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Isolating DNA from *Wolbachia* and *Drosophila* with the QIAwave DNA Blood and Tissue Kit

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Helene Hartman¹, J. Dylan Shropshire¹

¹Department of Biological Sciences, Lehigh University

Shropshire Lab



J. Dylan Shropshire

Lehigh University

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We use this protocol and it's working

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Abstract

This DNA extraction protocol is column-based and adapted from the manufacturer's recommendations for the QIAwave DNA Blood and Tissue Kit. It has been successfully used to isolate high-quality genomic DNA from a range of *Drosophila* tissues, including testes and ovaries. The protocol is also well-suited for research on maternally transmitted *Wolbachia* endosymbionts.

Materials

Materials

-  QIAwave DNA Blood & Tissue Kit **Qiagen Catalog #69556**
-  Pure Ethanol, 200 Proof **KOPTEC Catalog #V1001**
-  Centrifuge tubes, safe-lock, PCR clean, 1.5 mL **Eppendorf Catalog #022363212**
-  Pipette tips, filtered, PCR clean and sterile, 0.1 - 10 uL **Eppendorf Catalog #0030078519**
-  Pipette tips, filtered, PCR clean and sterile, 2 - 100 uL **Eppendorf Catalog #0030078543**
-  Pipette tips, filtered, PCR clean and sterile, 50 - 1,000 uL **Eppendorf Catalog #0030078578**
-  Homogenizing beads, 2.8 mm, ceramic **VWR International (Avantor) Catalog #10158-554**

Equipment

- Bead-mill homogenizer (Benchmark, IPD9600)
- Centrifuge (Eppendorf, 5420)
- Dry-bath incubator (Eppendorf, F2.0 Thermomixer)
- Freezer (any)
- Fridge (any)
- Pipette, 10 µL (Eppendorf, 4861000708)
- Pipette, 100 µL (Eppendorf, 4861000716)
- Pipette, 1000 µL (Eppendorf, 4861000732)
- Vortex mixer (Any)

Protocol materials

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Safety warnings

-  This protocol involves the use of various chemicals and reagents that require careful handling and strict adherence to safety guidelines to ensure safe laboratory practices. Please review the Material Safety Data Sheets (MSDS) for each reagent before beginning the protocol and take appropriate precautions. All steps should be performed while wearing gloves, a lab coat, and eye protection.

Before start

1. Collect samples for DNA extraction in
 -  Centrifuge tubes, 1.5 mL, safe-lock, PCR clean **Eppendorf Catalog #22363212** . Include 3
 -  Homogenizing beads, 2.8 mm, ceramic **VWR International (Avantor) Catalog #10158-554** in each tube.
2. Preheat dry-bath incubator (e.g., Eppendorf, F2.0 Thermomixer) to  56 °C .
3. Clean workspace with  10 % (v/v) bleach and  70 % (v/v) ethanol.
4. Unpack  QIAwave DNA Blood & Tissue Kit **Qiagen Catalog #69556** . The kit should contain spin columns, collection tubes, Buffer ATL, Buffer AL, Buffer AW1, Buffer AW2, Buffer AE, and Proteinase K. Create aliquots of each solution, as appropriate.
5. Make sure ethanol has been added to Buffer AW1 and Buffer AW2.
6. Check Buffer ATL and Buffer AL for precipitates. Dissolve precipitates at  56 °C .



Lyse Cells

2h 5m

1 Transfer

2m

 Centrifuge tubes, 1.5 mL, safe-lock, PCR clean **Eppendorf Catalog #22363212** containing sample to a bead-mill homogenizer.

Homogenize at  1500 rpm, Room temperature for  00:02:00 .

2 Create lysis mixture containing the following, per sample in a

1m

 Centrifuge tubes, 1.5 mL, safe-lock, PCR clean **Eppendorf Catalog #22363212** :

-  180 μ L Buffer ATL
-  20 μ L Proteinase K



3 Add 200 μ L lysis mixture to each sample.

1m



4 Vortex briefly to mix.

1m



5 Incubate at 56 °C for a minimum of 02:00:00 in a dry-bath incubator.

2h

 Overnight incubation is also acceptable.



(Optional) If using a Thermomixer, set to shake at  300 rpm .



Four samples in a Thermomixer set to 56°C and 300 rpm.

Capture DNA

6m

6 While waiting for the lysis incubation, complete the following:

- Set up 1 set of spin columns.
- Set up 1 set of

3m

☒ Centrifuge tubes, 1.5 mL, safe-lock, PCR clean **Eppendorf Catalog #22363212** .





- Premix  200 μL Buffer AL and  200 μL 200 proof ethanol per sample. Make extra.

7 Add  400 μL of Buffer AL/ethanol mixture to each sample.

Immediately vortex for  00:00:10 to mix.

1m

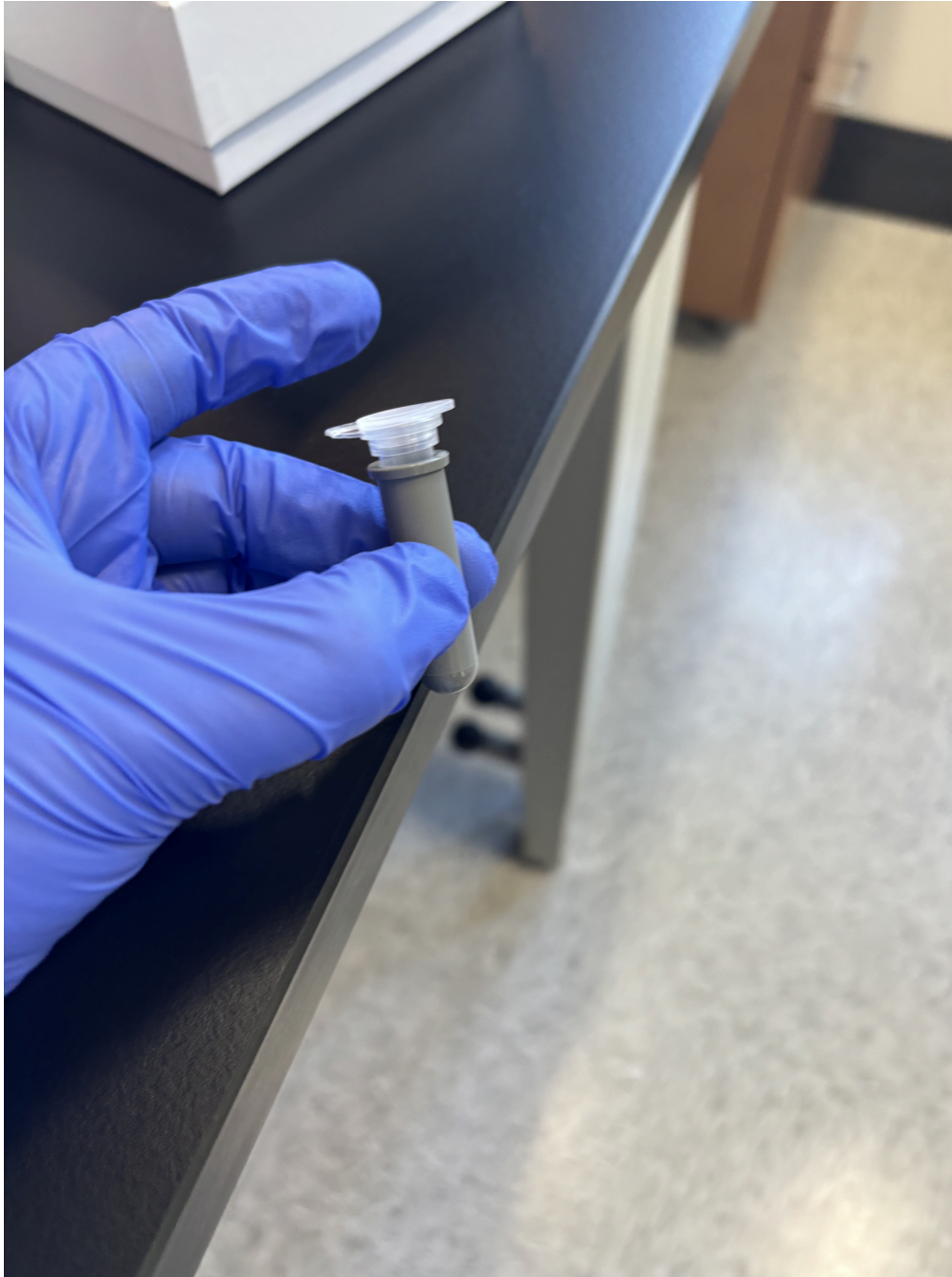


Note

It is important that the samples are immediately mixed after the Buffer AL/ethanol mixture is added. Failing to do so can impact DNA yield.

8 Transfer samples to spin columns in collection tubes.

1m



Spin column within a waste collection tube.

- 9 Centrifuge for  00:01:00 at  16000 rcf, Room temperature to capture the DNA on the column membranes.

1m



Wash DNA

10m

- 10 Empty collection tubes by pouring into a waste container.



11 Add  500 μ L Buffer AW1 to each sample.

1m

12 Centrifuge for  00:01:00 at  16000 rcf, Room temperature to wash the membranes.

1m



13 Empty collection tubes by pouring into a waste container.

1m



14 Add  500 μ L Buffer AW2 to each sample.

1m



15 Centrifuge for  00:01:00 at  16000 rcf, Room temperature to wash the membranes.

1m



16 Transfer the columns to new collection tubes.

1m

17 Centrifuge for  00:03:00 at  16000 rcf, Room temperature to dry the membranes.

3m



Elute DNA

4m

18 Transfer the columns to clean

1m

 Centrifuge tubes, 1.5 mL, safe-lock, PCR clean **Eppendorf Catalog #22363212** .

19 Add  40 μ L buffer AE to the membrane of each column.

1m

Note

Take care to apply Buffer AE directly to the membrane. Salts are likely to accumulate in the sample if the buffer is applied to the plastic.



20 Incubate at  Room temperature for  00:01:00 .

1m



- 21 Centrifuge for  00:01:00 at  16000 rcf, Room temperature to elute DNA from the membrane.

1m



Safety information

As the column prevents complete closure of the centrifuge tube lid, position the lid inward toward the center of the centrifuge. Secure the centrifuge rotor lid properly to prevent tube lid breakage during centrifugation.

- 22 Use immediately or store at:

-  -80 °C for long-term storage
-  -20 °C for use within a few weeks
-  4 °C for use within a few days

