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Isolating DNA from *Wolbachia* and *Drosophila* with the Puregene Tissue Kit

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol is a modified version of the Puregene Tissue Kit manufacturer's protocol. It has been successfully used to isolate high-quality genomic DNA from various *Drosophila* tissues, including testes and ovaries. This method has also been used to extract DNA from the maternally transmitted *Wolbachia* endosymbiont of *Drosophila*.

Materials

Materials

- Puregene Tissue Kit (4 g) **Qiagen Catalog #**158063
- Isopropanol **Fisher Scientific Catalog #**BP2618500
- [M] 70 % (v/v) ethanol from Pure Ethanol, 200 Proof **KOPTEC Catalog #**V1001 and molecular-grade H_2O
- 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
- Glycogen, UltraPure **Invitrogen - Thermo Fisher Catalog #**10814010
- 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)
- Vacuum centrifuge (Eppendorf, Vacufuge Plus)

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- Homogenizing beads, 2.8 mm, ceramic **VWR International (Avantor)** **Catalog #**10158-554
- Centrifuge tubes, 1.5 mL, safe-lock, PCR clean **Eppendorf** **Catalog #**22363212
- Puregene Tissue Kit **Qiagen** **Catalog #**158063
- Isopropanol, molecular biology grade **Fisher Scientific** **Catalog #**BP-2618-1
- Glycogen, molecular biology grade **Invitrogen** **Catalog #**10814-010

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 55 °C.
3. Clean workspace with [M] 10 % (v/v) bleach and [M] 70 % (v/v) ethanol.
4. Unpack Puregene Tissue Kit **Qiagen** **Catalog #**158063. The kit should contain cell lysis solution, protein precipitation solution, DNA hydration solution, and Proteinase K. Create aliquots of each solution, as appropriate.

Lysis

3h 6m

1 Transfer
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 .
2m

2 Create lysis mixture containing the following, per sample in a
Centrifuge tubes, 1.5 mL, safe-lock, PCR clean **Eppendorf Catalog #**22363212 :
- 300 µL cell lysis solution
- 1.5 µL Proteinase K

Prepare a bit extra to account for pipetting errors.
1m

3 Add 301.5 µL lysis mixture to each sample.
2m

4 Mix by inverting the tubes 25 times.
1m

> Note
>
> To process large batches, arrange tubes in a tube rack. Place a second rack on top, sandwiching the tubes. Invert the entire stack to simultaneously invert all tubes.

5 Incubate at 55 °C for between 03:00:00 and 16:00:00 in a dry-bath incubator.
(Optional) If using a Thermomixer, set to shake at 300 rpm .
3h

Protein precipitation

5m

6 Transfer samples to an ice block.
Incubate for 00:01:00 to quickly cool the samples.
1m

7 Add 100 µL protein precipitation solution to each sample.
Immediately vortex each sample for 00:00:20 .
1m

Note

It is important that the samples are immediately mixed after the protein precipitation solution is added. Failing to do so can impact quality of the precipitation.

8 Centrifuge for 00:03:00 at 16000 rpm, Room temperature to pellet the protein. 3m
If the protein pellet is not tight, incubate On ice for 00:05:00 and repeat the centrifugation.

DNA precipitation 4m 15s

9 Add 300 µL 1m
Isopropanol, molecular biology grade **Fisher Scientific Catalog #**BP-2618-1 to a clean
Centrifuge tubes, 1.5 mL, safe-lock, PCR clean **Eppendorf Catalog #**22363212 .
Carefully transfer the supernatant from the prior step to the isopropanol by pipetting.

10 (Optional) Dilute [M] 20 µg/µL 30s
Glycogen, molecular biology grade **Invitrogen Catalog #**10814-010 to [M] 0.5 µg/µL . Add 1 µL to each sample.

Note

Glycogen acts as a carrier molecule, improving DNA precipitation yield, particularly in low-biomass samples.

11 Mix by inverting the tubes 50 times. 1m

12 Centrifuge for 00:01:00 at 16000 rcf, Room temperature to pellet the DNA. 1m

13 Carefully discard the supernatant by pouring into a waste container. 45s
Drain the tube by inverting on a paper towel.
Take care that the pellet remains in the tube.

Note

A single, rapid inversion is essential. Allowing the liquid to settle back to the bottom can dislodge the DNA pellet, leading to loss of yield.

Wash with ethanol

16m

14 Add 300 µL of [M] 70 % (v/v) ethanol to each sample. 30s

15 Mix by inverting the tubes 50 times. 1m

16 Centrifuge for 00:01:00 at 16000 rcf, Room temperature to pellet the DNA. 1m

17 Carefully discard the supernatant by pouring into a waste container. 30s

18 Washing the pellet once more: go to step #14 . 3m

19 Transfer samples to a vacuum centrifuge.
Dry the DNA pellets for 00:10:00 . If using a Vacufuge plus, use the D-AL setting. 10m

Note

Overdrying the DNA pellet can significantly reduce its solubility. It is important to stop the drying process before the pellet becomes completely dry.

DNA hydration

1h 0m 35s

20 Add between 15 µL and 100 µL DNA hydration solution to each sample. 30s

Note

The choice of elution volume depends on the starting material.
For low biomass samples, a lower volume is preferred.

21 Vortex for 00:00:05 to break up the DNA pellet. 5s

22 Choose 1: 1h

- Incubate at 65 °C for 01:00:00 to dissolve the DNA.
- Incubate at Room temperature Overnight to dissolve the DNA.

23 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