

Video Article

Injection of dsRNA into Female *A. aegypti* Mosquitos

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Abstract

Reverse genetic approaches have proven extremely useful for determining which genes underly resistance to vector pathogens in mosquitoes. This video protocol illustrates a method used by the James lab to inject dsRNA into female *A. aegypti* mosquitoes, which harbor the dengue virus. The technique for calibrating injection needles, manipulating the injection setup, and injecting dsRNA into the thorax is illustrated.

Video Link

The video component of this article can be found at <https://www.jove.com/video/215/>

Protocol

1. Make microinjection needles by pulling glass capillary tubes. We use a needle puller from Sutter Instrument CO, (Model P-2000). The needle puller program specifications are:
heat: 275
fil: 3
vel: 37
del: 250
pul: 125
2. Load a known volume (e.g., 1 μ l) of water in the needles and manually mark the scale with a fine sharpie.
3. Set up a post-injection cup for the mosquitoes. First cut the outmost tip off of a 1000 μ l pipette tip. Then, cut a hole in the side of a cup and insert the remaining part of the pipette tip in the cup. Put a net or lid on top of the cup.
4. Fill two Styrofoam boxes with ice. Place ice box #1 under a stereomicroscope and place a glass watchglass on the ice.
5. Anesthetize the adult mosquitoes with CO₂ and place cup with adults in ice box #2.
6. Prepare needle for injection of dsRNA. Put needle filled with dsRNA on the plastic hose of injection block (for description of injection block see below).
7. Move an anesthetized mosquito from the cup to the watch glass with forceps. Break injection needle tip on mosquito cuticle. Discard this mosquito.
8. For injections, the needle is inserted into the thorax of the mosquito. With your free hand, adjust valve on injector from "resting position" to "injection position" and compress plunger until desired amount of dsRNA has been injected.
9. Place injected mosquito into post-injection cup.

Injection block set up

1. Fix a 50 ml syringe to a cinder block.
2. Attach an adjustable 1-way valve to the end of the syringe with at least 2 air outlets.
3. Attach an airtight plastic hose to 1 air outlet.
4. Insert a micropipette adapter into the free end of the hose.

Disclosures

The authors have nothing to disclose.