Video Article

Injection of An. stephensi Embryos to Generate Malaria-resistant Mosquitoes

Olle Terenius[1], Jennifer Juhn[1], Anthony A. James[2]

[1]Department of Molecular Biology and Biochemistry, University of California, Irvine (UCI)

[2]Department of Molecular Biology and Biochemistry, Department of Microbiology and Molecular Genetics, University of California, Irvine (UCI)

Correspondence to: Olle Terenius at olle.terenius@uci.edu, Anthony A. James at aajames@uci.edu

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Abstract

The introduction of exogenous genes into the genomes of mosquitoes requires microinjection techniques tailored to the specific species of interest. This video protocol demonstrates a method used by the James laboratory to microinject DNA constructs into Anopheles stephensi embryos for the generation of transformed mosquitoes. Techniques for preparing microinjection needles, collecting and preparing embryos and performing the microinjection are illustrated.

Video Link

The video component of this article can be found at http://www.jove.com/video/216/

Protocol

Preparation in advance of microinjection:

1. Blood feed mosquitoes: for injection from Monday to Wednesday feed females on the previous Friday. To inject Thursday and Friday, feed females on Monday of the same week.

2. Prepare the Quartz needles using program 2 and isotonic buffer (see Materials).

3. "Laying tube" for the embryos is the regular Drosophila culture vial. Wet cotton wool in the bottom, with a wet disk of filter paper covering it.

4. Prepare plastic cover slip by sticking double-side tape to one end. Trim tape to cover slip so that it ends at edge of cover slip.

5. Prepare oil for desiccation.

6. Prepare a Petri dish with isotonic buffer to transfer embryos for hatching.

Set up of forced laying:

1. Collect 6-10 blood fed females with the use of an aspirator and transfer them to the *Drosophila* culture vial with cotton and filter paper wet with isotonic buffer.

2. The mosquitoes are then put back into insectery conditions in the dark and allowed to lay eggs for 1 h and 15 min.

3. Let adults fly into the cage and remove the filter paper disk with embryos.

4. To line up eggs, do it under the dissecting scope. Collect bunches of eggs with a fine paintbrush (Sable, No 0000) and transfer to a prepared square of 3MM Whatman paper soaked with isotonic buffer. Keep paper all time wet. Don't let eggs get desiccated. Remove the exochorion with the brush, it helps to better stick eggs to the tape.

5. Using fine forcep No. 5 or a fine paintbrush, pick up darker grey embryos and arrange in line on square of 3MM Whatman wet with isotonic buffer. Line up from 20-30 embryos. All embryos must be in the same orientation as injection has to be at posterior pole. The anterior end of the embryos is slightly wider than the posterior. Then, using stripes of 3MM Whatman paper, dry the filter where the eggs are lined up by pressing hard on both sides of the egg line, not touching the eggs.

6. To transfer the eggs, invert the slide containing the double sided tape (Medicine), and gently press against the eggs. The posterior end of the eggs have to be very close to the edge of the double sided tape. The wetness of the paper is critical for this, if it is too wet they won't stick. I do desiccation of the embryos from **5-10** second, but desiccation time depends from humid and temperature in microinjection room.

7. **Desiccation** is crucial for eggs: little bit too much and embryos will not hatch. Without desiccation DNA is not going into embryo.

8. Cover desiccated embryos with halocarbon oil to prevent further desiccation.

Microinjection of the embryos:

The most important aspect of good injection is the quality of the needle (see Note).

1. Fill a needle with the DNA solution to be injected by using a microloader (Eppendorf #5242 956.003). Very little injection soliution is needed, 1 to 2 ml.

2. Connect the needle to the Eppendorf Transjector, which controls the injection time and pressure, as well as the backpressure. Microinjection is performed using a microscope with a moving stage (Leica) at x 10 magnification and micromanipulator (Leica). If necessary, a raised microscope stage may be prepared by stacking 4-5 microscope slides. Place the cover slip carrying the embryos onto raised stage. Embryos are injected at a 150° angle; penetration should be at the posterior pole. For injection, I keep the needle stationary and move the stage of the microscope. When the needle is new, the tip is sealed and usually breaks in the first injection. I always work out the pressure needed to expel a small droplet of DNA into oil in between each injection.

3. I set the injection time to 0.2 - 0.9 sec and 1000 hPa when the needle is new. I vary the pressure and time until a small droplet is seen coming out from the needle into the oil. The backpressure needs to be set around 100. As the needle tip gets worn, the injection pressure has to be reduced to keep the injection volume low (about 300 hPa). After this, the needle tip is too large and is killing most of the the embryos.

4. After injection, remove the oil with tissue paper and cover the slide with embryos and place in the Petri dish with isotonic buffer. Place the dishes in the insectory and leave them there until the embryos hatch. They start to hatch after 2-6 days. Transfer the larvae to distilled water with the pinch of ground fish food.

Notes

DNA for injection: Plasmid DNA solution for injection was isolated using EndoFree plasmid kit. Construct plasmid and Helper plasmid mix together in right concentration, precipitated with isopropanol. The pellet was washed with 70% ethanol before resuspending in injection buffer. Before injection, clean DNA using Millex-GV column.

Izotonic buffer:

1.68 M NaCl - 88.3 ml	OR	5 M NaCl - 29.67 ml
1.68 M NaCl - 2.9 m		1 M KCl - 4.87 ml
1 M Hepes -10.7 ml		1 M Hepes - 10.7 ml
1.12 M $CaCl_2$ - 2.2 ml		1.12 M $CaCl_2$ - 2.2 ml
dH_2O - 896 ml		dH_2O - 952.56 ml

Adjust to pH 7.2