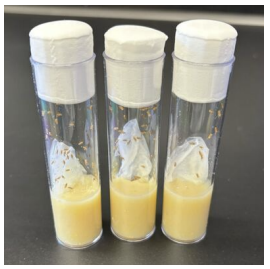
Nov 21, 2024

Long-term maintenance of diverse *Drosophila* species with and without *Wolbachia* endosymbionts



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We use this protocol and it's working

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Abstract

This protocol provides a step-by-step guide for maintaining diverse *Drosophila* species in the laboratory. It assumes that the user has prepared food and established fly colonies. The protocol begins by outlining the preparation of food vials. This includes adding Kimwipes soaked in 0.5% propionic acid to the vials to provide a pupation substrate and increase humidity. We have found this addition to be particularly beneficial for more challenging species. We also detail the process of transferring flies between vials, maintaining accurate records, and establishing a fly emergency room for struggling lines. Using these procedures, we have successfully maintained nearly 90 *Drosophila* species, including those from the Sophophora and Drosophila subgenera, as well as mushroom specialists like *Scaptomyza pallida* with the addition of mushrooms.

Materials

Materials

- Dissection Probe **Amazon Catalog #**B00M04W6NI OR
  Paint Brushes, Small **Amazon Catalog #**B092CN8XJ1
- Droso-Plugs, narrow vials **Genesee Scientific Catalog #**59-200
- Drosophila vials, narrow, polystyrene, reload system **Genesee Scientific Catalog #**32-109RL with flies.
- Drosophila vials, narrow, polystyrene, reload system **Genesee Scientific Catalog #**32-109RL with food.
- Flystuff Narrow Fly Vial Reload Tray **Genesee Scientific Catalog #**59-207
- Kimtech Science™ Kimwipes™ Delicate Task Wipes **Kimberly-Clark Catalog #**34155
- Lab Tape, Rainbow Pack, 19mm x 13m **Fisher Scientific Catalog #**15-901-5R
- Mouse Pad **Amazon Catalog #**B08W47P44Y
- [M] 0.5 % (v/v) propionic acid from
  Propionic acid, ACS reagent, ≥99.5% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**402907 and
  diH_2O.
- Rubber Band, 2 × 1/8 **Genesee Scientific Catalog #**64-104
- Sharpie Permanent Marker **Amazon Catalog #**B00G4CJ8GK

Equipment

- *Drosophila* incubator (Percival, DR-36VL)
- Fridge (any)
- Freezer (any)

Protocol materials

- Kimtech Science™ Kimwipes™ Delicate Task Wipes **Kimberly-Clark Catalog #**34155
- Dissection Probe **Amazon Catalog #**B00M04W6NI
- Paint Brushes, Small **Amazon Catalog #**B092CN8XJ1
- Rubber Band, 2 × 1/8 **Genesee Scientific Catalog #**64-104
- Mouse Pad **Amazon Catalog #**B08W47P44Y
- Sharpie Permanent Marker **Amazon Catalog #**B00G4CJ8GK
- Glad Pressn Seal **Genesee Scientific Catalog #**88-142
- Droso-Plugs, narrow vials **Genesee Scientific Catalog #**59-200
- Propionic acid, ACS reagent, ≥99.5% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**402907
- Drosophila vials, narrow, polystyrene, reload system **Genesee Scientific Catalog #**32-109RL
- Flystuff Narrow Fly Vial Reload Tray **Genesee Scientific Catalog #**59-207
- Lab Tape, Rainbow Pack, 19mm x 13m **Fisher Scientific Catalog #**15-901-5R

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 [M] 0.5 % (v/v) propionic acid solution.

1. Using 10 mL serological pipet, add 2.5 mL of propionic acid to a 500 mL graduated cylinder.
2. Add diH_2O to the 500 mL mark.
3. Pour solution into appropriately labeled wash bottle.
4. Label bottle with the date the solution was made and your initials.

Clean the workspace with [M] 10 % (v/v) bleach and [M] 70 % (v/v) ethanol.

Clean a dissecting probe or brush with [M] 10 % (v/v) bleach and [M] 70 % (v/v) ethanol.

Prepare vials with closures

1h 6m

1 Remove a Flystuff Narrow Fly Vial Reload Tray **Genesee Scientific Catalog #**59-207 with Drosophila vials, narrow, polystyrene, reload system **Genesee Scientific Catalog #**32-109RL containing fly food from the fridge.

1m

Note

We use the following protocol to produce fly food:

Protocol

NAME

Preparing food for *Drosophila melanogaster* and its relatives

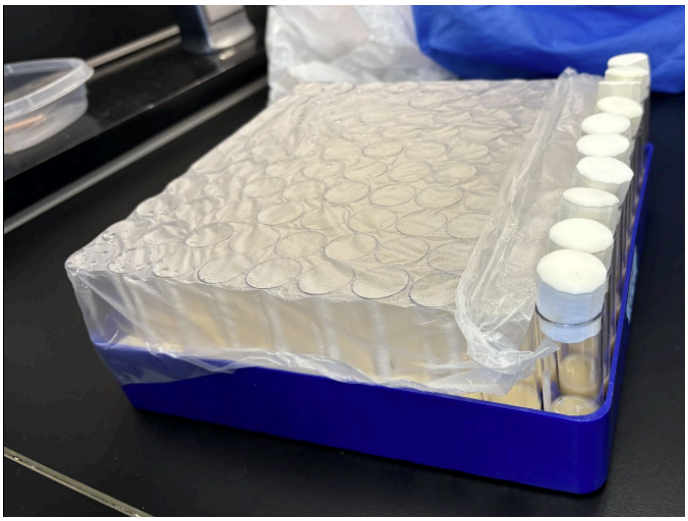
CREATED BY

J. Dylan Shropshire

Preview

2 If vials were sealed with Glad Pressn Seal **Genesee Scientific Catalog #**88-142, peel it back and insert clean Droso-Plugs, narrow vials **Genesee Scientific Catalog #**59-200 or another closure into each vial.

5m



Note

If using previously autoclaved plugs, mark with a Sharpie Permanent Marker **Amazon Catalog #**B00G4CJ8GK. In our experience, plugs can only be autoclaved once. A black dot indicates that plugs should be discarded after use and not re-used.

We use the following protocol to clean and sterilize Droso-Plugs:

Protocol

NAME

Cleaning and sterilizing Droso-Plugs for *Drosophila* maintenance

CREATED BY

J. Dylan Shropshire

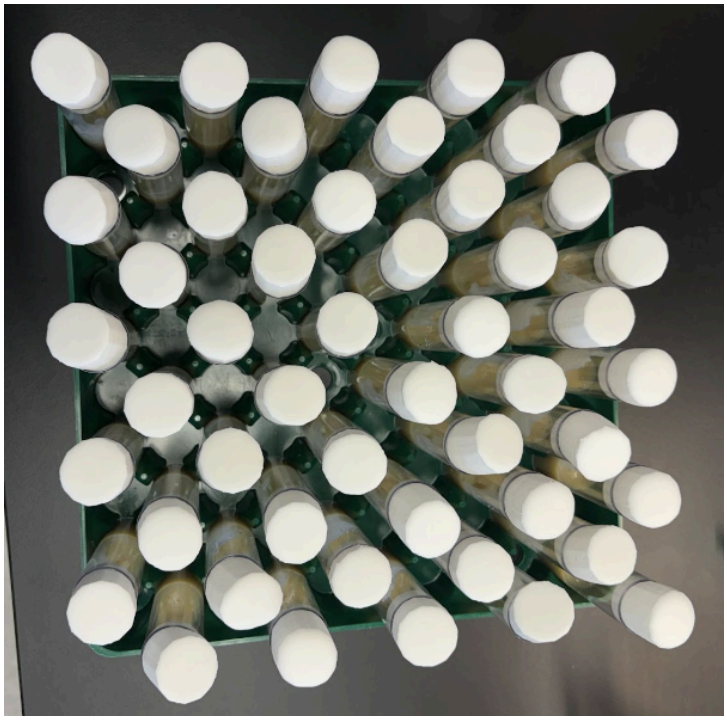
Preview

3 Allow food vials to warm to room temperature. 1h

Note

Warming the vials is very important.
Exposure to temperature extremes can impact *Wolbachia* abundance in flies.
Flipping flies into cold food vials could result in loss of *Wolbachia*.

Warming the vials can be sped up by moving half of the vials in a tray to a separate tray so there is an empty space next to each vial.
You can also move the vials to a 23 °C to 25 °C incubator to warm them up faster.



Prepare vials with Kimwipes 10m

4 1m

Note

We add Kimwipes soaked in [M] 0.5 % (v/v) propionic acid to fly vials to provide an attractive pupation substrate and add additional humidity to the vial.

Not all flies require this preparation. It is unlikely this will ever hurt a line, but it may not be necessary for all lines.

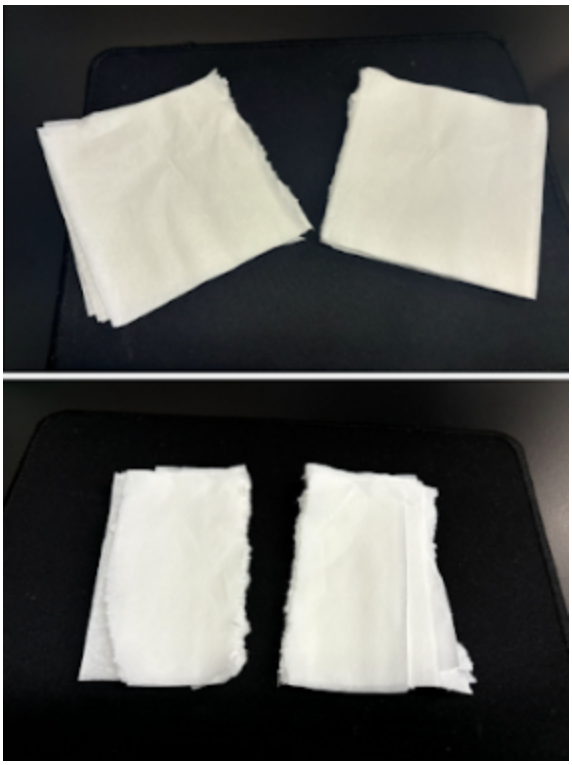
As a rule of thumb, use this preparation when an established stock already has it or if a stock does not have it and looks unhealthy.

Remove several

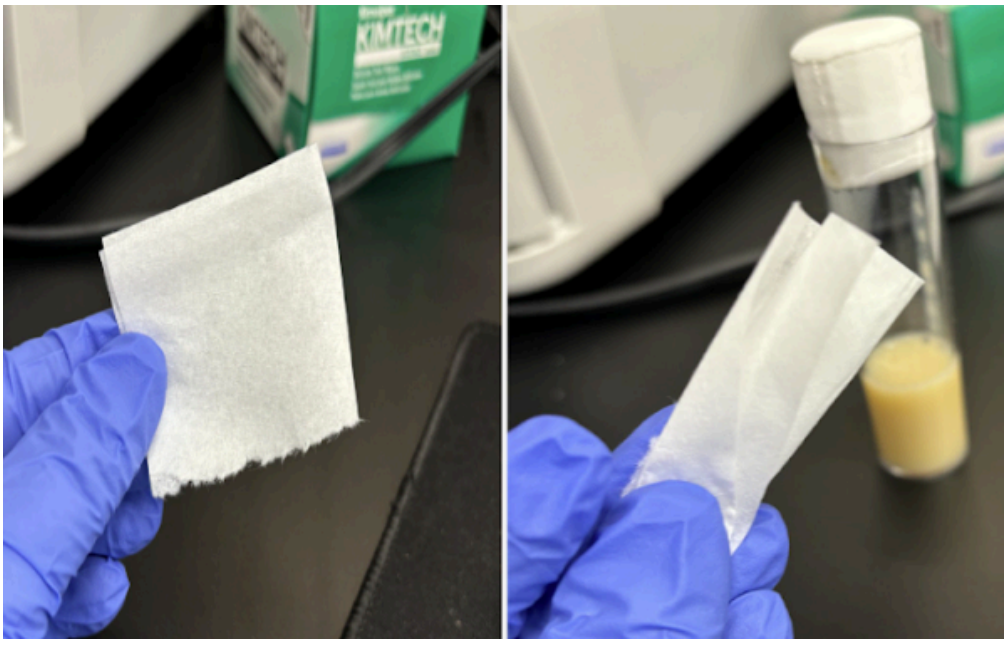
Kimtech Science™ Kimwipes™ Delicate Task Wipes **Kimberly-Clark Catalog #**34155

from the box and place on top of one another.

5 Rip pile of Kimwipes in half and then into quarters. 1m



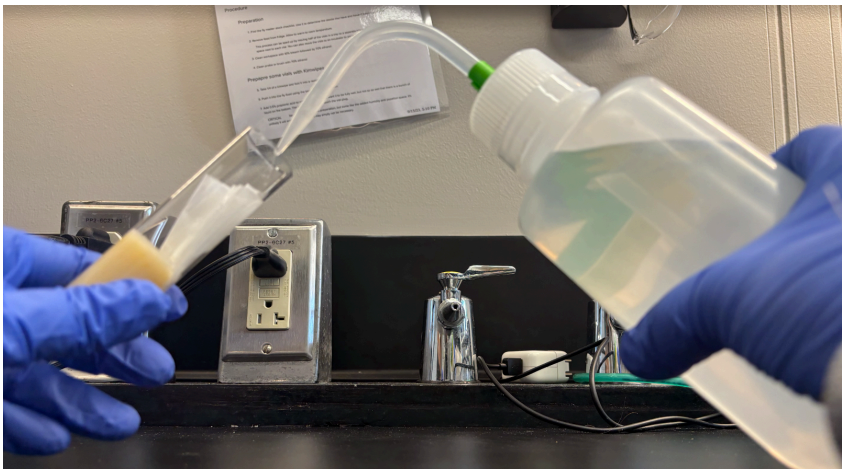
6 Fold quarter in half and then into an accordion-style rectangle. 2m



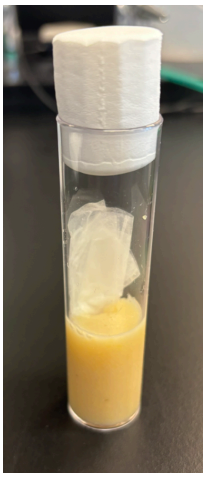
7 Push the folded Kimwipe into the fly food using the back of a Dissection Probe **Amazon Catalog #**B00M04W6NI or Paint Brushes, Small **Amazon Catalog #**B092CN8XJ1. 2m

8 Add [M] 0.5 % (v/v) propionic acid to wet the Kimwipe. 1m

The Kimwipe should be fully wet but not so wet that there is liquid pooling atop the food.



9 Push the moist Kimwipe against the side of the vial so that it does not block flies from food. 2m



Note

The Kimwipe must **not** touch the vial plug.
If the Kimwipe touches the plug, it can pull moisture into the plug and into the air, drying the vial.

10 Label Kimwipe prepped tray with: 1m

- The date of prep
- Your initials
- The bottle of propionic acid used

The top label displays the date fly food was prepared and the initials of the person who prepared the fly food. The bottom label displays the date and initials for the person who prepared the Kimwipe vials. The propionic acid bottle used to prepare the vials is also indicated on the label. In this case the propionic acid bottle was labeled "Insect 7".

Flip flies

5m 30s

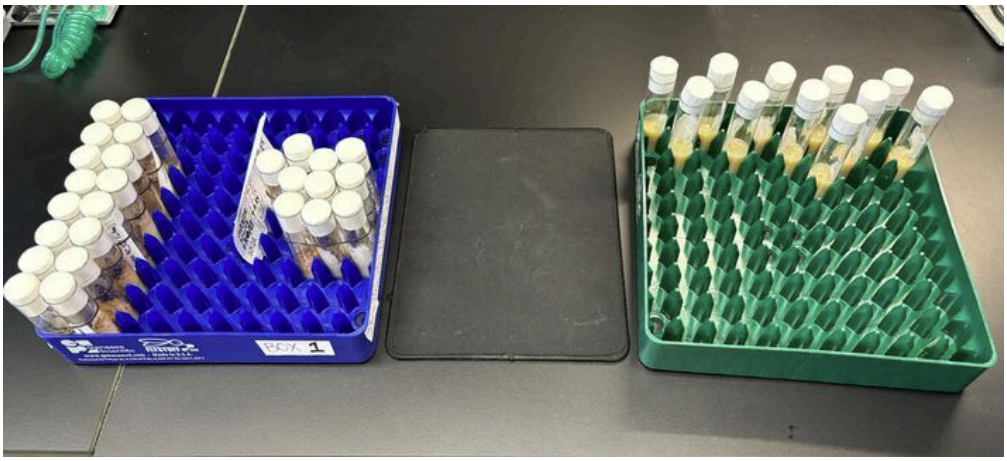
11 Open the stock maintenance sheet in your lab notebook. 1m

Note

We use digital lab notebooks with the following columns in the sheet:
- Box name (Each tray of stocks receives a unique name.)
- Vials needed (The number of vials needed to maintain the stocks.)
- Week of (The week that stocks are maintained.)
- Date transferred (The date that stocks are maintained.)
- Notes on stocks (Any notes on the health of the stocks.)
- Initials (The initials of the person who performed stock maintenance.)

Every time stocks are maintained, the table is duplicated with the dates, notes, and initials replaced.

12 Set a tray of fly stocks to your non-dominant side. 1m
Set a tray of fresh vials to your dominant side.
Put a Mouse Pad **Amazon Catalog #**B08W47P44Y between them.



Note

While flipping, the vial will be repeatedly tapped against the work surface to move flies away from the opening. The mouse pad is used to soften the impact. It is optional.

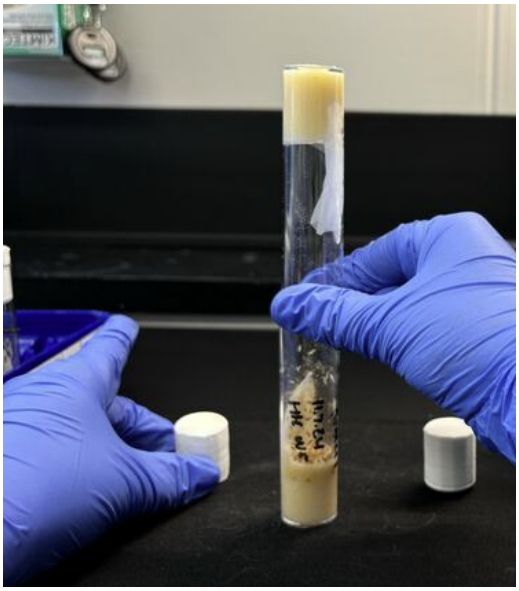
13 Grab the oldest healthy vial and put it in your non-dominant hand.
Grab a new clean vial in your dominant hand.

5s

Note

Most healthy lines will have 2-3 vials in the stock, at minimum.
Use a Rubber Band, 2 × 1/8 **Genesee Scientific Catalog #**64-104 to keep vials of the same stock together.

14 In a quick motion: 5s
1. Tap the fly-containing vial on the mousepad to get the flies to the bottom.
2. Quickly remove the closures from both vials.
3. Flip the new vial on top of the old vial so that the openings touch.



15 Flip both vials over so that the fly-containing vial is on top. 5s
Gently tap on the mousepad to transfer the flies into the new clean vial.

Note

To prevent dislodging food from the fly-containing vial during transfer, avoid using excessive force when tapping the vial. Gentle tapping is sufficient to encourage the flies to move.

16 After the flies have been transferred, tap down again briefly to transfer them to the bottom.
Quickly replace the closure.

5s

17 Take the tape label affixed to the old vial and transfer it to the new vial with flies.
Alternatively, label the vial with a sharpie marker.
If expanding a stock, add a new label.

5s

18 Put the old vial into a discard tray.

5s

19 Repeat steps go to step #13 to go to step #18 for each fly line. 30s
Between fly lines, inspect the immediate area for stray flies.

20 When finished with a tray, record the date and appearance of all stock in your lab notebook. 30s
Move the flies to a *Drosophila* incubator or store at room temperature.

21 When finished for the day, move the discard tray of vials to -20 °C Overnight 1m
to kill the flies.
Treat as biohazardous waste.

22 Clean the workspace with [M] 10 % (v/v) bleach and [M] 70 % (v/v) ethanol. 1m

Maintaining a fly emergency room (ER)

23 If a line of flies looks unhealthy or their numbers are diminishing, remove it from the stock tray and place in a new tray designated as a fly ER.

24 Monitor lines in the ER on a routine basis (i.e., every other day).
Observing the line should reveal why the line is unhealthy.
Take appropriate steps to improve the health of the stock, such as adjusting humidity, quickly transferring emerged flies to new vials after adult emergence, or adjusting the temperature.