

# Changing fly Stocks

## AUTHORS

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## ABSTRACT

Changing fly stocks properly is essential for maintaining healthy fly stocks. This protocol gives step by step instructions for changing *Drosophila* stocks.

## INTRODUCTION

*Drosophila melanogaster* serves as an important genetically tractable model system to study a variety of biological questions. Most fly strains with transgenes or mutations are kept as live stocks and flipped on a regular bases. For genotypes that are unstable or not in use but need to be kept for years, cryopreservation of ovaries or embryos may be used. The rate of *Drosophila* development is temperature dependent. Generation time is approximately 10 days at 25°C and 20 days at 18°C. Most stocks are kept at 18C. However, some stocks may not grow well at 18C. Keep another copy at RT or 25C until you are sure the stock can reproduce well at 18C. For maintaining fly stocks, the adults needs to be transferred to fresh vials every few generations, usually 2-3 weeks for 25°C stocks and 4-5 weeks for 18°C stocks. This protocols gives step by step instructions for changing *Drosophila* stocks. It also covers how to deal with sick stocks.

## MATERIALS

### REAGENTS

- Food vials (*see* REAGENT SETUP)
- DI water
- Ethanol

### EQUIPMENT

- Dissecting Microscope
- Cotton Balls
- CO<sub>2</sub> pad
- CO<sub>2</sub> gun
- Waste container with ethanol
- Paper towels
- Paint Brush
- Labeling tape
- Sharpie

### REAGENT SETUP

- **Preparation of food vials**
  - We have two types of food, brown and yellow. The brown food is usually drier than the yellow food, so adults are less likely to get stuck to the food. You may need to add water

to the brown food more often than the yellow food. The generation time in brown food is around 2 days longer than in yellow food. Do not use food older than one and half months.

- Make sure the food is not too wet nor too dry/old. (When the food is too wet, when you tilt the vial you can see water droplet running down the side of the vial, or when you gently tap the vial you can see the food move.)
- Place cotton balls in the top of vials. (Some people like to flip the vials upside down without putting in cotton balls but I think it doesn't save much time. Do not put in cotton balls in the vials and leave the tray out for more than a week. The plastic seal and bag help to keep the moisture. Also mites can still get in the vials through the cotton balls. Some people like to put in yeast before putting in cotton balls but if you do that, make sure you don't put too much yeast and you use these vials that day because flies don't like old yeast.)
- Check corner vials for mites or mite eggs if the tray has been opened previously. (If you left food, even those with cotton ball and plastic bag around the fly station for several days, you might have mites in your food.)

## PROCEDURE

1. Remove balancer before putting flies to stocks if possible to prevent stock from accumulating mutations. Do not keep double balanced stocks if possible. Mark unhealthy stocks to make sure enough flies are flipped. Label the fly stocks with the complete genotype using reusable tapes. Make good annotation of stock by listing the genotype and markers for easy checkup for contamination. **Table 1** shows an example annotation. **Table 2** and **Figure 1** shows common markers in fly stocks.

**Critical Step** Keep two independent copies of important stocks in separate locations to prevent loss of stocks by accident.

**Table 1**

location	genotype	eye color	marker	From	Notes
T1A1	w; slbo-Gal4/CyO	mW	Cy1	Wei	outcross 1 generation from Dani's fly

**Table 2**

Balancer	Marker	phenotype
FM7C	B <sup>1</sup>	Heterozygotes have a deep anterior nick in the adult eye.
	y	Yellow body color
	w	White eye
CyO	Cy <sup>1</sup>	Wings curled upward, may not be fully penetrant at 18C
	Sco	Missing bristles, especially from posterior thorax
TM3	Sb <sup>1</sup>	Bristles short and stubby
	Ser	Wings notched

	e	Darker body color
TM6B	Hu	Extra humeral bristles are formed.
	Tb <sup>1</sup>	larvae and pupae are shorter
	e	Darker body color
TM2	Ubx	Haltere larger and rounder than normal
	e	Darker body color

Figure 1



- Wipe down the area with ethanol, pick up objects and be sure to clean under them. This is done to ensure that no mites or other contaminants get into your stocks.

**Critical Step** Change mites paper every month and remove unattended flies from the incubator and fly room. Empty the fly morgue when it gets full. Remove the fly waste bag when it gets full. When this becomes a routine it will save a lot of effort on checking for mites in each vial and rescuing sick stocks.

- Perform a quick check for mites in the vials. If you see vials with very few flies and it looks very dry and has a lot of white spots you should suspect mite infestation. If you have mite problem you should check every vial before flipping. Check for mites under a microscope. Mites are small round arthropods who like to eat sick flies. When looking for them tilt your vial at a slight angle to prevent glare from the microscopes light. Mite eggs are tiny round and can be seen on the wall of vials.
- This step can be performed using option A-D depending on whether or not mites were found in the vial, and the condition of the vial. **Table 2** lists all the possible conditions. For how to nurse/rescue sick stocks, see **Troubleshooting**.

**Critical Step** To avoid skipping vials and mixing different vials together make sure all vials are clearly labeled and quickly check the phenotype. Also, once the vials are flipped place them in a new tray. It is important to keep two copies of each stock just in case a mistake occurs you still have an extra copy.

**Table 2**

condition	Two balancer or unhealthy stock	mites	# females available	CO2 pad	CO2 gun	# female to transfer	nurse/rescue
1	0	0	>15	0	1	~10	
2	0	0	8-15	0	1	all	
3	0	0	4-8	0	0	all	
4	0	0	<4	0	0	all	1
5	1	0	>8	0	1	all	
6	1	0	<8	0	0	all	1
7	0	1	>4	1	1	5-10	
8	0	1	<4	1	1	all	1
9	1	1	>8	1	1	all	
10	1	1	<8	1	1	all	1

- A. Condition 1: No Mites >30 healthy adult flies (~15 females).
- i. Obtain a freshly prepared vial of food with yeast and loose the cotton ball.
  - ii. Obtain the desired stock and pull the cotton ball part way out to make inserting the CO<sub>2</sub> gun easier. Turn the knob on the CO<sub>2</sub> valve to begin the flow of CO<sub>2</sub>. Before inserting the gun into the vial check the intensity of the CO<sub>2</sub> on your hand to make sure it will not push the flies into the wet food.
  - iii. Anesthetize the flies with a small amount of CO<sub>2</sub> (look for when the flies start to “slow down”). When doing so, orient the vial horizontal so the flies won’t fall all the way into the food or to the cotton ball.  
**Critical Step** When using CO<sub>2</sub> the less you can use and the shorter time the flies are anesthetized the better.
  - iv. Remove the cotton ball and tap the desire amount of flies into the new vial.
  - v. Secure the new vial with its cotton ball.  
**Critical Step** Make sure to create a nice seal to prevent flies from escaping. If any flies fall off the CO<sub>2</sub> pad and not into the fly morgue, immediately kill them so no further stocks are contaminated.
  - vi. Tap the rest of the adults into the fly morgue (waste container with ethanol) and secure the old vial with its cotton ball.
  - vii. Make sure the new vial is properly labeled and put back to its own location.
- B. Condition 2 and 5: no mites, 15-30 adult flies (~8-15 females).
- i. Obtain a freshly prepared vial of food with yeast and loose the cotton ball.
  - ii. Obtain the desired stock and pull the cotton ball part way out to make inserting the CO<sub>2</sub> gun easier. Turn the knob on the CO<sub>2</sub> valve to begin the flow of CO<sub>2</sub>. Before inserting the gun into the vial check the intensity of the CO<sub>2</sub> on your hand to make sure it will not push the flies into the wet food.
  - iii. Anesthetize the flies with a small amount of CO<sub>2</sub> (look for when the flies start to “slow down”). When doing so, orient the vial horizontal so the flies won’t fall all the way into the food or to the cotton ball.
  - iv. Remove the cotton ball and tap all flies into the new vial.
  - v. Secure the new vial with its cotton ball.
  - vi. Secure the old vial with its cotton ball.
  - vii. Make sure the new vial is properly labeled and put back to its own location.
- C. Condition 3-4 and 6: no mites, <15 adult flies.
- i. Obtain a freshly prepared vial of food with yeast and loose the cotton ball.
  - ii. Obtain the desired stock. If food is not wet, gently tap all the flies down so they are not close to the cotton ball, continue tapping while removing the cotton ball and then quickly connect the new vial on top of the old vial. If food is wet, invert the vial and let the flies climb up, keep the vial tilted upside down while removing the cotton ball and then quickly connect the new vial.
  - iii. Reorient the vials and tap all the flies out of the old vial into the new one, keep tapping when removing the old vial from the top and secure this vial with the new cotton ball.

- iv. Secure the old vial with its cotton ball, add water if vial is dry but still have larvae.
- v. Make sure the new vial is properly labeled and put back to its own location.  
**Critical Step** Make sure there are at least 4 healthy females or at least 8 double balanced/unhealthy females and a few males (females have lighter pigmentation in the terminal of the abdomen and a bigger abdomen than males), then flip directly without CO<sub>2</sub> (It is gentler to the flies and helps them to reproduce better). If there is not enough females see TROUBLESHOOTING.

D. Condition 7-10: mites

- i. Obtain a freshly prepared vial of food with yeast and loose the cotton ball.
- ii. Obtain the desired stock and pull the cotton ball part way out to make inserting the CO<sub>2</sub> gun easier.
- iii. Anesthetize the flies with a small amount of CO<sub>2</sub> (look for when the flies start to “slow down”) and orient the vial horizontal so the flies won’t fall all the way into the food or to the cotton ball.
- iv. Remove the cotton ball and tap all the flies onto the CO<sub>2</sub> pad (don’t tap too hard because mites/dead flies will all fall out)
- v. If you have a lot of flies, get the new/healthy ones and make sure there are no mite eggs attached. If you have few flies, remove mites/dead flies and brush off the mite egg from the old flies.  
**Critical Step** When flipping stocks it is always better to use the newest and healthiest flies to help propagate the line. Old flies tend to have mite eggs attached to their bodies. You can identify age difference in flies by comparing eye color (the older the darker), abdomen shape and size (older flies have curved shrunk abdomens while newer flies look “fuller”), wing shape (older flies tend to have notches on their wings compared to newer flies or have their wings stuck to their bodies because of food), and also older flies may develop a fungus on their head and body.
- vi. When you have separated your desired flies place them towards the edge of the CO<sub>2</sub> pad and place your new vial flush with its edge so that there is no gap between the vial and the CO<sub>2</sub> pad.
- vii. Brush the desired flies into the new vial and seal with the new cotton ball. Dump the remaining flies on the CO<sub>2</sub> pad into the fly morgue. Clean up the CO<sub>2</sub> pad to make sure no mites/mite egg remains.  
**Critical Step** Anesthetize on pad and sort out mites/mite eggs. Make sure there are at least 4 healthy females or at least 8 double balanced/unhealthy females and a few males (females have lighter pigmentation in the terminal of the abdomen and a bigger abdomen than males). If not enough females, see TROUBLESHOOTING.
- viii. If the old vial has mites remove the vial and place it in a new tray. Additionally, create a quarantine area on your own bench with new mite paper. Clean station well when finished to prevent spread of mites. When cleaning incubator trays spray with EtOH and Benzyl Benzyl before taping the mite paper.

5. Keep the old vial in the left side and the new vial in the right side. After 5-10 days, quickly check the new vial to make sure stocks are growing properly. If mite contamination is a concern in the lab/stock room in the past month, throw away the old vial as soon as the new vial appears to have an adequate amount of progeny.

**Critical Step** For 18°C stocks, change every month. This will save you time on sorting flies on pad.

## TIMING

Step 1, establishing proper fly stock and record: it takes at least one fly generation time to remove balancers if necessary.

Step 2, cleaning station: 1 min

Step 3, checking for mites: 5-30 sec for each stock

Step 4, flipping stocks: 30-60 sec for each stock or 30-60 min for a tray.

Step 5, check the flipping: 5 min for a tray.

## TROUBLESHOOTING

Where there are few to no females due to the following conditions, for conditions 1 and 2 continue straight to rescuing, for conditions 3 and 4 one step has to be performed before continuing to rescuing:

1. Not enough flies were flipped into the current vial
2. Stock is very old at time of flipping
3. Stocks may be intrinsically sick due to mutations or balancers  
To prevent this from happening, outcross sick files to remove accumulating mutations that make the fly sick, or remove unnecessary balancers from the stock.
4. Mite Infestation  
Remove any mites and or mite eggs and discard old vials as soon as possible.

Rescuing:

For rescuing stocks, if there are still females left add the flies to a vial with new yeast and some yeast paste. Place a piece of red tape on top of the vial as a reminder to check back in 5 days. Perform a follow-up check on the new vial 5 days later add more adults from the old vial if necessary. If there are no females left but only males left cross them with corresponding balancer flies to reestablish the stock.

How to amplify flies by crossing them to balancer flies:

Step 1. Amplify the chromosome using balancer chromosomes.

Step 2. Remove balancer chromosomes if possible.

Examples:

Transgene or mutant A located on X chromosome:

FM7C; Sco/CyO X A/Y → collect virgin and male A/FM7C; CyO/+ X A/Y; CyO/+ → collect virgin and male A X A/Y

Transgene or mutant B located on 2nd chromosome:

Sco/CyO X B → collect virgin and male B/CyO X B/CyO → collect virgin and male B X B

Transgene or mutant C located on 3rd chromosome:

TM3/TM6B X C → collect virgin and male C/TM3 X C/TM3 → collect virgin and male C X C

How to outcross flies to remove accumulated mutations that causes sickness:

Step 1. Outcross to wildtype flies.

Step 2. Allow recombination to happen between mutant chromosome and wildtype chromosome in the female.

Step 3. Establish lines from individual males.

Step 4. Select for healthier line.

Examples:

Transgene or mutant A located on X chromosome: W1118 X A/FM7C → collect virgin A/+ X FM7C/Y; Sco/CyO → collect individual male and cross with balancer virgins FM7C; Sco/CyO X A/Y; CyO/+ → collect virgin and male A/FM7C; CyO/+ X A/Y; CyO/+ → keep the line that produces healthier progenies.

Transgene or mutant B located on 2nd chromosome: W1118 X B/CyO → collect virgin B/+ X Sco/CyO → collect individual male and cross with balancer virgins Sco/CyO X B/CyO → collect virgin and male B/CyO X B/CyO → keep the line that produces healthier progenies.

Transgene or mutant C located on 3rd chromosome: W1118 X C/TM3 → collect virgin C/+ X TM3/TM6B → collect individual male and cross with balancer virgins TM3/TM6B X C/TM3 → collect virgin and male C/TM3 X C/TM3 → keep the line that produces healthier progenies.

## ANTICIPATED RESULTS

If all the stocks are properly flipped you should expect there to be around 30-40 flies in each vial. There should be an even distribution of male and female flies and the flies should appear healthy and have no mites.