

Submission ID #: 71872

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Project Page Link: <https://review.jove.com/account/file-uploader?src=21921008>

Title: An Efficient Rearing and Microinjection Protocol for Genetic Modification of the Stable Fly, *Stomoxys calcitrans*

Authors and Affiliations:

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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.

If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and you will have to perform the procedure using one eye.

Leica DM750M

If a dissection or stereo microscope is required for your protocol, please list all shots from the script that will be visualized using the microscope (shots are indicated with the 3-digit numbers, like 2.1.1, 2.1.2, etc.).

Scope shots: **2.8.2, 2.8.3, 3.7.1, 3.7.2**

Videographer: Please film the above-mentioned scope shots using the scope kit.

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**

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

If **Yes**, please provide the **full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author willing to participate.

Tyler Forrest Chan, PhD candidate in GENE Genetics and Genomics Interdisciplinary Program at Texas A&M University

Current Protocol Length

Number of Steps: 20

Number of Shots: 44

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Answers are slightly edited in accordance with journal guidelines. Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Tyler Chan:** My research focuses on studying the application of molecular genetic tools like transposases and CRISPR or Cas9 to control harmful insect populations.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Tyler Chan:** Previous protocols were difficult to replicate; this protocol increases the efficiency and reliability of generating transgenic stable flies.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Tyler Chan:** This protocol allows researchers to transform embryos with nucleic acids and observe their development through larvae, pupae, and adults.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.4. **Tyler Chan:** Additionally, this injection protocol can be used to inject nucleases, such as Cas9, for gene knockouts.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Tyler Chan:** Future studies building on this work can investigate RNAi gene suppression, Cas9 knockouts, gene drive constructs, and other advanced tools for pest control.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

What value do you expect this video article to have for other researchers working in your field?

1.6. **Tyler Forrest Chan, PhD Candidate, Texas A&M University:** (authors will present their testimonial statements live)

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

How does the video format of your article help you to share your work with the broader scientific community?

1.7. **Tyler Forrest Chan, PhD Candidate, Texas A&M University:** (authors will present their testimonial statements live)

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

Ethics Title Card

This research has been approved by the Institutional Biosafety Committee at Texas A&M University

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Embryo Collection and Microinjection Preparation

Demonstrator: Tyler Chan

- 2.1. To begin, fill a 250-milliliter Griffin beaker with approximately 100 milliliters of room-temperature tap water [1]. Place a 20-centimeter-by-20-centimeter square of black cotton fabric in the water until it is moistened [2]. Adjust the fabric so that its edges extend 1 to 2 centimeters beyond the rim of the beaker [3].
 - 2.1.1. WIDE: Talent filling a 250-milliliter Griffin beaker with room-temperature tap water. ***Videographer:** Please obtain CU shot as well for this shot.*
Videographer's NOTE: 2.1.1 & 2.1.2 - One shot
 - 2.1.2. Talent placing a 20-centimeter by 20-centimeter square of black cotton fabric into the water until it is fully moistened.
 - 2.1.3. Shot of adjusting the black cotton fabric such that the edges extend 1 to 2 centimeters beyond the rim of the beaker.
- 2.2. Transfer the beaker to an already prepared adult stable fly cage and leave it for 25 minutes to collect eggs [1]. Using a wash bottle, rinse the eggs onto a 106-micrometer stainless-steel sieve [2].
 - 2.2.1. Talent transferring the beaker to an adult cage for egg collection.
 - 2.2.2. Talent rinsing eggs onto a 106-micrometer stainless-steel sieve using a wash bottle.
- 2.3. Allow the embryos to rest undisturbed on the sieve for an additional 30 minutes [1].
 - 2.3.1. Shot of embryos in the sieve kept undisturbed.
- 2.4. While the embryos are aging, prepare 150 milliliters each of the oxidizing, washing, and rinsing solutions [1]. Use sufficient volume to cover the embryos with approximately 1-centimeter of solution [2].
 - 2.4.1. Shot of the oxidizing, washing, and rinsing solutions arranged on the workbench.
 - 2.4.2. Close-up shot of the prepared solution containers showing sufficient solution volume for embryo immersion
- 2.5. Immerse the sieve containing embryos in the oxidizing bath for 2 minutes [1] and gently agitate the embryos using a Pasteur pipette [2].

- 2.5.1. Shot of the sieve containing embryos being placed into the oxidizing bath
Videographer's NOTE: 2.5.1 & 2.5.2 - One shot
- 2.5.2. Shot of embryos being gently agitated with a Pasteur pipette.

- 2.6. Remove the sieve from the oxidizing bath and tap off excess solution [1]. Transfer the sieve to the washing solution [2]. Agitate by aspiration and dispensing for 2 minutes [3].
 - 2.6.1. Shot of the sieve being removed from the oxidizing bath and tapping off excess solution. **Videographer's NOTE: 2.6.1 , 2.6.2 , 2.6.3 - One shot**
 - 2.6.2. Shot of the sieve being transferred to the washing solution.
 - 2.6.3. Shot of the embryos being agitated by aspiration and dispensing in the washing solution.

- 2.7. Transfer the sieve containing embryos to the rinsing solution [1] and agitate as previously demonstrated for 2 minutes [2]. Remove the sieve from the rinsing solution and tap off excess water [3].
 - 2.7.1. Shot of the sieve containing embryos being placed into the rinsing solution.
Videographer's NOTE: 2.7.1, 2.7.2, 2.7.3 - One shot
 - 2.7.2. Shot of the embryos being agitated by aspiration and dispensing in the rinsing solution.
 - 2.7.3. Shot of removing the sieve from the rinsing solution and tapping off excess water.

- 2.8. Place a sterile glass coverslip on the sieve [1]. Under a dissection microscope, use a fine-tipped nylon paintbrush to transfer embryos from the sieve to the edge of the coverslip, while preventing bending, distortion, or tearing of the embryos [2]. Position the embryos close together without allowing them to touch, with the wider posterior pole facing the edge of the coverslip [3].
 - 2.8.1. Talent placing a sterile glass coverslip on the sieve.
 - 2.8.2. SCOPE: Shot of transferring embryos from the sieve to the edge of the coverslip using a fine-tipped nylon paintbrush under a dissection microscope.
Videographer's NOTE: 2.8.2 & 2.8.3 - One shot
 - 2.8.3. SCOPE: Shot of arranging embryos close together without contact, with the wider posterior pole facing the edge of the coverslip.

- 2.9. Prepare a desiccation chamber by covering the bottom of a 1-liter flask with 100 milliliters of calcium sulfate desiccant [1]. Seal the flask with a rubber stopper [2].
- 2.9.1. Talent covering the bottom of a 1-liter flask with 100 milliliters of calcium sulfate desiccant. **Videographer's NOTE: 2.9.1 & 2.9.2 - One shot**
- 2.9.2. Talent sealing the flask with a rubber stopper.
- 2.10. Remove excess water from the coverslip [1]. Place the coverslip containing dechorionated embryos onto the desiccant [2]. Reseal the flask with a rubber stopper and desiccate the embryos for 8 minutes [3].
- 2.10.1. Shot of removing excess water from the coverslip.
- 2.10.2. Shot of placing the coverslip containing dechorionated embryos onto the desiccant.
- 2.10.3. Shot of resealing the flask with a rubber stopper.
- 2.11. Remove the coverslip from the desiccation chamber [1] and place it on the microscope stage under a 20x objective for microinjection [2].
- 2.11.1. Shot of removing the coverslip from the desiccation chamber.
- 2.11.2. Shot of placing the coverslip on the microscope stage under a 20 times objective for microinjection.

3. Embryo Microinjection

- 3.1. Pull quartz microinjection needles from 100-millimeter filamented glass capillaries using the specific parameters [1]. Store the needles in a dust-free location [2].
- 3.1.1. Shot of pulling quartz microinjection needles from filamented glass capillaries.
- 3.1.2. Shot of placing the prepared quartz microinjection needles in a dust-free storage location.
- 3.2. Prepare 20 microliters of injection mix by combining 10 micrograms each of piggyBac helper plasmid and donor plasmid containing the genetic cargo between the left and right piggyBac inverted terminal repeats with 4 microliters of injection buffer [1]. Dilute the mixture with nuclease-free water to a final concentration of 500 nanograms per microliter [2].
- 3.2.1. Shot of combining the piggyBac helper plasmid, donor plasmid, and injection buffer in a microcentrifuge tube to prepare the injection mix. **Videographer's NOTE: 3.2.1 & 3.2.2 - One shot**
- 3.2.2. Shot of diluting the injection mix with nuclease-free water.
- 3.3. Transfer the injection mix to a low-volume 0.2-micrometer polytetrafluoroethylene or

- PTFE (*P-T-F-E*) syringe filter [1]. Place the filter into the opening of a 0.5-milliliter microcentrifuge tube [2]. Centrifuge at 11,000 g for 10 minutes or until all injection mix has passed through the filter [3]. Store any unused injection mix at minus 20 degrees Celsius [4].
- 3.3.1. Talent transferring the injection mix to a low-volume 0.20-micrometer polytetrafluoroethylene syringe filter. **Videographer's NOTE: 3.3.1 & 3.3.2 - One shot**
 - 3.3.2. Talent placing the syringe filter into the opening of a 0.5-milliliter microcentrifuge tube.
 - 3.3.3. Talent placing the microcentrifuge tube containing the filter into the centrifuge.
 - 3.3.4. Talent storing the unused injection mix at minus 20 degrees Celsius.
 - 3.4. Using a micro gel-loading pipette tip, back-fill a quartz microinjection needle with approximately 2 microliters of injection mix and avoid introducing air bubbles during loading [1].
 - 3.4.1. Shot of back-filling a quartz microinjection needle with injection mix without visible air bubbles using a micro gel-loading pipette tip.
 - 3.5. Gently tap the needle tip against the edge of a glass slide until the injection mix flows through the needle tip [1].
 - 3.5.1. Shot of gently tapping the needle tip against the edge of a glass slide until the injection mix flows through the tip. **Videographer's NOTE: 3.5.1, 3.6.1, 3.7.1, 3.7.2 - One shot**
 - 3.6. Inject the embryo through the posterior pole immediately after desiccation [1-TXT].
 - 3.6.1. Talent injecting the embryo through the posterior pole immediately after desiccation. **TXT: Inj. pressure: ~700 hPa; Back pressure: 300 hPa**
 - 3.7. Gently pierce the embryo [1]. Inject approximately one-tenth of the embryo volume with the injection mixture [2].
 - 3.7.1. SCOPE: Microinjection needle gently piercing the embryo.
 - 3.7.2. SCOPE: Injection mixture being delivered into the embryo.
 - 3.8. Transfer the coverslip to a Petri dish lined with a sterile, moistened 90-millimeter black filter paper immediately after injection [1]. Incubate at room temperature for 48 hours [2].
 - 3.8.1. Talent transferring the coverslip to a Petri dish lined with a moistened 90-millimeter black filter paper.
 - 3.8.2. Talent placing the Petri dish into the incubator.
 - 3.9. Examine the embryos for hatching every 12 to 16 hours [1]. Transfer newly hatched larvae and the glass coverslip to an inoculated and conditioned agar plate [2].

- 3.9.1. Talent examining the embryos for hatching.
- 3.9.2. Talent transferring newly hatched larvae and the glass coverslip to an inoculated and conditioned agar plate.

Results

4. Results

- 4.1. Of the 431 injected embryos, 18 ZsGreen-positive larvae [1] and 8 ZsGreen-negative larvae hatched 48 to 76 hours post-injection [2]. Overall, 26 of 431 injected embryos hatched [3], and 16 of 26 hatched larvae survived to adulthood [4].
 - 4.1.1. LAB MEDIA: Table 3. *Video editor: Emphasize the values in the ZsGreen(+) L1 column.*
 - 4.1.2. LAB MEDIA: Table 3. *Video editor: Emphasize the values in the ZsGreen(-) L1 column.*
 - 4.1.3. LAB MEDIA: Table 3. *Video editor: Emphasize the values in the ZsGreen(+) L1 column and ZsGreen(-) L1 column.*
 - 4.1.4. LAB MEDIA: Table 3. *Video editor: Emphasize the values in the ZsGreen(+) Adults column and ZsGreen(-) Adults.*
- 4.2. Of the 18 ZsGreen-positive G0 larvae, 12 survived to pupation [1] and 10 eclosed as adults [2]. Six ZsGreen-positive adults produced ZsGreen-positive offspring [3].
 - 4.2.1. LAB MEDIA: Table 3. *Video editor: Emphasize the values in the ZsGreen(+) Pupae column.*
 - 4.2.2. LAB MEDIA: Table 3. *Video editor: Emphasize the values in the ZsGreen(+) Adults column.*
 - 4.2.3. LAB MEDIA: Table 3. *Video editor: Emphasize the values in the # Adults producing ZsGreen(+) offspring column.*
- 4.3. These offspring were self-crossed and maintained fluorescence for six generations at the time of writing [1].
 - 4.3.1. LAB MEDIA: Figure 9A–E. *Video editor: Emphasize the specimens labeled “Hsp83:zsGreen” in all panels*
- 4.4. Among the eight ZsGreen-negative larvae, six survived to adulthood [1]. None of the non-fluorescent adults produced fluorescent offspring [2].
 - 4.4.1. LAB MEDIA: Table 3. *Video editor: Emphasize the value 6 under the column “ZsGreen(-) Adults”.*
 - 4.4.2. LAB MEDIA: Table 3. *Video editor: Emphasize the column “# Adults producing ZsGreen(+) offspring.”*

1. Micrometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/micrometer>
IPA: /'maɪkroʊˌmi:tər/
Phonetic Spelling: MY-kroh-mee-ter
2. Sieve
Pronunciation link: <https://www.merriam-webster.com/dictionary/sieve>
IPA: /sɪv/
Phonetic Spelling: siv
3. Embryos
Pronunciation link: <https://www.merriam-webster.com/dictionary/embryo>
IPA: /'ɛmbriəʊz/
Phonetic Spelling: EM-bree-ohz
4. Pasteur
Pronunciation link: No confirmed link found
IPA: /pæ'stɜːr/
Phonetic Spelling: pa-STUR
5. Pipette
Pronunciation link: <https://www.merriam-webster.com/dictionary/pipette>
IPA: /paɪ'pet/
Phonetic Spelling: pie-PET
6. Aspiration
Pronunciation link: <https://www.merriam-webster.com/dictionary/aspiration>
IPA: /ˌæspə'reɪʃən/
Phonetic Spelling: as-puh-RAY-shun
7. Posterior
Pronunciation link: <https://www.merriam-webster.com/dictionary/posterior>
IPA: /poʊ'stɪriər/, /pɑː'stɪriər/
Phonetic Spelling: poh-STEER-ee-er, pah-STEER-ee-er
8. Desiccation
Pronunciation link: <https://www.merriam-webster.com/dictionary/desiccation>
IPA: /ˌdɛsɪ'keɪʃən/
Phonetic Spelling: dess-ih-KAY-shun
9. Calcium
Pronunciation link: <https://www.merriam-webster.com/dictionary/calcium>
IPA: /'kælsiəm/
Phonetic Spelling: KAL-see-um
10. Sulfate
Pronunciation link: <https://www.merriam-webster.com/dictionary/sulfate>
IPA: /'sʌlˌfeɪt/
Phonetic Spelling: SUL-fayt
11. Desiccant
Pronunciation link: <https://www.merriam-webster.com/dictionary/desiccant>
IPA: /'dɛsɪkənt/
Phonetic Spelling: DESS-ih-kunt

12. Dechorionated
Pronunciation link: No confirmed link found
IPA: /di:'kɔ:riə,nertɪd/
Phonetic Spelling: dee-KOR-ee-uh-nay-tid
13. Desiccate
Pronunciation link: <https://www.merriam-webster.com/dictionary/desiccate>
IPA: /'dɛsɪ,kert/
Phonetic Spelling: DESS-ih-kayt
14. Microinjection
Pronunciation link: <https://www.merriam-webster.com/dictionary/microinjection>
IPA: /,maɪkroʊɪn'dʒɛkʃən/
Phonetic Spelling: my-kroh-in-JEK-shun
15. Quartz
Pronunciation link: <https://www.merriam-webster.com/dictionary/quartz>
IPA: /kwɔrts/
Phonetic Spelling: kworts
16. Capillaries
Pronunciation link: <https://www.merriam-webster.com/dictionary/capillary>
IPA: /'kæpə,lɛrɪz/, /'kæpə,lɛrɪz/
Phonetic Spelling: KAP-uh-lair-eez
17. Plasmid
Pronunciation link: <https://www.merriam-webster.com/dictionary/plasmid>
IPA: /'plæzmɪd/
Phonetic Spelling: PLAZ-mid
18. piggyBac
Pronunciation link: No confirmed link found
IPA: /'pɪɡɪbæk/
Phonetic Spelling: PIG-ee-bak
19. Nuclease
Pronunciation link: <https://www.merriam-webster.com/dictionary/nuclease>
IPA: /'nu:kli,ɛs/, /'nju:kli,ɛs/, /'nu:kli,ɛrɪz/, /'nju:kli,ɛrɪz/
Phonetic Spelling: NOO-klee-ays, NYOO-klee-ays, NOO-klee-ayz, NYOO-klee-ayz
20. Polytetrafluoroethylene
Pronunciation link: No confirmed link found
IPA: /,pɑ:li,tɛtrə'fluɔroʊ'ɛθəli:n/
Phonetic Spelling: pol-ee-tet-ruh-floor-oh-ETH-uh-leen
21. PTFE
Pronunciation link: No confirmed link found
IPA: /,pi:ti:ɛf'i:/
Phonetic Spelling: P-T-F-E
22. Microcentrifuge
Pronunciation link: No confirmed link found
IPA: /,maɪkroʊ'sentrə,fju:dʒ/
Phonetic Spelling: my-kroh-SEN-truh-fyooj

23. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /'sentrəˌfjuːdʒ/
Phonetic Spelling: SEN-truh-fyooj
24. Inoculated
Pronunciation link: <https://www.merriam-webster.com/dictionary/inoculate>
IPA: /ɪˈnɑːkjəˌleɪtɪd/
Phonetic Spelling: ih-NOK-yuh-lay-tid
25. Agar
Pronunciation link: <https://www.merriam-webster.com/dictionary/agar>
IPA: /ˈɑːɡər/
Phonetic Spelling: AH-ger
26. Larvae
Pronunciation link: <https://www.merriam-webster.com/dictionary/larva>
IPA: /ˈlɑrv iː/, /ˈlɑrv aɪ/
Phonetic Spelling: LAR-vee, LAR-vye
27. Pupae
Pronunciation link: <https://www.merriam-webster.com/dictionary/pupa>
IPA: /ˈpjuː piː/, /ˈpjuː paɪ/
Phonetic Spelling: PYOO-pee, PYOO-pye
28. Pupation
Pronunciation link: <https://www.merriam-webster.com/dictionary/pupation>
IPA: /pjuːˈpeɪʃən/
Phonetic Spelling: pyoo-PAY-shun
29. Eclosed
Pronunciation link: <https://www.merriam-webster.com/dictionary/eclose>
IPA: /iˈkloʊzd/
Phonetic Spelling: ee-KLOHZD
30. Fluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
IPA: /flʊˈresəns/, /flʌˈresəns/
Phonetic Spelling: floo-RESS-uhns, flaw-RESS-uhns
31. Fluorescent
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescent>
IPA: /flʊˈresənt/, /flʌˈresənt/
Phonetic Spelling: floo-RESS-unt, flaw-RESS-unt