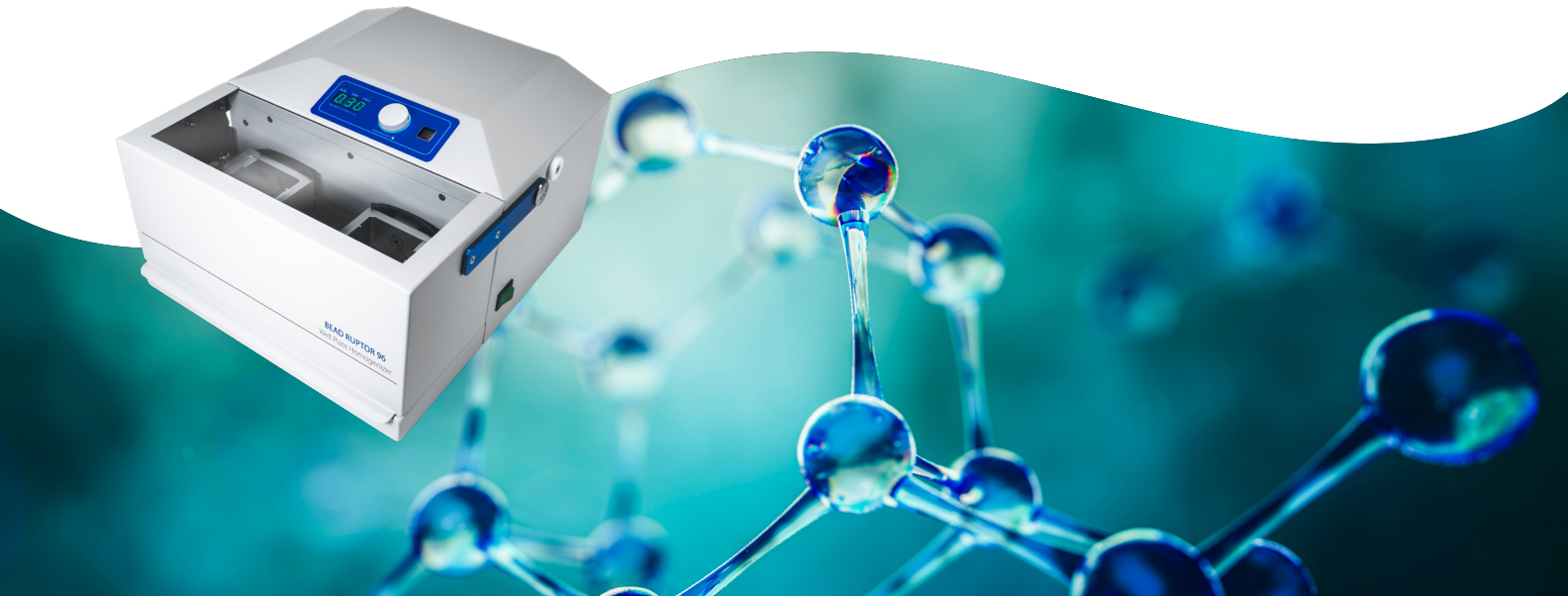
**Omni Bead Ruptor™ 96 bead mill homogenizer**



## Materials and methods

**Equipment**

- Omni Bead Ruptor 96 bead mill homogenizer (Cat # 27-0001)
- Microtube or Screw Cap Tube Holders (Cat # 27-106)
- Omni Bead Ruptor 96 Well Plate Adapters (Cat # 27-101)
- Hard Tissue Grinding Mix 2.4 mm Metal (1.5 mL) (Cat # 19-610)
- 2 mL 96 Deep Well Plate (Cat # 27-520)
- Deep Well Plate Sealing Mat (Cat # 27-530)
- 2.4 mm Stainless Steel Beads Bulk, 500 g (Cat # 19-640)

## Procedure

**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 mosquito were added to a 1.5 mL microcentrifuge tube pre-filled with 3 x 2.4 mm stainless steel beads (Cat # 19-610). Precisely 350 µL of commercially available lysis buffer specialized for insect DNA extraction was added and the sample was homogenized on the Omni Bead Ruptor 96 bead mill homogenizer at 30 Hz for 3 minutes. Post homogenization, DNA was purified using a commercially available insect DNA extraction kit following manufacturer's instructions. Eluted DNA was stored at -20 °C.

**Single mosquito DNA extraction in 96 well plates**

A single mosquito was thawed and placed in a 2 mL 96 deep well plate (Cat # 27-520) containing 3 x 2.4 mm stainless steel beads (Cat # 19-640). One milliliter of 0.3 M sucrose, 0.3 M NaCl and 60 mM of Tris-HCl, pH 7.4 was added to each sample well and the plate was sealed with a Deep Well Plate Sealing Mat (Cat # 27-530). The sample was then homogenized on the Omni Bead Ruptor 96 bead mill homogenizer 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 then 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, used for downstream methods, while the cellular debris portioned in the lower phase was discarded.

**DNA quantification**

Eluted DNA from the 50 mg *Aedes* extraction was quantified, via $A_{260}/A_{280}$ spectrophotometry (Table 1). One microliter of DNA was further analyzed on an Agilent 2100 Bioanalyzer using the DNA 7500 kit following manufacturer's instructions (Figure 1).

**Endpoint PCR**

Endpoint PCR was performed on DNA from the 50 mg *Aedes aegypti* and single mosquito extraction. Eluted DNA was diluted to 1 pg/µL and 1 µL was added to 19 µL MolTaq PCR reactions. Primers were used to amplify two separate genes of interest in single-plex reactions: 18S rRNA gene and *Aedes aegypti* serine protease gene (Serpin 5b) (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. All amplicons were diluted 1:1 with TBE Urea Sample Buffer (G Biosciences, Cat # 786-474) and electrophoresis was performed for 1 hour at 140 V. After electrophoresis, amplicons were stained with 0.05 % ethidium bromide (Bio-Rad, Cat # 161-0433) for 20 minutes followed by visualization on a Bio-Rad Gel Doc EZ system.

## Results

DNA concentration, purity, and integrity obtained from silica spin column purification following dissociation of 50 mg of mosquitoes is shown in Table 1 and Figure 1, respectively. Not surprisingly the $A_{260}/A_{280}$ ratio was observed as 2.04 indicating the possible co-purification of RNA. The Bioanalyzer analysis also indicated that most of the purified DNA was in the range of 3000-6000 base pairs.

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

| Sample | Average nucleic acid concentration (ng/µL) | Average $A_{260}$ | Average $A_{280}$ | Average $A_{260}/A_{280}$ |
|---|---|---|---|---|
| *Aedes aegypti* | 1302.3 | 26.047 | 12.741 | 2.04 |

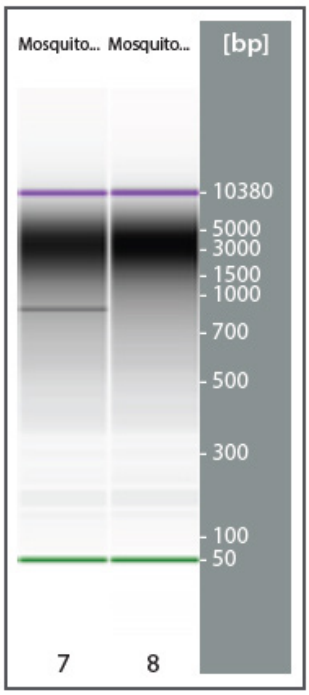



Figure 1: Bioanalyzer-generated electropherogram from the analysis of purified DNA obtained from 50 mg of *Aedes aegypti*.

Table 1: Primers used for endpoint PCR amplification of Serpin 5b and 18S rRNA genes.

| 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 |

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 aegypti* serine protease gene, Serpin 5b (Figures 2-3). For the 50 mg mosquito extraction, abundant amplicons were observed for both the 18S and Serpin 5b gene targets. For the single mosquito DNA purification, the 18S amplicon was detected while the Serpin 5b targeted PCR did not yield a detectable result. This is presumably due to the lower concentration of DNA obtained from the single organism.

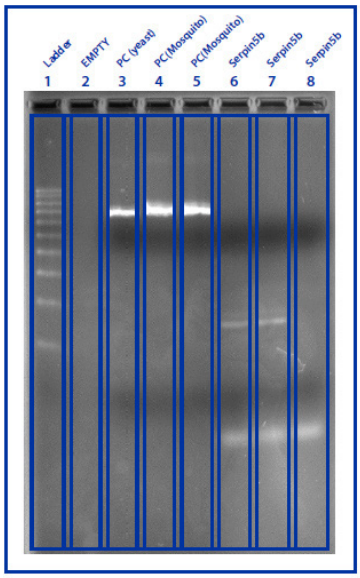



Figure 2: Gel visualization of PCR products targeting 18S and Serpin 5b genes from the 50 mg mosquito extraction. PC indicates positive control.

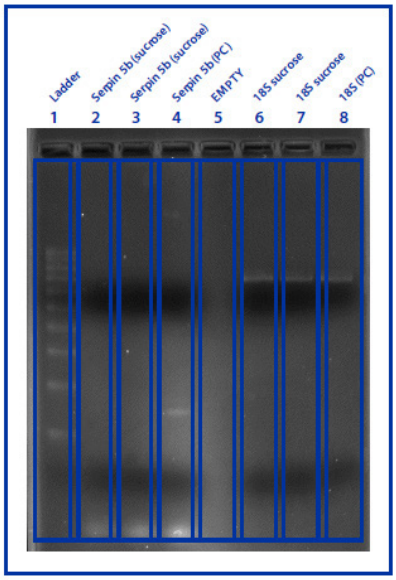



Figure 3: Gel visualization of PCR products targeting 18S and Serpin 5b genes from the single mosquito extraction. PC indicates positive control.

## Conclusion

Field sampling of mosquito followed by sample dissociation and DNA purification is a routine process 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 Omni Bead Ruptor 96 bead mill homogenizer, when operated with two 96 well plates, allows up to 192 samples to be processed simultaneously in SBS format , which is compatible with liquid handling systems. In this application, we have demonstrated a method of DNA extraction using two different approaches. The first used multiple mosquitoes (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.

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

## 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. Eisen, L., & Moore, C. G. (2013). Aedes (Stegomyia) aegypti in the continental United States: a vector at the cool margin of its geographic range. *Journal of medical entomology*, 50(3), 467-478. https://doi.org/10.1603/ me12245

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