Journal of Visualized Experiments

A magnetic-beads-based mosquito DNA extraction protocol for high throughput sequencing

--Manuscript Draft--

Article Type:	Invited Methods Collection - JoVE Produced Video
Manuscript Number:	JoVE62354R1
Full Title:	A magnetic-beads-based mosquito DNA extraction protocol for high throughput sequencing
Corresponding Author:	Yoosook Lee, Ph.D. University of Florida Institute of Food and Agricultural Sciences Vero Beach, FL UNITED STATES
Corresponding Author's Institution:	University of Florida Institute of Food and Agricultural Sciences
Corresponding Author E-Mail:	yoosook.lee@ufl.edu
Order of Authors:	Tse-Yu Chen
	Adam Vorsino
	Kyle Kosinski
	Ana Romero-Weaver
	Eva Buckner
	Joanna Chiu
	Yoosook Lee, Ph.D.
Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$2,400)
Please specify the section of the submitted manuscript.	Biology
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	Vero Beach, FL, USA
Please confirm that you have read and agree to the terms and conditions of the author license agreement that applies below:	I agree to the Author License Agreement
Please provide any comments to the journal here.	
Please indicate whether this article will be Standard Access or Open Access.	Open Access ($3900)

TITLE:

A Magnetic-Bead-Based Mosquito DNA Extraction Protocol for Next-Generation Sequencing

AUTHORS AND AFFILIATIONS:

Tse-Yu Chen[1], Adam E. Vorsino[2], Kyle J. Kosinski[1], Ana Romero-Weaver[1], Eva A. Buckner[1], Joanna C. Chiu[3], Yoosook Lee[1]

[1]University of Florida, Institute of Food and Agricultural Sciences, Florida Medical Entomology Laboratory, Vero Beach, FL

[2]U. S. Fish and Wildlife Service, Pacific Islands Fish and Wildlife Office, Honolulu, HI

[3]University of California Davis, Department of Entomology and Nematology, Davis, CA

Email addresses of co-authors:

Tse-Yu Chen	(papilioninae@ufl.edu)
Adam E. Vorsino	(adam_vorsino@fws.gov)
Kyle J. Kosinski	(kyle.kosinski@ufl.edu)
Ana Romero-Weaver	(aromeroweaver@ufl.edu)
Eva A. Buckner	(eva.buckner@ufl.edu)
Joanna C. Chiu	(jcchiu@ucdavis.edu)
Yoosook Lee	(Yoosook.lee@ufl.edu)

Corresponding authors:

Yoosook Lee	(Yoosook.lee@ufl.edu)

KEYWORDS:

DNA extraction, mosquito, insect

SUMMARY:

Described here is a DNA extraction protocol using magnetic beads to produce high quality DNA extractions from mosquitoes. These extractions are suitable for a downstream next-generation sequencing approach.

ABSTRACT:

A recently published DNA extraction protocol using magnetic beads and an automated DNA extraction instrument suggested that it is possible to extract high quality and quantity DNA from a well-preserved individual mosquito sufficient for downstream whole genome sequencing. However, reliance on an expensive automated DNA extraction instrument can be prohibitive for many laboratories. Here, the study provides a budget-friendly magnetic-bead-based DNA extraction protocol, which is suitable for low to medium throughput. The protocol described here was successfully tested using individual *Aedes aegypti* mosquito samples. The reduced costs associated with high quality DNA extraction will increase the application of high throughput sequencing to resource limited labs and studies.

INTRODUCTION:

Recent development of an improved DNA extraction protocol[1] has allowed many high-impact downstream studies involving whole genome sequencing[2–6]. This magnetic bead-based DNA extraction protocol provides reliable DNA yield from individual mosquito samples, which in turn reduces the cost and time associated with acquiring a sufficient number of samples from field collections.

Recent advances in population and landscape genomics are directly correlated with decreasing costs of whole genome sequencing. Though the previous DNA extraction protocol[1] increases efficiencies associated with high throughput sequencing, smaller labs/studies without the funds may opt out of using these new powerful landscape and population genomics tools due to costs of implementing the protocol (e.g., costs of specialized instruments).

Here, a modified DNA extraction protocol is presented that uses a similar magnetic bead extraction step as Neiman et al.[1] to obtain high purity DNA but does not rely on high-cost instruments for tissue lysis and DNA extraction. This protocol is suitable for experiments requiring >10 ng of high-quality DNA.

PROTOCOL:

1. General sample storage and preparations prior to DNA extraction

1.1. Hydrate the sample in 100 µL PCR-grade water for 1 h (or overnight) at 4 °C if the sample has been stored in >70% alcohol to soften the tissue.

2. Sample disruption

2.1. Set an incubator or shaking heat block at 56 °C.

2.2. Make proteinase K (PK) buffer/enzyme mix. 2 µL of Proteinase K (100 mg/mL) and 98 µL of Proteinase K Buffer (total 100 µL) is required for each individual mosquito extraction. To prepare a master mix for multiple specimens, increase the total amount (combined for all individual specimens) by ~15% to ensure the availability of adequate volume.

2.3. If samples were hydrated prior to extraction, discard water from each sample tube.

2.4. In a 1.5 mL microcentrifuge tube containing mosquito tissue, add 100 µL of PK buffer/enzyme mix.

2.5. Homogenize the tissue using microcentrifuge tube pestle.

2.6. Centrifuge the samples for 1 min at 9,600 x *g* at room temperature.

2.7. Incubate the sample for 2–3 h at 56 °C.

2.8. During incubation, prepare the other reagents using the DNA extraction step (step 3).

3. DNA extraction

3.1. Make a magnetic bead master mix. For each sample, mix 100 µL of lysis buffer, 100 µL of Isopropanol, and 15 µL of magnetic beads (total 215 µL). To prepare a master mix for multiple reactions, increase the total amount (combined for all individual specimens) by ~20% to ensure the availability of adequate volume.

3.2. After the incubation time (step 2.7), transfer each lysate to a clean microcentrifuge tube or a microplate well using pipette.

3.3. Add 100 µL lysate and 215 µL of the magnetic bead master mix to each sample.

3.4. Use a pipette to mix it well for 10–20 s and then let it stand for 10 min at room temperature. Gently shake the tube occasionally to maximize the binding of magnetic beads and DNA.

3.5. Place the tube/plate on the magnetic bead separator and wait until the solution is clear.

3.6. Discard the liquid from the tube/plate using pipette. When removing the supernatant, try not to touch or disturb the magnetic beads holding the DNA.

3.7. Move the tube/plate away from magnetic bead separator and add 325 µL of washing buffer 1 to each well.

3.8. Mix thoroughly by pipetting and incubate for 1 min at room temperature.

3.9. Repeat steps 3.5–3.6.

3.10. Move the tube/plate away from magnetic bead separator and add 250 µL of washing buffer 1 to each well.

3.11. Mix thoroughly by pipetting and incubate for 1 min at room temperature.

3.12. Repeat steps 3.5–3.6.

3.13. Move the tube/plate away from magnetic bead separator and add 250 µL of washing buffer 2 to each sample.

3.14. Mix thoroughly and incubate for 1 min at room temperature.

3.15. Repeat steps 3.5–3.6.

3.16. Move the tube/plate away from magnetic bead separator and add 250 µL of washing buffer 2 to each well.

3.17. Mix thoroughly and incubate for 1 min at room temperature.

3.18. Repeat steps 3.5–3.6.

3.19. Move the tube/plate away from the magnetic bead separator and add 100 µL of elution buffer to each well.

3.20. Mix thoroughly and incubate for 2 min at room temperature.

3.21. Move the tube/plate over on the magnetic bead separator and wait until the solution is clear.

3.22. Pipette the supernatant into a new clean 0.5 microcentrifuge tube or microplate well.

3.23. If microplate is being used, cover it with parafilm and store DNA samples at 4 °C (up to 1 week) or -80 °C.

3.24. Determine the DNA yields using any appropriate method.

NOTE: In this study, DNA fluorometer reading and absorbance at 260/280 nm were utilized.

REPRESENTATIVE RESULTS:

The average DNA yield per individual mosquito head/thorax tissue was 4.121 ng/µL (N = 92, standard deviation 3.513) measured using a fluorometer when eluted using 100 µL of elution buffer. This is sufficient for the 10–30 ng genomic DNA input requirements necessary for whole genome library construction[1,7]. The quantity of DNA can vary between 0.3–29.7 ng/µL depending on the mosquito body size and preservation conditions. Some of the high variability is due to the characteristic of magnetic beads used in the study. Different brands of magnetic beads may produce more consistent concentrations, as indicated in the previous report[1]. It should also be noted that different mosquito species differ in size and that the DNA yield is dependent on those

individual and species size ranges. If the DNA concentration is below the typical ranges, one may adjust the incubation period in the proteinase K reaction (step 2.7), but this extension should be no longer than 16 h. Moreover, switching the proteinase K and/or lysis buffer brands can have significant impact on DNA yield[1].

The typical microvolume fluorometer reading of the final DNA in elution buffer with 0.5 mM EDTA is shown in **Figure 1**. The average absorbance ratio for 260/280 nm was 2.3 (standard deviation = 0.071). The data was consistent from the samples used in the previous studies for whole genome sequencing[3,4].

Typical reagent and consumable cost for extraction is around $9.50/sample. These cost estimates include reagents listed in the **Table of Materials**, and other consumables such as pipette tips, tubes, and gloves needed for extraction. The reagent and consumable cost are equivalent to any typical magnetic-bead-based extraction method (**Table 1**). The cost may vary somewhat depending on bulk purchase discounts available for any given product. The major cost benefit of this protocol comes from not requiring the automated DNA extraction instrument.

FIGURE AND TABLE LEGENDS:

Figure 1: Fluorometer output. Typical DNA fluorometer output of mosquito DNA eluted in elution buffer with 0.5 mM EDTA.

Table 1: Cost analysis for different extraction methods. The table lists the costs and the number of samples processed in one working day for different extraction methods.

DISCUSSION:

The protocol described here can be adapted for other insect species. The original version of the protocol introduced in Nieman et al.[1] has been tested on multiple species, including *Aedes aegypti, Ae. busckii, Ae. taeniorhynchus, Anopheles arabiensis, An. coluzzii, An. coustani, An. darlingi, An. funestus, An. gambiae, An. quadriannulatus, An. rufipes, Culex pipiens, Cx. quinquefasciatus, Cx. theileri, Drosophila suzukii, Chrysomela aeneicollis Tuta absoluta,* and *Keiferia lycopersicella*[2,8–10]. It is expected that this protocol will work for these as well as other arthropods.

Ideally, samples should be stored in 70%–80% ethanol at 4 °C prior to DNA extraction. Typically, 80% ethanol is used for storing mosquito samples in Africa. High temperature conditions could accelerate evaporation of ethanol and make ethanol content lower than what is intended (70%). Sample stored in 100% ethanol, denatured alcohol, 75%–90% isopropanol or rubbing alcohol have also resulted in successful whole genome sequencing using the Nieman et al.[1] protocol, and it is expected that this protocol would perform equally well. The DNA extraction protocol described here has also been tried on samples stored dry in silica and frozen at -20 °C. However, there was a higher (15%–30%) chance of failure when stored for >6–12 months, suggesting that

these were less than ideal storage conditions.

As DNA extraction without physical sample disruption has a higher chance of failure (33%)[1], this protocol incorporates a manual physical disruption technique (step 2.5). Sample disruption can also be achieved using steel beads on a tissue lysis instrument[1].

The reagents and consumable cost for DNA extraction using this protocol is comparable with other extraction methods available (**Table 1**). However, the protocol described here does not require an automated DNA extraction instrument. Due to manual labor involved in processing DNA extraction, one person can typically process 12–16 samples and finish with DNA quantification in a day. This is significantly less than what automated DNA extraction setup can process per day. Thus, when choosing a protocol, the sample processing capacity should be taken into account. However, the lower cost threshold this protocol offers should enable many resource limited labs and studies to leverage in high-throughput sequencing technology.

In both the Nieman et al.[1] and this protocol, elution buffer can be replaced with the typical TE buffer, 10 mM Tris-Cl, or water depending on the downstream analysis. An elution buffer containing some EDTA may impact enzyme efficiency. Typical enzymatic shearing-based gDNA library preparation protocols include conditioning solutions or enhancers added to compensate for enzyme inhibition by EDTA. It should also be considered that EDTA may change the absorbance at 230 nm wavelength[11]. For example, higher concentrations of EDTA would create a higher absorbance value between 220–240 nm wavelength than that shown in **Figure 1** (data not shown).

This protocol can be easily adapted to laboratories with minimal molecular biology tools. The protocol described here attempts to reduce the cost thresholds associated with genome extraction and thereby increase the application of high throughput sequencing to resource limited labs and studies.

ACKNOWLEDGMENTS:

We acknowledge funding support from the Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement 1U01CK000516), CDC grant NU50CK000420-04-04, the USDA National Institute of Food and Agriculture (Hatch project 1025565), UF/IFAS Florida Medical Entomology Laboratory fellowship to Tse-Yu Chen, NSF CAMTech IUCRC Phase II grant (AWD05009_MOD0030), and Florida Department of Health (Contract CODQJ). The findings and conclusions in this article are those of the author(s) and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

DISCLOSURES:

The authors have nothing to disclose.

REFERENCES:

1. Nieman, C. C., Yamasaki, Y., Collier, T. C., Lee, Y. A DNA extraction protocol for improved DNA yield from individual mosquitoes. *F1000Research*. **4**, 1314 (2015).
2. Lee, Y. et al. Genome-wide divergence among invasive populations of *Aedes aegypti* in California. *BMC Genomics*. **20** (1), 204 (2019).
3. Schmidt, H. et al. Abundance of conserved CRISPR-Cas9 target sites within the highly polymorphic genomes of *Anopheles* and *Aedes* mosquitoes. *Nature Communications*. **11** (1), 1425 (2020).
4. Schmidt, H. et al. Transcontinental dispersal of *Anopheles gambiae* occurred from West African origin via serial founder events. *Communications Biology*. **2**, 473 (2019).
5. Norris, L. C. et al. Adaptive introgression in an African malaria mosquito coincident with the increased usage of insecticide-treated bed nets. *Proceedings of the National Academy of Sciences of the United States of America*. **112** (3), 815–820 (2015).
6. Main, B. J. et al. The genetic basis of host preference and resting behavior in the major african malaria vector, *Anopheles arabiensis*. *Plos Genetics*. **12** (9), e1006303 (2016).
7. Yamasaki, Y. K. et al. Improved tools for genomic DNA library construction of small insects. *F1000Resesarch*. **5**, 211 (2016).
8. Tabuloc, C. A. et al. Sequencing of *Tuta absoluta* genome to develop SNP genotyping assays for species identification. *Journal of Pest Science*. **92**, 1397–1407 (2019).
9. Campos, M. et al. Complete mitogenome sequence of Anopheles coustani from São Tomé island. *Mitochondrial DNA. Part B, Resources*. **5** (3), 3376–3378 (2020).
10. Cornel, A. J. et al. Complete mitogenome sequences of *Aedes* (*Howardina*) *busckii* and *Aedes* (*Ochlerotatus*) *taeniorhynchus* from the Caribbean Island of Saba. *Mitochondrial DNA. Part B, Resources*. **5** (2), 1163–1164 (2020).
11. Lucena-Aguilar, G. et al. DNA source selection for downstream applications based on dna quality indicators analysis. *Biopreservation and Biobanking*. **14** (4), 264–270 (2016).

Figure

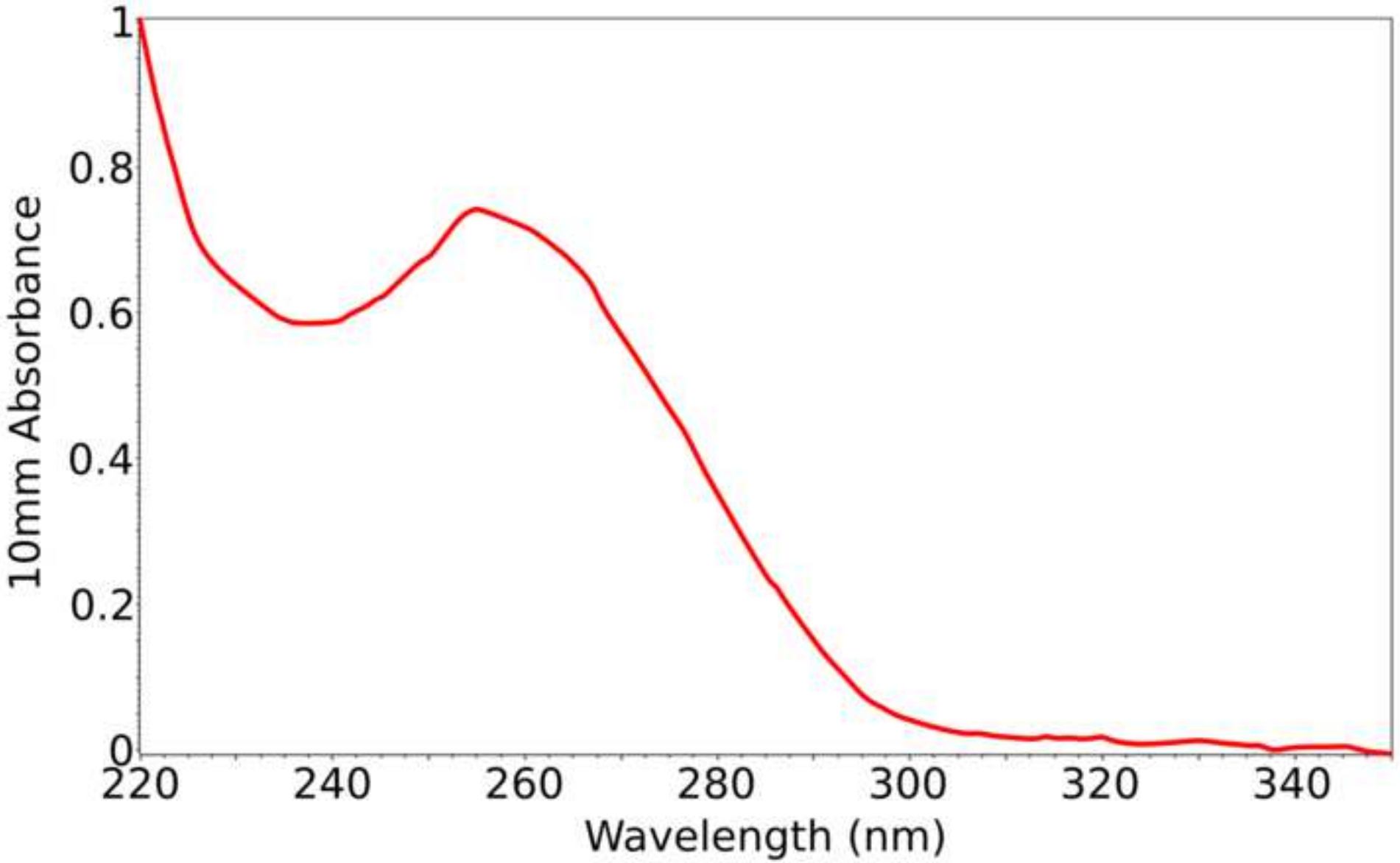

Table 1:

	This protocol	other manual magnetic bead-based extraction	Other automated magnetic bead-based extraction
Sample processing cost (reagent + consumables)	$9.50/sample	$12/sample	$9.60/sample
Tissue disruption Instrument	$0	$0	$2000-$20,000
DNA extraction instrument	$250	$250	$60,000
DNA Fluorometer	$4000	$4000	$4000
Typical sample processing per one working day	12-16	12-16	96-192

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
AE Buffer	Qiagen	19077	Elution buffer
AL Buffer	Qiagen	19075	Lysis buffer
AW1 Buffer	Qiagen	19081	Washing buffer 1
AW2 Buffer	Qiagen	19072	Washing buffer 2
MagAttract Suspension G	Qiagen	1026901	magnetic bead
Magnetic bead separator	Epigentek	Q10002-1	
Nanodrop	ThermoFisher	ND-2000	microvolume spectrophotometer
PK Buffer	ThermoFisher	4489111	Proteinase K buffer
Proteinase K	ThermoFisher	A25561	
Qubit	Invitrogen	Q33238	fluorometer

Response to editor/reviewer comments to JoVE62354

We thank reviewers and editors for constructive comments. We responded to each comment below.

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. E.g. line 146: “enzymatic-shearing..” instead of “..-sheering..”?
    a. Done.
2. Add a single space between the quantity and its unit. E.g. “20 µL” instead of “20µL”, “20 mL” instead of “20mL”, “10 s” instead of “10s”, “4 oC” instead of “4oC”, etc.
    a. Done
3. Line 78: Please specify what 10K represents? Also write it as 10,000, and express it in g.
    a. Done
4. Please provide details about the “magnetic bead separator”. Is the same item referred to as “magnet” (line 93, 96, 99, 102)?. Please use consistent terminology.
    a. Done. Used magnetic bead separator instead of magnet.
5. What are AL, AW1, AW2, AE buffers? Are they part of any commercial kits?
    a. Yes. We provided the product info as part of the JoVE Materials table.
6. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials. E.g. MagAttract, Beadbeater, TissueLyzer, Nanodrop etc.
    a. The names are changed to genetic descriptions that is including the buffer names you listed in comment #5.
7. Please highlight up to 3 pages of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.
    a. Done. Total protocol is <1.5 page. For Steps 3.9-3.17 are repetition of the same process with varying buffer kind and amount so narrative can be changed a little (e.g “repeat this washing steps first with washing buffer 1 and twice with washing buffer 2)”).
8. Please expand the representative results to include text explaining the Representative Results in the context of the technique you have described, e.g., how do these results show the technique, suggestions about how to analyze the outcome, etc. The paragraph text should refer to all of the figures. Data from both successful and sub-optimal experiments can be included.
    a. The Representative Results section was expanded to include more datapoints and relevant suggestions if the concentration deviates from the normal range.
9. Please discuss how this method is cost-effective compared to the other known methods.
    a. A new table of cost analysis is added and relevant results and discussion section is added.
10. Figure 1. Please check the Y-axis label. Also, do not embed the figure in the text. Instead upload it separately through editorial manager.

11. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage (YEAR).] For more than 6 authors, list only the first author then et al. Do not abbreviate the journal names.
    a. I used the JoVE EndNote format and included the full journal names.
12. Please sort the Materials Table alphabetically by the name of the material.
    a. Done

Reviewers' comments:

Reviewer #1:

No major or minor concerns.

Reviewer #2:

Major Concerns:

1. Why is the reported value for DNA yield 4x higher than the original protocol (Nieman et al)?
    a. The Nieman et al. used *Anopheles gambiae* mosquitoes and this paper used *Aedes aegypti* samples. The sample size is also different in calculating average. We noticed that body size has major impact on the DNA yield but we did not systematically explored this. Also for typical field collection sample processing, body size is not something you can control. We increased our dataset to calculate the average, minimum and maximum concentration and provided in the revised manuscript. We also added notes on the characteristics of magnetic beads can also increase variability in DNA yield.
2. Please include values for absorbance at 280 nm, which represents protein contaminants.
    a. We provided the ratio of 260/280 data in the revised manuscript, as that is what is typically checked for DNA quality for whole genome sequencing.
3. Which downstream test did the authors perform to confirm the quality of the extracted DNA?
    a. We provided the Nanodrop and Qubit for DNA quantity and quality.
4. Please include standard deviation for DNA yield.
    a. Done
5. Authors state in Results that "absorbance below 230 nm may change depending on the concentration of EDTA". This sentence belongs to Discussion. Further, other chemicals also absorb below 230 nm, such as guanidine, ethanol and other organic compounds. Lastly, a reference would be missing for this information.
    a. Sentence has been edited moved to the Discussion section.