5.2 Rapid Larval Midgut Extraction

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Introduction

Once mastered, this technique allows the researcher to quickly separate the midgut from the rest of the body, and is therefore suitable for procedures which require the pooling of large amounts of tissue, such as protein and/or RNA extraction.

Materials

- Dissection dish (a Petri dish that has its bottom coated with a fine layer of Sylgard® (silicone, Dow-Corning Corporation, Midland, Michigan)
- Stereoscope
- Fine forceps
- Microdissection scissors
- Dissecting needles or minutien pin mounted on a long wooden stick
- Clean microscope slides and cover slips

Protocol

1. Place larvae on ice for 10-15 minutes in order to immobilize them.
2. Transfer each larva to a dissection dish containing 70% ethanol.
3. Pin the head capsule to the bottom of the dish using a minutien pin (sometimes placing the larva ventral-side up can facilitate this task).
4. Using sharp microdissection scissors, clip-off abdominal segments 8-10.
5. Use the sides of two sets of forceps to carefully ‘squeeze’ the gut out of the larval body. Start by applying pressure at the head/thorax junction (**Figure 5.2.1 A**), and work your way across the rest of the thorax and the abdomen using a stepwise motion. The gut should progressively protrude out of the distal end of the abdomen (**Figure 5.2.1 B**).
6. After the gut has been extracted, it might need to be carefully cleaned of attached fat-body and/or large tracheal trunks. Clean guts should immediately be transferred to the appropriate reagents for protein or RNA isolation.

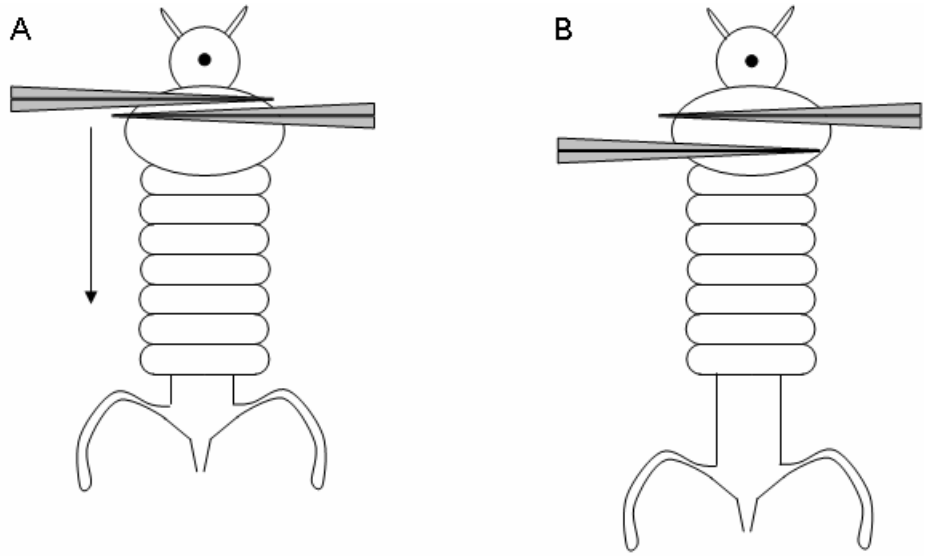

Figure 5.2.1. Larval mosquito midgut extraction.