Submission ID #: 69323
Scriptwriter Name: Poornima G
Project Page Link: https://review.jove.com/account/file-uploader?src=21143563

Title: Generation of Genome-Edited Marmosets

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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**

    1.4.3

    1.4.4

    1.4.5

    1.5.1

    1.5.2

    1.5.3

    1.5.4

    1.9.9

    1.10.1

    1.10.3

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. **No**

Current Protocol Length
Number of Steps: 21
Number of Shots: 43 (5 Scope)

Introduction

INTRODUCTION:

1.1. **Toshiaki Watanabe:** The development of auto-transplantation methods brought innovation for making genetically modified marmosets, because it dramatically increased the birth rate.

What are the current experimental challenges?

1.2. **Toshiaki Watanabe:** Since marmosets have a long generation time, we would like to analyze non-mosaic animals in the founder generation.

CONCLUSION:

What advantage does your protocol offer compared to other techniques?

1.3. **Toshiaki Watanabe:** By using our protocol, non-mosaic animals can be obtained at high rates. It accelerates the study using genetically modified marmosets.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee at the National Center for Child Health and Development

Protocol

2. GV Oocyte Retrieval from Ovaries Removed Surgically

Demonstrator: Toshiaki Watanabe and Yuwei Xie

2.1. To begin, intramuscularly inject 75 international units of human chorionic gonadotropin into the marmoset in the afternoon on the day before ovary excision **[1]**. Collect oocytes from excised ovaries of anesthetized marmosets instead of performing follicular aspiration **[2-TXT]**.

2.1.1. Talent administering an intramuscular injection of human chorionic gonadotropin to the marmoset.

2.1.2. Talent positioning the anesthetized marmoset for the procedure. **TXT: Anesthesia: 3% Isoflurane (100% O_2)**

2.2. Prepare the drops of maturation medium before excising the ovaries **[1]**. Ligate the blood vessels connected to the superior and inferior portions of the ovaries prior to dissection **[2]**.

2.2.1. Talent pipetting maturation medium to prepare drops in a Petri dish. **TXT: Maturation medium: 5% FBS; 0.15 IU/mL hFSH; 10 IU hCG**

2.2.2. Talent ligating blood vessels at the superior（part1） /（part2）

and inferior ends of the ovaries before dissection.

2.3. After dissecting the ovaries, use a stereomicroscope to remove adipose and other unnecessary tissues from the excised ovaries **[1]**.

2.3.1. SCOPE: Show the excised ovary under the stereomicroscope as the talent carefully removes adipose and connective tissues.

2.4. In a 60-millimeter dish containing 4 milliliters of Waymouth's-HEPES medium, hold the ovaries with forceps **[1]**. Using an 18-gauge needle, mechanically disrupt the ovarian tissue by scratching **[2]**, and isolate the oocytes from the follicles **[3]**.

2.4.1. Talent placing the ovaries into a 60 millimeter dish containing Waymouth's-HEPES medium.

2.4.2. Talent using an 18 gauge needle to gently scratch the ovary surface.

2.4.3. SCOPE: Close-up of oocytes being released from disrupted follicles into the medium.

2.5. When oocytes remain enclosed within follicles, use the tip of a 26-gauge needle to break the follicles **[1]**. Carefully manipulate the needle to avoid damaging the oocytes **[2]**.

2.5.1. Talent using a fine 26 gauge needle to rupture intact follicles.

2.5.2. Close-up of oocytes being released undamaged after follicle disruption.

2.6. Then, using a mouth pipette with a glass capillary attached, collect the oocytes from the medium in 35-millimeter dishes under a stereomicroscope equipped with a heating stage **[1]**. Transfer the collected oocytes into a 200-microliter drop of maturation medium **[2]**.

2.6.1. SCOPE: using a mouth pipette with glass capillary to collect oocytes.

~~2.6.2. Close-up of transferring collected oocytes into a 200 microliter drop of maturation medium.~~

2.7. Next, transfer the oocytes into a 50-microliter drop of maturation medium **[1]**, and then move them to another 50-microliter drop for washing **[2]**.

2.7.1. ~~Talent placing oocytes into a 50 microliter drop of maturation medium.~~ In 2.6.1

2.7.2. Talent transferring oocytes to a second 50 microliter drop for washing.

2.8. Classify the washed oocytes into metaphase I or metaphase II, germinal vesicle A , B, and C types according to their maturation stage **[1]**. Transfer each class of oocytes to separate drops based on their developmental stage **[2]**.

2.8.1. Talent examining the sample under a microscope.

2.8.2. Talent transferring oocytes into separate drops labeled for each developmental stage.

2.9. To allow germinal vesicle oocytes to mature into metaphase II oocytes, place them overnight in an incubator at 37 degrees Celsius with 5 percent carbon dioxide and 5 percent oxygen **[1]**.

2.9.1. Talent placing culture dishes containing germinal vesicle oocytes into the incubator and closing the incubator door.

3. Sperm Collection and In Vitro Fertilization

Demonstrator: Toshiaki Watanabe and Yuwei Xie

3.1. Prepare three 1.5-milliliter tubes each containing 500 microliters of TYH medium and one 5 milliliter tube containing 1 milliliter of TYH medium **[1]**. Equilibrate the TYH medium at 37 degrees Celsius, with 5 percent carbon dioxide and 5 percent oxygen, for at least 3 hours **[2]**. Keep the tubes partially open to allow air exchange during equilibration **[3]**.

3.1.1. Talent labeling three 1.5 milliliter tubes and one 5 milliliter tube with TYH medium.

3.1.2. Talent placing all tubes into the incubator set at 37 degrees Celsius, 5 percent carbon dioxide, and 5 percent oxygen.

3.1.3. Close-up shot of the tubes with lids partially open for air exchange.

3.2. Now, place the male marmoset in a restrainer **[1]**. Stimulate the penis with a vibrator to induce ejaculation **[2]** and collect the semen in 500 microliters of TYH medium **[3]**. Check sperm count and motility under a microscope **[4]**.

3.2.1. Talent positioning the male marmoset securely in the restrainer.

3.2.2. Talent using a vibrator to stimulate the penis.

3.2.3. Talent collecting the ejaculate into a 1.5 milliliter tube containing TYH medium.

3.2.4. Shot of Talent examining the sample under the microscope.

3.3. Centrifuge the 1.5-milliliter tube containing semen at 1,110 *g* for 5 minutes at room temperature **[1]**.

3.3.1. Talent placing the 1.5 milliliter tube into the centrifuge and starting the spin cycle.

3.4. Discard the supernatant quickly, leaving a small volume of medium with the pellet in the tube **[1]** and add 500 microliters of TYH medium **[2]**. For capacitation, incubate the tube at 37 degrees Celsius, with 5 percent carbon dioxide and 5 percent oxygen, for 30 minutes **[3]**.

3.4.1. Talent removing the supernatant carefully using a pipette.

3.4.2. Talent adding 500 microliters of TYH medium to the sperm pellet. (within 3.4.1)

3.4.3. Talent placing the tube in the incubator.

3.5. Next, centrifuge the 1.5-milliliter tube again at 1,110 *g* for 5 minutes at room temperature to remove eluate from seminal plasma **[1]**. Discard the supernatant quickly, leaving a small amount of medium in the tube **[2]**.

3.5.1. Talent placing the tube in the centrifuge.

3.5.2. Close-up Shot of removing most of the supernatant, leaving a small residual volume.

3.6. Now, gently transfer the sperm pellet to the bottom of the 5-milliliter tube containing 1 milliliter of TYH medium **[1]**. Incubate the sample at 37 degrees Celsius, with 5 percent carbon dioxide and 5 percent oxygen, for 30 minutes while keeping the lid slightly open **[2]**.

3.6.1. Talent transferring the sperm pellet into the 5 milliliter tube.

3.6.2. Talent placing the 5 milliliter tube into the incubator with the lid partially open.

3.7. Collect 700 to 800 microliters of the upper medium from the 5-milliliter tube to obtain active sperm **[1]**. Transfer this medium to a new 1.5 milliliter tube **[2]** and centrifuge the new tube at 1,110 *g* for 5 minutes at room temperature **[3]**.

3.7.1. Talent aspirating the upper layer of medium from the 5 milliliter tube.

3.7.2. Talent transferring the aspirated medium to a clean 1.5 milliliter tube (within 3.7.1).

3.7.3. Talent placing the tube in the centrifuge and running it.

3.8. Discard the supernatant quickly, leaving a small amount in the tube **[1]** and add 500 microliters of TYH medium to gently resuspend the sperm **[2]**.

3.8.1. Talent removing the supernatant carefully with a pipette.

3.8.2. Talent adding TYH medium and gently swirling the tube to resuspend sperm. (within 3.8.1)

3.9. Count the number of active sperm using a hemocytometer **[1]**.

3.9.1. Talent adding the sample onto a hemocytometer grid.

3.10. Add approximately 1×10^5 active sperm to 50 microliters of TYH medium containing the oocytes **[1-TXT]**.

3.10.1. Talent pipetting active sperm into the TYH medium drop containing oocytes. **TXT: If the sperm volume exceeds 10 µL, centrifuge, resuspend, and concentrate**

3.11. Incubate the oocyte and sperm mixture at 37 degrees Celsius, with 5 percent carbon dioxide and 5 percent oxygen, overnight for 10 to 20 hours **[1]**.

3.11.1. Talent placing the dish containing sperm and oocytes in the incubator and closing the door.

3.12. After incubation, transfer the fertilized eggs to new drops of TYH medium equilibrated at 37 degrees Celsius, 5 percent carbon dioxide, and 5 percent oxygen **[1]**. Using a mouth pipette with a glass capillary of matching diameter, remove the sperm adhering to the zona pellucida **[2]**. Finally, confirm fertilization by checking for two pronuclei or extrusion of the second polar body **[3]**. 3.12 was not filmed by the photographer because the author needed some time for culturing to prepare the material.

3.12.1. Talent transferring fertilized eggs into fresh TYH medium drops. 3.12.1.avi, 0:28-0:38, 0:47-0:50.

3.12.2. SCOPE: using a mouth pipette and glass capillary to remove sperm from the zona pellucida. 3.12.2.avi, 1:41:1:50.

3.12.3. SCOPE: Close-up of a fertilized egg showing two pronuclei or the second polar body. 20251225_10.jpg.

Results

4. Results

4.1. Ovarian dissection yielded more oocytes **[1]** than follicular aspiration in the same animal **[2]**.

4.1.1. LAB MEDIA: Table 1. *Video editor: Highlight the last row for animal I7469F with "both ovary dissection*".

4.1.2. LAB MEDIA: Table 1. *Video editor: Highlight the row 5 for animal I7469F with "follicular aspiration"*

4.2. The efficiency of maturation to metaphase I or II *(1 or 2)* oocytes was highest for class GVA at 69.1% **[1]**, followed by class B at 57.1% **[2]**, and class C at 42.6% **[3]**.

4.2.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the bar labeled "GVA*,"

4.2.2. LAB MEDIA: Figure 3A. *Video editor: Highlight the bar labeled "GVB,"*

4.2.3. LAB MEDIA: Figure 3A. *Video editor: Highlight the bar labeled "GVC"*

4.3. The fertilization success rate after Cas9-gRNA complex injection averaged around 50% **[1]**.

4.3.1. LAB MEDIA: Figure 3B.

4.4. Of the 24 fertilized embryos, 19 developed to the 4-cell stage, representing a developmental rate of nearly 80% **[1]**.

4.4.1. LAB MEDIA: Figure 3C.

4.5. A 50 to 100 base pair deletion was detected in 3 out of 4 embryos after Cas9-gRNA complex injection **[1]**.

4.5.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the three lanes labeled "deleted".*

1. Intramuscularly
   Pronunciation link: https://www.merriam-webster.com/dictionary/intramuscular

IPA: /ˌɪntrəˈmʌskjələr/
Phonetic Spelling: in·truh·muh·sku·lur

2. Chorionic
Pronunciation link: https://www.merriam-webster.com/dictionary/chorionic
IPA: /ˌkɔːriˈɑːnɪk/
Phonetic Spelling: kor·ee·on·ik
3. Gonadotropin
Pronunciation link: https://www.merriam-webster.com/dictionary/gonadotropin
IPA: /ˌgoʊˌnædəˈtroʊpɪn/
Phonetic Spelling: goh·nad·uh·troh·pin
4. Marmoset
Pronunciation link: https://www.merriam-webster.com/dictionary/marmoset
IPA: /ˈmɑːrməˌsɛt/
Phonetic Spelling: mar·muh·set
5. Oocytes
Pronunciation link: https://www.merriam-webster.com/dictionary/oocyte
IPA: /ˈoʊəˌsaɪt/
Phonetic Spelling: oh·uh·site
6. Anesthetized
Pronunciation link: https://www.merriam-webster.com/dictionary/anesthetized
IPA: /əˈnɛsθəˌtaɪzd/
Phonetic Spelling: uh·nes·thuh·tyzd
7. Isoflurane
Pronunciation link: https://www.merriam-webster.com/dictionary/isoflurane
IPA: /ˌaɪsoʊˈflʊreɪn/
Phonetic Spelling: eye·soh·floor·ayn
8. Follicular
Pronunciation link: https://www.merriam-webster.com/dictionary/follicular
IPA: /fəˈlɪkjələr/
Phonetic Spelling: fuh·lih·kyuh·lur
9. Aspiration
Pronunciation link: https://www.merriam-webster.com/dictionary/aspiration
IPA: /ˌæspəˈreɪʃən/
Phonetic Spelling: as·puh·ray·shun
10. Ligate
Pronunciation link: https://www.merriam-webster.com/dictionary/ligate
IPA: /ˈlaɪˌgeɪt/
Phonetic Spelling: lye·gayt
11. Stereomicroscope
Pronunciation link: https://www.merriam-webster.com/dictionary/stereomicroscope
IPA: /ˌstɛrioʊˈmaɪkrəˌskoʊp/
Phonetic Spelling: stair·ee·oh·my·kruh·skohp

12. Adipose
    Pronunciation link: https://www.merriam-webster.com/dictionary/adipose
    IPA: /ˈædəˌpoʊs/
    Phonetic Spelling: ad·uh·pohs
13. Waymouth's-HEPES
    Pronunciation link: No confirmed link found
    IPA: /ˈweɪmaʊθs ˈhɛpɛs/
    Phonetic Spelling: way·mowths hep·ess
14. Follicles
    Pronunciation link: https://www.merriam-webster.com/dictionary/follicle
    IPA: /ˈfɑːlɪkəl/
    Phonetic Spelling: fah·luh·kul
15. Metaphase
    Pronunciation link: https://www.merriam-webster.com/dictionary/metaphase
    IPA: /ˈmɛtəˌfeɪz/
    Phonetic Spelling: meh·tuh·fayz
16. Germinal
    Pronunciation link: https://www.merriam-webster.com/dictionary/germinal
    IPA: /ˈdʒɝːmɪnəl/
    Phonetic Spelling: jer·muh·nul
17. Incubator
    Pronunciation link: https://www.merriam-webster.com/dictionary/incubator
    IPA: /ˈɪŋkjəˌbeɪtər/
    Phonetic Spelling: ing·kyuh·bay·ter
18. Centrifuge
    Pronunciation link: https://www.merriam-webster.com/dictionary/centrifuge
    IPA: /ˈsɛntrəˌfjuːdʒ/
    Phonetic Spelling: sen·truh·fyooj
19. Supernatant
    Pronunciation link: https://www.merriam-webster.com/dictionary/supernatant
    IPA: /ˌsuːpərˈneɪtənt/
    Phonetic Spelling: soo·per·nay·tunt
20. Capacitation
    Pronunciation link: https://www.merriam-webster.com/dictionary/capacitation
    IPA: /kəˌpæsɪˈteɪʃən/
    Phonetic Spelling: kuh·pass·uh·tay·shun
21. Hemocytometer
    Pronunciation link: https://www.merriam-webster.com/dictionary/hemocytometer
    IPA: /ˌhiːməsaɪˈtɑːmɪtər/
    Phonetic Spelling: hee·muh·sy·tah·muh·ter
22. Fertilization
    Pronunciation link: https://www.merriam-webster.com/dictionary/fertilization

IPA: /ˌfɜːtələˈzeɪʃən/
Phonetic Spelling: fer·tuh·luh·zay·shun

23. Zona pellucida
Pronunciation link: https://www.merriam-webster.com/dictionary/zona%20pellucida
IPA: /ˈzoʊnə pəˈluːsɪdə/
Phonetic Spelling: zoh·nuh puh·loo·suh·duh
24. Pronuclei
Pronunciation link: https://www.merriam-webster.com/dictionary/pronucleus
IPA: /proʊˈnuːkliaɪ/
Phonetic Spelling: proh·noo·klee·eye
25. Cas9
Pronunciation link: https://www.merriam-webster.com/dictionary/Cas9
IPA: /ˌkæsˈnaɪn/
Phonetic Spelling: kaz·nine
26. gRNA
Pronunciation link: No confirmed link found
IPA: /ˌdʒiː ɑːr ˈɛn eɪ/
Phonetic Spelling: gee·ar·en·ay
27. Embryos
Pronunciation link: https://www.merriam-webster.com/dictionary/embryo
IPA: /ˈɛmbrioʊ/
Phonetic Spelling: em·bree·oh
28. Metaphase II
Pronunciation link: https://www.merriam-webster.com/dictionary/metaphase
IPA: /ˈmɛtəˌfeɪz tuː/
Phonetic Spelling: meh·tuh·fayz too
29. Hemocytometer
Pronunciation link: https://www.merriam-webster.com/dictionary/hemocytometer
IPA: /ˌhiːməsaɪˈtɑːmɪtər/
Phonetic Spelling: hee·muh·sy·tah·muh·ter
30. Deletion
Pronunciation link: https://www.merriam-webster.com/dictionary/deletion
IPA: /dɪˈliːʃən/
Phonetic Spelling: dih·lee·shun