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Title: Preparing and Injecting Embryos of Culex Mosquitoes to Generate Null Mutations Using CRISPR/Cas9

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes**

   If **Yes**, can you record movies/images using your own microscope camera?

   Yes

   If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope.

   Olympus SZX 12 with camera port. The camera we have is a AmScope HD200VP-UM 1080p HDMI Digital Camera for Standalone and PC Imaging

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

At IBBR filming of interviews will need to occur outside the building to conform with IBBR Covid-19 policies.

☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? Filming will take place in two different rooms at the same location. The rooms are across the hall form each other.

Current Protocol Length

Number of Steps: 14
Number of Shots: 27

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Megan Meuti:** This protocol provides detailed instructions on how to prepare and inject mosquito embryos for CRISPR/Cas9 genome editing.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Rob Harrell:** The main advantages of this microinjection technique are that it has a high rate of mosquito survival and allows researchers to determine the function of a gene.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

OPTIONAL:

1.3. **Megan Meuti:** This protocol has been specifically tailored to inject *Culex* mosquito embryos. This genus of mosquitoes transmits several pathogens, including viruses that cause West Nile fever and St. Louis encephalitis, as well as filarial nematodes that cause elephantiasis and canine heartworm.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.4. **Rob Harrell:** Injecting freshly laid insect embryos and getting a high rate of survival takes lots of time, practice and patience. Therefore, we recommend injecting several embryos with a colored saline solution before injecting expensive materials to generate a mutation.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

2. Injecting *Culex* Embryos

2.1. Begin by backfilling the injection needles with the injection mix **[1]**. Choose a needle filler or gel loading tip that will fit easily into the back end of the selected injection needle. Make sure that there is a bit of space between the end of the needle filler and the injection needle **[2]**.

2.1.1. WIDE: Establishing shot of talent at the lab bench preparing to fill the needle.

2.1.2. Appropriate needle filler or gel loading tip.

NOTE: Shot number 2.1.2 also includes the footage for shot 2.2.1.

2.2. Carefully aspirate a single, small drop of the injection mix into the injection needle, near the spot where it begins to taper **[1-TXT]**, then attach the injection needle to the micro-injector **[2]**. Place the slide containing the embryos onto the stage of the microscope **[3]**.

2.2.1. Talent aspirating the drop of injection mix into the needle. **TEXT: 0.5 – 1 µL**

2.2.2. Talent attaching the needle to the micro-injector.

2.2.3. Talent placing the slide onto the microscope stage.

2.3. To open the needle, use a starting injection pressure of approximately 30 PSI and adjust it as necessary to deliver an appropriate volume of injection mix **[1]**.

2.3.1. Talent adjusting the injection pressure.

2.4. Under a dissection microscope, position the needle above the mosquito embryo **[1]** and carefully use the micromanipulator to lower the needle onto the embryo **[2]**. Once the needle is barely touching the embryo, quickly move the embryo perpendicular to the long axis of the needle **[3]**. *Videographer: This step is difficult and important!*

2.4.1. Talent at the microscope.

2.4.2. SCOPE: 61651 Culex injertion video.mp4. 00:56 - 01:04

2.4.3. SCOPE: 61651 Culex injertion video.mp4. 01:32 – 01:38

2.5. To determine if the needle was successfully opened, press the injection trigger **[1]** and see whether any bubbles or injection mix escape from the needle. If not, repeat moving the mosquito embryo against the needle until it is open and the injection mix escapes when the trigger is pressed **[2]**. *Videographer: This step is important!*

2.5.1. Talent pressing the trigger.

2.5.2. SCOPE: 61651 Culex injertion video.mp4. 01:39 - 01:50

2.6. Position the first mosquito embryo in the center of view, ensure that it is in focus **[1]**, and place the needle over the first egg **[2]**.

2.6.1. SCOPE: 61651 Culex injertion video.mp4. 01:52 – 01:59

2.6.2. SCOPE: 61651 Culex injertion video.mp4. 01:59 – 02:03

2.7. Progressively increase the magnification on the microscope, carefully lowering the needle to keep it just above the first embryo. Proceed until the microscope has reached its highest magnification and both the embryo and the needle are in focus **[1]**.

2.7.1. SCOPE: 61651 Culex injertion video.mp4. 01:50 – 01:54, 02:04 - 02:16

2.8. Move the embryo slightly to the left and lower the needle so that it is in the same plane of view as the embryo **[1]**. Use the microscope stage to gently touch the embryo to the tip of the needle. The narrow, posterior end of the mosquito embryo should deflect slightly **[2]**.

2.8.1. SCOPE: 61651 Culex injertion video.mp4. 02:26 – 02:30

2.8.2. SCOPE: 61651 Culex injertion video.mp4. 02:30 – 02:32

2.9. Using the microscope stage, move the posterior end of the embryo onto the needle and observe the needle penetrating the chorion of the mosquito egg **[1]**.
Videographer: This step is difficult and important!

2.9.1. Talent moving the microscope stage.

2.10. The needle should just penetrate the membrane in the approximate location of the pole cells within the embryo. Placing the injection mix in this region increases the likelihood that mutations will occur and be inherited in the germline **[1]**.
Videographer: This step is difficult and important!

2.10.1. SCOPE: 61651 Culex injertion video.mp4. 02:32 – 02:34

2.11. Pull the injection trigger 1 to 3 times **[1]** to shoot injection mix into the embryo. Deliver a small amount of injection mix, just enough that a small clearing in the embryoplasm can be detected **[2]**.

2.11.1. Talent pulling the injection trigger.

2.11.2. SCOPE: 61651 Culex injertion video.mp4. 02:34 – 02:38

2.12. Use the stage to move the embryo to the right, away and off the tip of the needle. If the injection went well, little to no fluid should leak from the embryo **[1]**.
Videographer: This step is important!

2.12.1. SCOPE: 61651 Culex injertion video.mp4. 02:38 – 02:47

2.13. Move the stage down so that the next embryo is positioned in front of the needle and repeat the injection **[1]**. If injection mix does not appear to be coming out or if a large

amount of fluid escapes from the embryo after the needle is removed, replace the injection needle and start again **[2]**. Destroy any un-injected or damaged eggs **[3]**.

2.13.1. SCOPE: 61651 Culex injertion video.mp4. 02:47 – 02:52

2.13.2. Talent replacing the needle.

2.13.3. SCOPE: 61651 Embryo removal.mp4. 00:34 – 00:41

2.14. After all embryos on the slide have been injected, wash off the Halocarbon oil by applying reverse osmosis water with a squirt bottle. Direct the stream of water onto the top of the glass coverslip above the injected embryos and allow the water to flow down the coverslip, making sure to not direct the stream onto the embryos **[1]**.

2.14.1. Talent rinsing the slide with water.

2.15. Cover the slide with 150 microliters of water and place it on wet filter paper inside of a square Petri dish **[1]**. Incubate the Petri dish in an environmental chamber set to 25 to 27 degrees Celsius and 70 to 80% relative humidity with a long day photoperiod **[2]**.

2.15.1. Talent adding water to the slide.

2.15.2. Talent putting the slide in the Petri dish.

NOTE: Shots 2.15.1 and 2.15.2. were combined together into a single shot and their order was inverted.

2.15.3. Talent putting the dish in the environmental chamber and closing the door.

Results

3. Results: Transmission of the Desired Mutation

3.1. This protocol was used to successfully inject embryos of the Northern house mosquito, *Culex pipiens (pronounce 'Q-lex pip-ee-ins')* **[1]**. Ensuring that the anterior end of the mosquito embryo extends beyond the strip of medical dressing greatly increased larval survival, and results in high quality offspring that are capable of developing to adulthood and reproducing **[2]**.

3.1.1. LAB MEDIA: Figure 2 A.

3.1.2. LAB MEDIA: Figure 2 B.

3.2. Sequencing revealed that approximately 10% of the screened mosquitoes showed a small insertion or deletion near the Cas9 cut site of the first guide RNA, suggesting that the pole cells were successfully targeted during microinjections **[1]**.

3.2.1. LAB MEDIA: Figure 3.

3.3. The F1 individuals successfully mated and produced viable offspring, some of which also contained the mutation **[1]**.

3.3.1. LAB MEDIA: Figure 3.

Conclusion

4. Conclusion Interview Statements

4.1. **Rob Harrell:** When microinjecting insect embryos, most people struggle with initially opening the needle and moving the embryo onto the needle. Remember that developing these skills takes time and practice.

4.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.4, 2.10.*

4.2. **Megan Meuti:** This protocol can be adapted to inject the embryos of other mosquitoes or insects. Beyond using this protocol to knock-out genes with targeted CRISPR/Cas9 genome editing, plasmids and transposons can also be injected to knock-in genes or generate random mutations.

4.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

4.3. **Megan Meuti:** My lab is currently using this technique to determine how mosquitoes measure daylength and regulate seasonal responses. However, CRISPR/Cas9 genome editing is being used by multiple researchers to better understand the biology of several organisms.

4.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.