Submission ID #: 61885
Scriptwriter Name: Anastasia Gomez
Project Page Link: https://www.jove.com/account/file-uploader?src=18874973

Title: Egg Microinjection and Efficient Mating for Genome Editing in the Firebrat Thermobia domestica

Authors and Affiliations:

Takahiro Ohde, Toshinori Minemura, Eiichi Hirose, Takaaki Daimon

Department of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwakecho, Sakyo-ku, Kyoto, Japan

Corresponding Authors:

Takahiro Ohde (ohde.takahiro.4n@kyoto-u.ac.jp)

Email Addresses for All Authors:

t.minemura430705@gmail.com
hirose6438@gmail.com
daimon.takaaki.7a@kyoto-u.ac.jp
ohde.takahiro.4n@kyoto-u.ac.jp

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?

   No

   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 SZX12

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**.

   ☒ 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? **No**

Current Protocol Length

Number of Steps: 15
Number of Shots: 30

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Takaaki Daimon:** Because Thermobia domestica is a basal insect, genetic studies on this species will illuminate mechanisms underlying the evolution of key innovations in insects such as powered flight and metamorphosis.

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

1.2. **Takahiro Ohde:** This protocol covers basic techniques, from culture maintenance to egg microinjection, to implement Thermobia as a laboratory model species.

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

OPTIONAL:

1.3. **Takahiro Ohde:** This method can also be used for application of other genetic tools such as RNAi-mediated gene knockdown and transgenesis in Thermobia.

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

Protocol

2. Egg Collection and Microinjection

2.1. Maintain wildtype and mutant populations in a large plastic container **[1-TXT]** with regular artificial fish food, water in plastic cups with a ventilation hole on the top, a folded paper for hiding the insects, and layered cotton for laying eggs **[2]**.

2.1.1. Plastic container. **TEXT: 460 mm x 360 mm x 170 mm** Vid NOTE: Focus is soft for the first half of the shot, and is corrected after approx. 1 minute (file: A003_10091337_C022.mov)

2.1.2. Container setup including the artificial fish food, water in plastic cups, a folded paper, and layered cotton for laying eggs.

2.2. Keep all *T. domestica* cultures inside 37-degree Celsius incubators and set the relative humidity inside each container to 60 to 80% **[1]**.

2.2.1. Talent putting the cultures in the incubator and closing the door.

2.3. To prepare the egg collection colonies, transfer about 20 male and 20 female adults to a middle-sized container with food, a water supply, a folded paper, and a small piece of layered cotton for egg laying **[1]**. Set up several colonies to obtain a large number of staged embryos within a short time period **[2]**.

2.3.1. Talent transferring adults to the container.

2.3.2. Several colonies.

2.4. On the day of injection, replace the cotton inside the containers with new cotton **[1]**. Eight hours later, collect the eggs from the layered cotton by separating the layers using forceps **[2]**.

2.4.1. Talent replacing the cotton.

2.4.2. Talent collecting eggs.

2.5. Align the eggs on the double-sided tape using a wet paint brush, keeping a 2-millimeter distance between the eggs. All eggs should be oriented so that the longitudinal axis of an egg faces the injection side **[1]**. Gently press down the eggs with a paint brush for firm holding during the injection **[2]**. *Videographer: This step is important!*

2.5.1. SCOPE: Talent aligning eggs.

2.5.2. SCOPE: Talent pressing down on the eggs.

2.6. Load 2 microliters of the gRNA-Cas9 *(pronounce 'G-R-N-A-kass-nine')* solution into a glass injection capillary with a microloader **[1]**. Make sure there are no air bubbles in

the solution before the injection. If necessary, tap the needle to remove the bubbles **[2]**.

2.6.1. Talent loading the solution into a capillary.

2.6.2. Solution with no air bubbles or talent tapping the needle to remove the bubbles.

2.7. Optimize the shape of the needle tip by breaking it slightly with forceps to prevent clogging and to get better durability throughout a series of injections **[1]**. *Videographer: This step is difficult and important!*

2.7.1. SCOPE: Talent breaking the needle tip.

2.8. Insert the needle at the midpoint of the longitudinal axis of an egg and inject a small amount of the solution **[1]**. Adjust the configuration of the electronic microinjector during injection, depending on the shape of the needle tip **[2]**. *Videographer: This step is difficult and important!*

2.8.1. SCOPE: Talent inserting the needle and injecting.

2.8.2. Talent adjusting the electronic microinjector.

2.9. Keep the injected eggs in an appropriately sized container with 60 to 80% relative humidity at 37 degrees Celsius **[1]**.

2.9.1. Talent putting eggs in a container.

3. Mating

3.1. Check the injected eggs periodically **[1]** and discard damaged eggs to avoid fungal growth **[2]**. If too much fungus is growing on an egg, clean the surface of the egg with 70% ethanol **[3]**.

3.1.1. Talent at the microscope.

3.1.2. SCOPE: Talent discarding a damaged egg. Vid NOTE: There is no fungus on the egg, but the cleaning with ethanol is performed anyway

3.1.3. SCOPE: Talent cleaning an egg.

3.2. Before hatching, dip the glass slide with the injected eggs into talcum powder to coat the surface of the double-sided tape. This will prevent the stacking of hatched nymphs **[1]**. Transfer the powder-coated glass slides to a middle-sized container with food, water, and a folded paper **[2]**.

3.2.1. Talent dipping the slide into talcum powder.

3.2.2. Talent transferring the slides to a container. Vid NOTE: Please use the retake of this shot, because the container in the original take had the incorrect content (filename: A003_10091502_C038.mov)

3.3. Transfer either a male or a female G0 *('G-zero')* adult that developed from an injected egg and wildtype adults to a small plastic dish with food, a folded paper, and a small piece of cotton for laying G1 eggs **[1,2]**. Keep the mating dishes in a larger container with 60 to 80% relative humidity **[3.3.2 REAL]**.

3.3.1. Talent transferring adults to a container.

3.3.2. Added shot: CU: a container with two adults inside.

3.3.2REAL: Mating dishes in a large container.

4. Genotyping

4.1. Isolate G1 nymphs into 24-well plates with an aspirator or a paint brush **[1]**. Maintain the supply of artificial regular fish food **[2]**. Place the 24-well plates in a larger container with a water supply for a humidity of 60 to 80% **[3]**.

4.1.1. Talent transferring G1 nymphs to a well.

4.1.2. Talent placing fish food in wells.

4.1.3. Talent placing the plate into a container.

4.2. Pinch and pull a cercus from a nymph or adult using forceps **[1]** and collect them into a 0.2-milliliter tube containing 50 microliters of ethanol **[2]**. Store the collected samples at -20 degrees Celsius for long-term storage **[3]**.

4.2.1. Talent pinching a cercus from a nymph or adult.

4.2.2. Talent putting the cerci into a tube.

4.2.3. Talent putting the sample into a freezer.

4.3. Place sample tubes with the lids open on a thermal block for 15 minutes at 70 degrees Celsius to evaporate the ethanol **[1]**.

4.3.1. Talent placing the sample tubes on a thermal block.

Results

5. Results: Dry Injection of *T. domestica* Eggs

5.1. Injection of gRNA-Cas9 ribonucleoprotein complex in embryos within the first 8 hours after egg laying results in indels at the gRNA targeted site. This causes biallelic mutations in some cells of the injected generation and mutant mosaic phenotypes are usually obtained **[1]**.

5.1.1. LAB MEDIA: Figure 5.

5.2. When this protocol was used to inject a gRNA that is designed to target the *white* gene, 32.6% of G0 nymphs displayed partial loss of pigmentation in their compound eyes and dorsal regions **[1]**.

5.2.1. LAB MEDIA: Figure 5. *Video Editor: Emphasize 5 B.*

5.3. Assessment of the germline transformation of G0 adults and mutated G1 individuals was done by genomic PCR followed by a heteroduplex mobility assay **[1]**. In G1 samples, differential band patterns between wildtype and mutated samples are clearly distinguishable **[2]**.

5.3.1. LAB MEDIA: Figure 4 B.

5.3.2. LAB MEDIA: Figure 4 B. *Video Editor: Emphasize the U and M lanes.*

Conclusion

6. Conclusion Interview Statements

6.1. **Takahiro Ohde:** Genome editing in Thermobia provides a solid approach to analyze genetic mechanisms underlying primitive traits of insects. It helps to explore the secrets behind their outstanding success on Earth.

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