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Eradicating *Wolbachia*: A tetracycline-based approach for *Drosophila melanogaster* and other fruit flies

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Brandon Cooper¹, J. Dylan Shropshire²

¹Division of Biological Sciences, University of Montana; ²Department 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

Wolbachia, a maternally inherited endosymbiont, can significantly influence the biology of its arthropod hosts, including *Drosophila melanogaster*. Its presence can confound experimental results. To overcome this challenge, we present a protocol for eliminating *Wolbachia* from *Drosophila melanogaster* and other fruit fly species using tetracycline antibiotics. By treating flies with tetracycline during development, we successfully eradicate *Wolbachia* from the host lineage, enabling precise and controlled experiments. This protocol provides a valuable tool for the *Drosophila* research community. *Wolbachia* researchers can also benefit from this protocol to compare *Wolbachia*-bearing and *Wolbachia*-free insects.

Materials

Materials

- ☒ Centrifuge tubes, safe-lock, Eppendorf quality, 1.5 mL **Eppendorf Catalog #022363204**
- ☒ Pipette tips, filtered, PCR clean and sterile, 2 - 100 uL **Eppendorf Catalog #0030078543**
- ☒ Tetracycline hydrochloride **Fisher Scientific Catalog #BP912**
- diH₂O

Equipment

- Analytical balance (any)
- Drosophila* incubator (Percival, DR-36VL)
- Microwave (any)
- Pipette, 100 µL (Eppendorf, 4861000716)
- Probe thermometer (any)
- Vortex mixer (any)

Protocol materials

☒ Centrifuge tubes, safe-lock, Eppendorf quality, 1.5 mL **Eppendorf Catalog #022363204**

☒ Tetracycline hydrochloride **Fisher Scientific Catalog #BP912**

☒ Pipette tips, filtered, PCR clean and sterile, 2 - 100 uL **Eppendorf Catalog #0030078543**

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

Prepare standard fly food. We use the following protocol:

Protocol



NAME

Preparing food for *Drosophila melanogaster* and its relatives

CREATED BY

J. Dylan Shropshire

Preview

Sterilize the working surface with 10% bleach and 70% ethanol.



Preparing tetracycline-treated fly food.

- 1 Using an analytical balance and a pipette, add the following to a



Centrifuge tubes, safe-lock, Eppendorf quality, 1.5 mL **Eppendorf Catalog #022363204**

:

- 3 mg Tetracycline hydrochloride **Fisher Scientific Catalog #BP912**
- 50 μ L diH_2O

Vortex and invert the tube until the tetracycline to completely dissolve.

- 2 Microwave a vial containing 10 mL of fly food until boiling.



Take care not to allow the food to boil up the sides of the vial.
Stir as needed.

- 3 Allow food to cool to 55 $^{\circ}\text{C}$.



Use a probe thermometer to confirm the temperature.

- 4 Add the dissolved tetracycline to the food and mix thoroughly.



The final concentration of tetracycline is approximately 0.3 mg/mL .

- 5 Allow food to cool to Room temperature .



Use a probe thermometer to confirm the temperature.

Treat flies with tetracycline

8w 5d

- 6 Transfer *Wolbachia*-bearing flies onto the tetracycline-treated media.

- 7 After four days, clear vials of adults.

4d

(Optional) Store vials in a *Drosophila* incubator set to 23 $^{\circ}\text{C}$ or 25 $^{\circ}\text{C}$.

- 8 Monitor vials regularly.

1w

Once adults begin emerging, transfer to a freshly prepared vial of tetracycline-treated food.

- 9 Repeat [go to step #7](#) and [go to step #8](#) until flies have been maintained on tetracycline-treated food for three generations.

3w 1d

- 10 Test progeny for *Wolbachia*.





If the flies still have *Wolbachia*, continue tetracycline treatment for an additional generation and test their offspring for *Wolbachia*.

Note

We use the following protocol to test if flies have *Wolbachia* using PCR:

Protocol

NAME

PCR-based cytotyping to detect *Wolbachia* in insect tissues

CREATED BY

J. Dylan Shropshire

Preview

- 11 Rear *Wolbachia*-free flies on regular fly food for 3 generations before using them in experiments.

4w



Note

Tetracycline can interfere with the host's mitochondria. Providing time for the flies to recover is important to avoid confounding effects associated with mitochondrial disfunction.

Ballard JWO, Melvin RG. (2007) Tetracycline treatment influences mitochondrial metabolism and mtDNA density two generations after treatment in *Drosophila*. Insect Mol Biol. 16(6):799–802. PMID: 18093008