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

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# Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group?

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

**Current Protocol Length**

Number of Steps: 11
Number of Shots: 30

# Introduction

## 1. Introductory Interview Statements

**REQUIRED:**

1.1. **Yoosook Lee:** This budget-friendly magnetic-bead-based DNA extraction protocol makes high-throughput sequencing accessible to resource-limited labs and studies.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 3.1.2*

1.2. **Ana:** This protocol does not require expensive equipment for high-quality DNA extraction and can be helpful in certain diagnostic or research situations where obtaining a sufficient quantity of DNA with a quality suitable for high-throughput sequencing is challenging.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 3.8.1*

**OPTIONAL:**

1.3. **Kyle:** This protocol is easy to replicate after a one-time demonstration. It should be tried with a small number of samples first to get familiar with the workflow.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 3.1.1*

# Protocol

## 2. Sample Disruption

2.1. To begin, hydrate the mosquito tissue sample stored in greater than 70% alcohol by adding 100 microliters of PCR-grade water **[1]** and incubating for 1 hour at 4 degrees Celsius to soften the tissue **[2]**.

2.1.1. WIDE: Talent adding water to the sample.

2.1.2. Talent incubating sample at 4 °C.

2.2. After the incubation, discard the water **[1]** and add 100 microliters of Proteinase K buffer-enzyme mix **[2-TXT]**. Then, use a microcentrifuge tube pestle to homogenize the tissue **[3].** *Videographer: This step is important!*

2.2.1. Talent discarding the water.

2.2.2. Talent adding Proteinase K buffer-enzyme mix. **TEXT: See text for Proteinase K buffer/enzyme mix preparation**

2.2.3. Talent homogenizing the tissue.

2.3. After homogenization, centrifuge the tissue lysate **[1-TXT]** and incubate for 2 to 3 hours at 56 degrees Celsius **[2]**. *Videographer: This step is important!*

2.3.1. Talent placing the sample in the centrifuge. **TEXT: 9,600 x *g*, 1 min, RT**

2.3.2. Talent incubating sample at 56 °C.

## 3. DNA Extraction

3.1. To extract DNA, pipette 100 microliters of the tissue lysate into a new clean microcentrifuge tube **[1-TXT]**, then add 215 microliters of the magnetic bead master mix **[2-TXT]**. *Videographer: This step is important!*

3.1.1. WIDE: Talent transferring lysate to a new tube. **TEXT: Use microplate for multiple sample processing**

3.1.2. Talent adding magnetic bead master mix. **TEXT: See text for magnetic bead master mix preparation**

3.2. Using a pipette, mix the lysate and the magnetic beads mixture for 10 to 20 seconds **[1]**, then let it stand for 10 minutes at room temperature **[2]**. During the incubation,

gently shake the tube occasionally to maximize the binding of the DNA to the magnetic beads **[3]**.

3.2.1. Talent mixing the lysate and the magnetic bead mixture.

3.2.2. Shot of tube incubating at room temperature.

3.2.3. Talent shaking the tube.

3.3. Next, place the tube on the magnetic bead separator **[1]** until the solution becomes clear **[2]**, then using a pipette, gently aspirate the liquid from the tube without disturbing the magnetic beads holding the DNA **[3]**. *Videographer: This step is important!*

3.3.1. Talent placing the tube on the magnetic bead separator.

3.3.2. Shot of clear solution in the tube.

3.3.3. ECU: Talent aspirating the supernatant without touching the magnetic beads.

3.4. Move the tube away from the magnetic bead separator **[1]** and wash the beads by adding 325 microliters of washing buffer 1 **[2]**. Mix thoroughly by pipetting **[3]** and incubate for 1 minute at room temperature **[4]**. *Videographer: This step is important!*

3.4.1. Talent moving the tube away from the magnetic bead separator. *Videographer: Obtain multiple usable takes because this will be reused in 3.5.3 and 3.7.1.*

3.4.2. Talent adding washing buffer 1.

3.4.3. Talent mixing by pipetting.

3.4.4. Shot of tube incubating at room temperature.

3.5. After the incubation, place the tube on the magnetic bead separator **[1]** and remove the supernatant as previously demonstrated **[2]**. Then, move the tube away from the magnetic bead separator **[3]** and wash the beads once more with washing buffer 1 **[4-TXT]**. *Videographer: This step is important!*

3.5.1. Talent placing tube on the magnetic bead separator.

3.5.2. Talent removing the supernatant.

3.5.3. *Use 3.4.1. Talent moving the tube away from the magnetic bead separator.*

3.5.4. Talent adding 250 µL of washing buffer 1. **TEXT: Second wash: 250 µL**

3.6. After the second wash with buffer 1, wash the beads twice with 250 microliters of washing buffer 2 in the same manner **[1]**.

3.6.1. Talent adding washing buffer 2 to the tube.

3.7. After the last wash, move the tube away from the magnetic bead separator **[1]** and add 100 microliters of elution buffer **[2]**. Mix thoroughly by pipetting **[3]**, then incubate the tube for 2 minutes at room temperature **[4]** before placing it back on the magnetic separator **[5]**. *Videographer: This step is important!*

3.7.1. *Use 3.4.1. Talent moving the tube away from the magnetic bead separator.*

3.7.2. Talent adding elution buffer.

3.7.3. Talent mixing by pipetting.

3.7.4. Shot of tube incubating at room temperature.

3.7.5. Talent placing the tube on the magnetic bead separator.

3.8. When the solution becomes clear, transfer the supernatant into a new clean 0.5-milliliter microcentrifuge tube and store appropriately **[1]**. *Videographer: This step is important!*

3.8.1. Talent transferring the supernatant into a new tube.

# Results

**4. Results: DNA Quality and Cost Per Sample**

4.1. Shown here is a typical microvolume fluorometer reading of the mosquito DNA in an elution buffer containing 0.5 millimolar EDTA. The average 260- to 280-nanometer absorbance ratio is 2.3 **[1]**.

4.1.1. LAB MEDIA: Figure 1.

4.2. This table **[1]** shows the costs **[2]** and the number of samples processed in one working day **[3]** for different extraction methods **[4]**. The typical reagent and consumable cost for extraction by this protocol is around $9.50 per sample **[5].** This cost is equivalent to any typical magnetic-bead-based extraction method **[6]**.

4.2.1. LAB MEDIA: Table 1.

4.2.2. LAB MEDIA: Table 1. *Video Editor: Emphasize the row headings: Sample processing cost, tissue disruption instrument, DNA extraction in instrument, and DNA fluorometer.*

4.2.3. LAB MEDIA: Table 1. *Video Editor: Emphasize the row heading: Typical sample processing per one working day.*

4.2.4. LAB MEDIA: Table 1. *Video Editor: Emphasize all column headings*

4.2.5. LAB MEDIA: Table 1. *Video Editor: Emphasize the sample processing cost for this protocol ($9.50/per sample).*

4.2.6. LAB MEDIA: Table 1. *Video Editor: Emphasize sample processing costs for other manual and automated magnetic bead-based extraction ($12/per sample and $9.60/sample).*

4.3. The major cost-benefit of this protocol comes from not requiring the automated DNA extraction instrument **[1]**.

4.3.1. LAB MEDIA: Table 1. *Video Editor: Emphasize the DNA extraction instrument cost for automated magnetic bead-based extraction ($60,000)*

# Conclusion

**5. Conclusion Interview Statements**

5.1. **Ana:** Make sure to change pipette tips between samples to prevent cross-contamination of DNA when working with multiple samples.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 3.7.3*

5.2. **Kyle:** We used the high-quality DNA extracted using this method for whole-genome sequencing. We are currently using this sequencing data to identify novel mutations in insecticide resistance or immune genes of concern in public health and disease control.

5.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

5.3. **Yoosook Lee:** Having affordable solutions for high throughput sequencing opens exciting doors for population genomics and landscape genomics aimed at understanding mosquito dispersal and gene flow, which influences the success of many novel genetic control strategies.

5.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera