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A simple method to achieve consistent larval density in bottle cultures.

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Whenever fitness is being measured on adult flies, consistent larval density in culture is critical. It is critical also when flies are under selection for fitness-related traits. The ideal method is to count larvae into culture vials. The larvae are picked so that they are all first instar. This is a common method of controlling density. Sometimes, demands of time or of numbers of flies required make this a difficult proposition. Here we describe a technique that is quick, simple, and inexpensive to achieve consistent larval density in bottle culture.

First, collect eggs. Collecting from many flies in a cage is ideal, because you can retrieve many eggs in a short period. This will ensure that most of the larvae will be at roughly the same developmental stage, thus reducing adverse effects of competition on younger and smaller larvae. It will also make virgin collection, if necessary, simpler as most flies will emerge over a shorter period.

Next, via a funnel, wash the eggs into a plastic 50 ml tube using 1× PBS (phosphate buffered saline) from a washbottle, dislodging them from the egg-laying medium with a soft brush. The tube must have a conical bottom end. Wash the remaining eggs from the funnel sides and allow them to settle. Pour off the supernatant leaving 5-10 ml of solution remaining with the eggs. Add more PBS for washing. If live yeast paste was used on the egg-laying medium to promote laying, several washes may be necessary to achieve a clear solution. If you have avoided yeast in the tube altogether, one wash will be sufficient. After the final wash, allow eggs to resettle to the bottom of the tube.

Next, eggs are aspirated from solution using a 100 µl micropipette, with a yellow/100 µl pipette tip cut sharply 6 mm from the end. This enlarged bore size allows eggs to enter freely and the tip can be used many times. The plunger is depressed, the tip lowered just into the mass of eggs and then the plunger is released quickly (*i.e.*, let go) as the tip is moved further into the egg mass to ensure maximum uptake. This should result in a well-packed volume of eggs and consistency can be achieved with only a little practice. The eggs can then be expelled onto the culture medium surface.

Setting the micropipette at 13 µl, we achieved a mean egg density of 223 ± 14.3 (95% CI). A doubling of the volume results in something less than a doubling of the egg number.

This method can also be used when comparing balanced mutant stocks with wildtype or other controls. For instance, assume the number of eggs delivered per 13 µl aspiration is 240 and that the experimental stock suffers balancer lethality so that one quarter of the eggs will not develop. To adjust the number of eggs upward to achieve equal adult numbers, multiply the number of eggs required by one third, so that 320 eggs will give 240 adults. In this case we set the micropipette to 21 µl, which seemed to work well.

There is one note of caution to remember; it is critical that the equipment is washed thoroughly between aspiration of eggs from different strains, otherwise culture contamination can easily occur. We apply water at high pressure from the tap followed by careful visual inspection.