Larval rapid fat body isolation

Fat bodies prepared in this way are good for Western blotting, RNA, enzyme assays, and metabolomics.

1. Squirt PBS on a slide. Get a straight razor blade and two #5 forceps that are not bent. Rinse larvae in a sieve and use a paintbrush to transfer them to the pool of PBS. You can put up to 30 or 40 larvae on at a time.

2. Bisect larvae near the posterior end using the razor blade, discarding the posterior spiracles and a small amount of hindgut. The sooner you do this, the fewer larvae crawl away.

3. Invert larvae (turn them inside out) using the forceps.

4. Once all are inverted, transfer to one or two Eppendorf tubes containing 500 µL PBS using forceps.

** You can insulin stimulate or culture organs at this point **

5. Using a 1 ml pipette and plunging slowly, pipette the inverted larvae up and down 5-10 times. If your tip is too narrow, cut the end off until the opening is about 2 mm. You should see floating fat body and other organs dislodged from the carcass, then stop pipetting.

6. Spin the tubes for 1 minute at > or equal to 13,000 rpm.

Depending on the diet and stage, fat bodies will now be either floating on top of the PBS (easiest) or as a white layer sitting on top of the pelleted carcasses/organs (more difficult). There may be both floating and pelleted fat bodies.

7A- Floating fat bodies: Draw off fat bodies with a 1 ml pipette onto a clean glass slide.

-Visually inspect and remove any testes, trachea, discs, etc. using forceps and transfer fat bodies with a fresh tip to a fresh tube.

7B- Pelleted fat bodies: Gently pipette the PBS up and down over the fat bodies, which dislodge more easily than the carcass pellet. If the carcass pellet dislodges, repeat the spin and try again. When you see the fat bodies swirling around with gentle pipetting, draw off fat bodies with a 1 ml pipette onto a clean glass slide being careful not to suck up any extra material from the carcass pellet.

-Visually inspect and remove any testes, trachea, discs, etc. using forceps and transfer fat bodies with a fresh tip to a fresh tube.

8. Spin another 1 minute at > or equal to 13,000 rpm.

9A- Floating fat bodies: Place a 200 µl tip under the layer of fat bodies and remove supernatant from below.
9B- Pelleted fat bodies: Place a 200 µl tip above and aspirate the supernatant. Final volume ~ 50-100 µl.