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Timed collection of Drosophila Virgins

For most genetic crosses, ensuring that the female flies are virgin is absolutely essential. Generally, there are two approaches. First, you can visually select young females that have large abdomens, poorly developed wings, and a dark spot on their abdomen. This approach works well, but can be wasteful, especially if flies are in short supply.

 Second, one can time collections to ensure that most females will be virgin. This is the preferred solution in many cases. The key is that newly hatched females do not accept mates for ~8-10 hours at 25 degrees, and ~16 hours at 18 degrees.

Day 0 – 5-7pm: Empty all the adult flies from the vials or bottles. If there are any adults stuck in the food or inaccessible, use a paintbrush to remove them. Many pupae should be darkening at this stage, indicating that they will hatch soon. Move the vials or bottles to 18 degrees overnight.

Day 1 – 8am: Less than 16 hours after you moved the flies to 18 degrees, dump all the newly hatched adults from the cultures and sort out every female. Store these in a fresh vial. Discard the males. Ensure that all adults are removed from the cultures and move these flies to 25 degrees. Store the virgins at 18 degrees.

Day 1 – ~2pm: In the middle of the day (less than 8 hours after your previous collection), sort out the females and put them into a fresh tube. Store these virgins at 18 degrees. Discard the males, ensure that all adults are removed, and return the flies to 25 degrees.

Day 1 – 6pm: At the end of the day (less than 8 hours after your previous collection), sort out the females and put them into a fresh tube. Store these virgins at 18 degrees. Discard the males, ensure that all adults are removed, and return the flies to 18 degrees overnight.

Day 2 – repeat.

If there’s doubt about whether the flies truly are virgin, watch the vials for 3-4 days before using. If there are larvae, throw out the entire vial.