

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

### Preparation

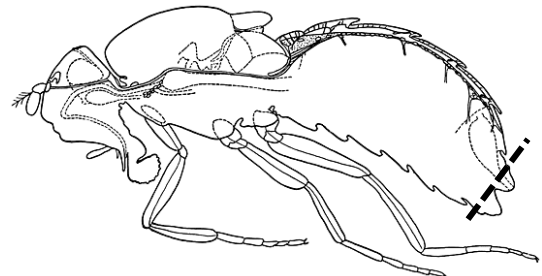
1. Warm artificial hemolymph to room temperature if dissecting for heart rate analysis. PBS is used for heart collection.
2. Anesthetize flies with FlyNap for 3 minutes in an empty vial using black swabs.
3. Prepare dissecting surface with a thin layer of Vaseline. Surface can be a glass slide or small 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.
5. Prepare pooter: Obtain 24 inches of 1/8<sup>th</sup> in rubber tubing. Using capillary tubes and Bunsen burner, flame and pull to make a small diameter capillary tube. Cut a gel loading tip on each end such that the larger end fits snugly in the rubber tubing and the pulled capillary tube fits snugly in the small-bore end. This aids in sucking out fat body without disturbing the heart. Two pooters may be needed, one with the small capillary tube and one with a gel loading tip.

### Dissection

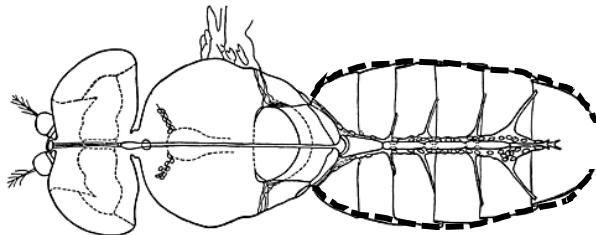
1. Using forceps, transfer fly dorsal side down with wings spread to the side so abdomen directly contacts the Vaseline.
2. Add artificial hemolymph or PBS to cover flies.
3. Cut off legs using micro scissors.
4. Cut off the posterior tip of abdomen so the scissor can be inserted, taking care to not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



Miller (1950)

cut here: - - - - -

5. Cut off the ventral cuticle and remaining legs 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 without touching the heart its self (this may damage the heart and cause unintended arrhythmias). Suck excess fat body with the fine 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 move 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 30 minutes before recording video. This is also a good prep for fixing and staining the heart.

#### Mounting:

1. Obtain a new clean slide and place a rubber gasket well on the surface. (Gasket is 20x20mm, 2.5 mm deep with 1cm well. Electron Microscopy Sciences Cat. #: 70336-46. Detailed instructions for use with package). Using nail polish, seal the outer edges where the gasket meets the slide glass.
2. Using a #1 22x22mm coverslip (VWR Cat. #: 48366-067), place a thin, straight line of petroleum jelly on the cover slip and place in a clean dish with ADH covering the coverslip.
3. From dissecting dish, grab the fly gently by the thorax and place on the coverslip, ventral side up with head and thorax in petroleum jelly. Abdomen must be hanging over clean glass.
4. If abdomen is not lying flat on the glass, use forceps to drag a small piece of petroleum jelly to the posterior end of the abdomen to tack it down.
5. Place 1 – 5 flies on each coverslip being sure to take note of genotype and sex of each fly. Best practice is to segregate by sex and genotype per coverslip.
6. Add ADH to the well such that the ADH level surpasses the top of the well.
7. Using forceps, carefully remove coverslip and flip the fly side down on to the well such that the anterior end of the fly is pointing to the etched portion of the slide.
8. Use a Kimwipe to remove excess ADH overflowing from the well and clean BOTH coverslip and slide before viewing under the microscope.

#### Video Capture:

1. Place slide on the stage of the Zeiss inverted microscope with Luminera camera attached. At this point, allow the hearts to recover for 30 minutes. In this time set up the microscope, microscope light and StreamPix7 software (Refer to StreamPix7 manual on computer desktop under “StreamPix7” folder).
2. Ensure you are using the 10x objective, Ph2 and LED light source. Focus in on the fly cuticle using the oculars.
3. Open StreamPix7, the camera will automatically open at this time.
4. Focus in on the fly’s 2<sup>nd</sup> heart chamber from the anterior of the abdomen.
5. On StreamPix7, open “Live Adjustments” in the top left corner.
  - a. Keep exposure to about 7.1. This will keep the Frames Per Second (FPS) to 140. Any higher and FPS will be sacrificed for better lighting.
  - b. Avoid auto exposure target.
  - c. Increase or decrease brightness to get a viewable heart
  - d. Keep contrast as low as possible without greying out the picture
  - e. Keep Gamma as low as possible to keep picture clear and without pixilation

- f. Set Gain to 1.
  - g. SOHA software depends on changes in pixel density and color. The crisper the image, the better analysis.
  - h. May have to do multiple rounds of focusing and live adjustments to get the heart clear and heart walls visible.
6. After 30 minutes of acclimation, select the "Record" button. StreamPix7 will prompt a name for the video. Use the SOHA naming convention:  
Genotype\_Set\_MolarFood/AgeSex\_Camera\_Time\_Repetition.,  
Ex: Vw1118\_01\_1M1F\_Luminera\_10s\_Rep1
  7. Take 5 videos of 10 seconds each for each fly changing the name of each fly by changing the repetition number and move on to the next fly. Remember to keep track of the set number and change for each consecutive fly.
  8. Refer to the StreamPix7 manual for any issues.
  9. Data will be saved automatically in Samsung 850 PRO 512GB > StreamPix7\_Data > Camera1 > Date of capture (Ex: "08-30-17")

#### Video Storage:

1. Videos collected will be stored as uncompressed AVI files as well as a duplicate SEQ file. The AVI file can be moved to its appropriate folder for processing (Ex: "Vw1118\_01\_1M1F\_Luminera\_10s\_Rep1" will go to a "1 week 1M Vw1118" folder on within the Samsung 850 PRO 512GB drive.
2. Once AVI files are moved to appropriate folder, remaining folders labeled as the date (08-30-17) can be moved to TOSHIBA EXT (E) drive.

#### SOHA Analysis:

1. Directions for SOHA analysis is both on hard copy or digital copy and should be read and understood prior to conducting any SOHA experiment. Reference for SOHA is Fink et al, 2009. Biotechniques. Video file with directions also available on desktop in SOHA folder.
2. Open SOHA on desktop
3. Click File > Analyze Data > File with AVI videos > ok
4. Highlight top file > Right click > Mark
5. Scroll back and forth on the video to find maximum diastole for the first beat, mark each edge of the heart wall, click on the beat until you get to diastole and check the highlight box.
  - a. Ensure the marks are directly in line with each other
6. Scroll again to find systole for the first beat and mark both edges of the heart, check the highlight box.
7. Using the cursor, draw a box around the group of points, select highlight
8. Accept. You may reject if the video is not useful or quit