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Title: Embryo Microinjection Techniques for Efficient Site-Specific Mutagenesis in *Culex quinquefasciatus*

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Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **Yes.**

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

   No.

   If **No**, JoVE will need to record the microscope images using our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope.

   Olympus BX41

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

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Consult with author regarding shots needing PPE

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Michelle Bui:** Embryo microinjection techniques have paved the way for the development of numerous genetic based vector control tools. Although numerous insect species have protocols already well developed, Culex quinquefasciatus remains relatively understudied.

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

1.2. **Michelle Bui:** This microinjection protocol has been specifically designed to accommodate the unique biological traits of Culex quinquefasciatus including egg collection, egg raft separation, and post injection procedures.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 3.5.3*

OPTIONAL:

1.3. **Michelle Bui:** This method can likely be adapted to any Culex species of interest and can be used to study gene function, or to generate genetic-based control technologies for Culex species.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 2.1.3*

Protocol

2. Alignment of *C. quinquefasciatus* Pre-blastoderm Stage Embryos

2.1. Begin by separating the eggs from the rafts **[1]**. Use forceps or a paintbrush to press down on the raft and tease apart the individual eggs **[2]**. Align the eggs on a thin strip of double-sided sticky tape placed across the top of a glass slide, making an effort to point the anterior side of each egg in the same direction **[3]**. *Videographer: This step is important!*

2.1.1. WIDE: Establishing shot of talent at the lab bench preparing to separate the eggs.

2.1.2. SCOPE: Talent pressing down on the raft and releasing individual eggs.

2.1.3. SCOPE: Talent aligning the eggs on the tape.

2.2. Prepare the halocarbon mix by gently mixing halocarbon reagents with water **[1]** and incubating the mixture at 25 degrees Celsius overnight **[2]**. Cover aligned eggs with the halocarbon oil mixture **[3]**.

2.2.1. Talent mixing the halocarbon reagents and water. NOTE: Do not use tk1

2.2.2. Mixture incubating. NOTE: tk4 best

2.2.3. Talent covering the eggs with the halocarbon oil mix.

3. Embryo Microinjection

3.1. To generate needles for the microinjections, place an aluminosilicate capillary glass into a needle puller, following the manufacturer's instructions **[1]**. Set Heat to 516, Velocity to 100, Delay to 70, Pull to 97, and Pressure to 500. Then, activate the needle puller **[2]**.

3.1.1. Talent placing the capillary glass into the needle puller.

3.1.2. Talent adjusting the settings and then activating the needle puller. NOTE: Do not use tk1

3.2. Gently touch the tip of the pulled needle on the rotating diamond abrasive plate for around 10 seconds at a 25-degree angle to bevel the needle tip **[1]**. Embed pulled and beveled needles into lines of modeler's clay in a Petri dish **[2]**.

3.2.1. ECU: Talent beveling the needle tip. NOTE: Do not use first 30sec of tk1

3.2.2. Talent embedding the pulled needles into the clay.

3.3. Prepare the injection mixture consisting of genome modification reagents and store it on ice **[1-TXT]**. Use a microloader tip to load 2 microliters of injection mixture into the injection needle **[2]**.

3.3.1. Talent putting the injection mixture on ice. **TEXT: 200 ng/µL sgRNA and 200 ng/µL Cas9 mixture**

3.3.2. Talent loading the injection needle.

3.4. Place the filled injection needle into a micromanipulator linked to an electronic microinjector **[1]** and place the glass slide with the aligned eggs on the stage of a compound microscope **[2]**.

3.4.1. Talent positioning the needle in the micromanipulator.

3.4.2. Talent placing the slide on the microscope stage.

3.5. Using the micromanipulator, align the needle to aim at the posterior end of the embryo at a 25 to 35-degree angle **[1]**. Carefully insert the needle into the embryo and inject the mixture at a quantity of about 10% of the volume of the embryo **[2-TXT]**. Inject 20 eggs at a time, then stop and perform embryo recovery procedures **[3]**. *Videographer: This step is difficult and important!*

3.5.1. SCOPE: Needle aligning with the embryo. NOTE: Do not use tk2

3.5.2. SCOPE: Needle inserting and mixture injecting. **TEXT: 700-800 pL**

3.5.3. Talent at the microscope, injecting embryos. NOTE: Last two tks best

4. Embryo Recovery and Hatching

4.1. Within 20 minutes post-injection, carefully remove the halocarbon oil from the eggs by brushing them lightly with a clean paintbrush **[1]**. Lift the eggs with the paintbrush and place them into a cup of double-distilled water, taking care to keep the eggs on the surface **[2]**. *Videographer: This step is important!*

4.1.1. Talent removing the oil from the eggs.

4.1.2. Talent transferring the eggs into the cup of water.

4.2. Over the next 7 days, check the eggs daily for hatching and follow normal larval rearing procedures **[1]**. Screen the injected mosquitoes for the mutant phenotypes using a stereoscope **[2]**.

4.2.1. Talent checking the eggs for hatching.

4.2.2. Talent using the stereoscope.

Results

5. Results: Survival and Mutation Rates of Embryos Injected with Single and Multiplexed gRNAs Targeting White

5.1. This method has been used to successfully generate somatic and germline mutations of a gene critical for the development of dark eye pigmentation. CRISPR- Cas9 *(pronounce 'crisper-cas-9')* generated somatic mutations were scored by screening for loss of pigmentation in pupal stage eyes of injected individuals **[1]**.

5.1.1. LAB MEDIA: Table 1.

5.2. Somatic mutations were generally present as mosaic phenotypes, where some but not all of the cells have the mutant phenotype **[1]**.

5.2.1. LAB MEDIA: Table 1. *Video Editor: Emphasize column F-H .*

5.3. Germline mutation rates were determined by intercrossing mosaic G zero individuals and scoring for completely white-eyed offspring **[1]**. These experiments resulted in a 64 to 82% embryo survival rate **[2]**, a 37 to 57% somatic mutagenesis rate **[3]**, and a greater than 61% germline mutagenesis rate **[4]**.

5.3.1. LAB MEDIA: Table 1. *Video Editor: Emphasize columns E – I.*

5.3.2. LAB MEDIA: Table 1. *Video Editor: Emphasize column E, rows 2 - 5.*

5.3.3. LAB MEDIA: Table 1. *Video Editor: Emphasize column H, rows 2 - 5.*

5.3.4. LAB MEDIA: Table 1. *Video Editor: Emphasize column I, rows 2 - 5.*

5.4. By multiplexing sgRNAs to target multiple loci in the same gene, somatic and germline mutagenesis rates increased to as much as 86% **[1]**. In addition, for many generations, viable homozygous stocks of the white mutants have been successfully kept in the lab **[2]**.

5.4.1. LAB MEDIA: Table 1. *Video Editor: Emphasize rows 6 – 9.*

5.4.2. LAB MEDIA: Table 1.

Conclusion

6. Conclusion Interview Statements

6.1. **Michelle Bui:** To optimize survival and mutagenesis rates, all materials including halocarbon oil, injection fluid, and needles should be properly prepared before attempting microinjections. This will allow for a more streamlined and focused microinjection process.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 3.2.2, 3.3.1*