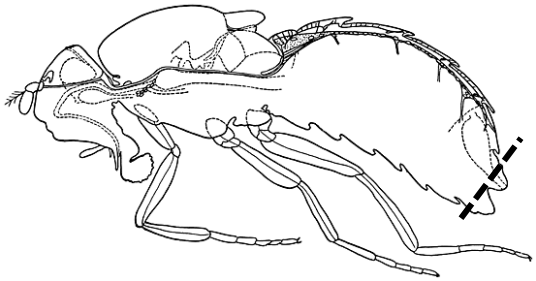
Heart prep for tissue collection and *in vivo* physiology (SOHA)

Preparation

1. Warm artificial hemolymph to room temperature if dissecting for heart rate analysis. PBS is used for heart collection.
2. Anesthetize flies with FlyNap for 3 minutes in an empty vial using black swabs.
3. Prepare dissecting surface with a thin layer of Vaseline. Surface can be a glass slide or small petri dish.
    -For heart imaging, keep a portion of glass clean and free of petroleum jelly.
4. Jewelers #5 fine forceps (Fine Science Tools 11252-20) and micro dissecting scissors (FST 15000-00) and a homemade pooter are also needed.

Dissection

1. Using forceps, transfer fly dorsal side down with wings spread to the side so abdomen directly contacts the Vaseline.
2. Add artificial hemolymph or PBS to cover flies.
3. Cut off legs using micro scissors.
4. Cut off the posterior tip of abdomen so the scissor can be inserted, taking care to not cut too far up on abdomen. (This will negatively affect the heart.)



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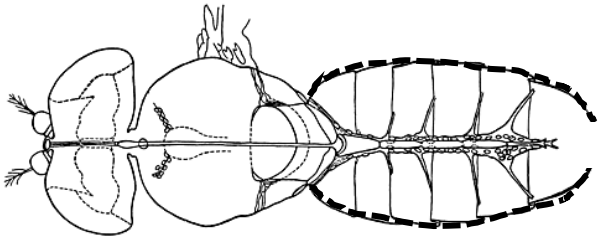
Miller (1950)

5. Cut off the ventral cuticle to expose internal organs.



Miller (1950)

6. Use pooter to suck out organs including gut, Malpighian tubule, and fat bodies. Fine forceps and/or additional cuts may be needed.
7. Use hooked probe to gently scrape fat body from lateral edges of the heart away. Suck excess fat body with pooter.

(Diagram)

If removing the heart, gently insert hook between dorsal side of the heart and ventral side of the cuticle at the posterior end, then drag? the probe to the anterior end to release the heart. Use #5 fine forceps to pluck heart out of the fly and place in PBS at -20°C. 10 hearts per 50 μl in a 500 μl tube works well for metabolomics.

If imaging the heart, zoom in to check if the heart is intact and beating. If so, equilibrate for 20 minutes before recording video. This is also a good prep for fixing and staining the heart.