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A beginners guide to tipping flies (Drosophila)

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We use this protocol and it's working

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Abstract

This protocol gives basic instructions and advice to beginners on tipping *Drosophila* flies between food vials when maintaining stock lines or during an experiment. It will be helpful to read this before commencing your stock care or experiment, however the best way to get better at tipping flies is with practice!

- 1 Order an appropriate number of Lewis vials (Lewis, 2014) approximately 1 week before you require them (or in accordance with your institutes guidelines).
- 2 Ensure your Lewis vials are at room temperature before tipping flies into vials. Remove vials from 4°C at least 2 hours prior to tipping.

Note

Adding flies to cold Lewis food will temporarily anaesthetise them, potentially causing them to stick to the food and consequently die.

- 3 Remove the cotton wool and sprinkle the surface of the Lewis medium with a little instant, dry yeast. This will promote egg laying.

Note

If leaving the yeast sprinkled vials unplugged then do not leave unattended- if an escapee female in the lab finds her way into one of these vials and lays eggs this could ruin the continuity of your stocks by mixing two genetically variant populations.

- 4 Using a tapping mat to reduce noise, gently tap the old stock vial so all individuals fall to the bottom. Quickly remove the cotton wool and tip the vial upside down onto a fresh, unplugged Lewis vial. Gently tap both vials, holding them securely together, until approximately 20 to 30 individuals have fallen into the new vial (check that you can see a good number of both females and males in your new vial). Separate the two vials, inverting the old vial back up the right way, while continuously tapping them down on the mat (to reduce escapees) and quickly re-plug both with cotton wool. Some people prefer to use a funnel during this process- have a play about and see which way you prefer. If no longer needed, the old vial can be disposed of in accordance with your institutes waste disposal guidelines.

Note

It can be tempting to add more than 20-30 individuals if you want lots of eggs but this will cause overcrowding- resulting in high competition for available food during the larval stage which can increase the development time of offspring. Furthermore if you add far too many individuals, a high frequency of social interactions, particularly from males attempting to copulate, will result in females being constantly bothered thus reducing the amount of time spent egg laying and resulting in a lower egg count than at lower population numbers.

- 5 Leave your adult flies in for however long you have decided is appropriate- generally flies are left in for 48 hours for egg laying but, depending on your experiment and the

species/genetic variant, it can be more suitable to leave them in for only 24 hours/up to 72 hours.

- 6 When you are ready to tip out your flies, firstly check to see if there are eggs in your vials- if there are not many you may want to consider leaving the flies in for another 24 hours. Once happy with your egg numbers, tap the vial down, quickly remove the cotton wool ball and invert the vial into a funnel placed over a fly morgue (a bottle filled with water and detergent), tapping the vial down into the funnel until all individuals have fallen out of the vial into the morgue.

Note

Ensure no flies are left in the vial. Sometimes individuals stick to the food and will not fall out when you tap down the vial. Use tweezers to remove these individuals/push them down into the food to ensure they cannot become free again and mix with the next generation.

- 7 Leave your offspring generation to develop. At 25°C this whole process from egg to adult eclosion should take around 11-13 days from the initial day you added the parent flies to the vial. The eggs take around 24 hours to hatch, the larvae will pass through three instar stages over approximately 5-6 days (this varies with population density-with a longer development at higher densities) and then pupate, staying in the pupae for approximately 4-5 days before eclosing. The number of offspring you get will vary depending on the line/mutant/species of fly you are using for your experiment but as a general estimate – if you leave 20-30 flies in a vial for 48 hours you will get around 50-100 eclosing offspring.