Submission ID #: 61999
Scriptwriter Name: Bridget Colvin
Project Page Link: https://www.jove.com/account/file-uploader?src=18909743

Title: Application of Mouse Parthenogenetic Haploid Embryonic Stem Cells as a Substitute of Sperm

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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 similar? **Y**

If you require a microscope for your technique but you can record movies/images through your microscope with your own camera, please indicate **Yes** here: **Y**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

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 self-record interview statements outside of the filming date. JoVE can provide support for this option.

4. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Number of Shots: **13**

Introduction

1. Introductory Interview Statements

NOTE: All interview statements uploaded to AWS and slated according to script.

REQUIRED:

1.1. **Eishi Aizawa:** This protocol facilitates the modification of genomic imprinting in haploid mouse embryonic stem cells as a substitute for sperm, enabling the generation of semi-cloned embryos and mice **[1]**.

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

REQUIRED:

1.2. **Eishi Aizawa**: The genetic modification of gametes generally requires difficult techniques. Using this methodology, parental genomes can be manipulated in haploid embryonic stem cells for subsequent introduction into mice as a sperm replacement option **[1]**.

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

OPTIONAL:

1.3. **Eishi Aizawa**: As this method enables the allele-specific modification of paternal genomes in embryos, it is particularly applicable to studies of genomic imprinting and fertilization **[1]**.

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

Introduction of Demonstrator on Camera

1.4. **Eishi Aizawa**: Demonstrating the procedure will be Charles-Etienne Dumeau, a mouse embryologist from my laboratory **[1][2]**.

1.4.1. LAB MEDIA: Author saying the above *Video Editor: please use audio from 1.4.1. with video from 1.4.2.*

1.4.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera *Videographer: Please film Charles-Etienne Dumeau at bench or similar in lab to be shown with with the audio from 1.4.1.*

Ethics Title Card

1.5. Procedures involving animal subjects have been approved under the license ZH152/17 in accordance with the regulations of the Cantonal Ethics Commission Zurich and ETH Zurich.

Protocol

2. Holding and Microinjection Pipette Preparation

2.1. To prepare holding and microinjection pipettes for the experiment, first use a micropipette puller to pull borosilicate glass capillaries with an elongated shape and a gradual taper **[1]**.

2.1.1. WIDE: Talent pulling capillary

2.2. To prepare holding pipettes, position a pulled capillary with an outer diameter of 60-100 micrometers in a microforge over the glass bead on the filament **[1]**.

2.2.1. LAB MEDIA: 2.2_to_2.4: 00:00-00:07

2.3. Switch on the filament and lower the capillary until it contacts the bead. When the capillary has attached to the bead, switch-off the filament and retract the capillary, causing the capillary to break with a straight end **[1]**.

2.3.1. LAB MEDIA: 2.2_to_2.4: 00:15-00:21

2.4. Next, position the broken capillary tip horizontally next to the glass bead on the filament and heat the filament to melt the end until the inner diameter of the capillary tip reaches 10-20 micrometers in diameter **[1]**.

2.4.1. LAB MEDIA: 2.2_to_2.4: 00:35-00:56 *Video Editor: can speed up*

2.5. Then position the capillary above the glass bead approximately 1 millimeter from the tip and heat the filament to allow the capillary to be bent to a 20-degree angle **[1]**.

2.5.1. LAB MEDIA: 2.2_to_2.4: 01:10-01:24 *Video Editor: can speed up*

2.6. To prepare a microinjection pipette, position a pulled capillary with an outer diameter of about 6 micrometers above the glass bead on the heated filament and lower the capillary until it contacts the bead. Once the capillary has attached, switch-off the filament and retract the capillary. The capillary will break with a straight end **[1]**.

2.6.1. LAB MEDIA: 2.5: 00:14-00:20

2.7. Then move the capillary over the glass bead approximately 1 millimeter from the capillary tip and heat the filament to allow the capillary to be bent at a 20-degree angle **[1]**.

2.7.1. LAB MEDIA: 2.6: 00:13-00:22

3. Intracytoplasmic Double Knockout Parthenogenetic Haploid Embryonic Stem Cell (DKO-phaESC) Injection

3.1. To perform an intracytoplasmic injection, place 5-microliter drops of PVP (P-V-P) solution **[1-TXT]** and 20-microliter drops of M2 medium on the upside-down lid of a 10-centimeter dish **[2-TXT]** and cover the drops with mineral oil **[3]**.

3.1.1. WIDE: Talent placing drops onto lid, with PVP container visible in frame **TEXT: PVP: polyvinylpyrrolidone**

3.1.2. Drops being added to lid, with medium container visible in frame **TEXT: See text for all medium and solution preparation details**

3.1.3. Drops being covered, with oil container visible in frame

3.2. Place the dish onto the stage **[1]** and install a holding pipette onto the micromanipulator **[2]**.

3.2.1. Talent placing dish onto stage

3.2.2. Talent installing holding pipette

3.3. Use a microloader tip to fill the microinjection pipette with fluorocarbon oil **[1]** and mount the pipette onto the piezo actuator **[2]**.

3.3.1. Talent loading tip with fluorocarbon oil

3.3.2. Talent mounting pipette onto actuator

3.4. Observing through the microscope, lower the microinjection pipette into a drop of PVP solution **[1]**. When the pipette is immersed, pipet up and down several times to coat the glass with the PVP **[2]**.

3.4.1. LAB MEDIA: 3.4_to-3.5: 00:00-00:05

3.4.2. LAB MEDIA: 3.4_to-3.5: 00:06-00:20 *Video Editor: can speed up*

3.5. Load a small volume of PVP solution into the microinjection pipette **[1]**

3.5.1. LAB MEDIA: 3.4_to-3.5: 00:40-00:45

3.6. Immerse the microinjection and holding pipettes in the M2 medium and focus on the pipette at the bottom of the drop **[1]**.

3.6.1. LAB MEDIA: 3.5.2_to_3.6: 00:08-00:16

3.7. Transfer approximately 2 microliters of the double knockout-parthogenetic ES (E-S) cell suspension into the M2 medium drop **[1]** and use a mouth pipette to transfer ten M-two oocytes into the same drop **[2-TXT]**.

3.7.1. Talent transferring suspension into drop

3.7.2. Talent using mouth pipette to transfer oocytes into drop **TEXT: See text for MII oocyte preparation details**

3.8. Rotate one oocyte and apply negative pressure through the holding pipette to hold the oocyte so that the perivitelline space faces the microinjection pipette **[1]**, taking care that the metaphase-two plate is not positioned in the path of the microinjection pipette **[2]**.

3.8.1. LAB MEDIA: 3.8_to_3.10.1: 00:00-00:11

3.8.2. Talent positioning oocyte for injection *Videographer: Important step*

3.9. Draw one double knockout-parthogenetic ES cell into the tip of the microinjection pipette **[1]** and confirm the rupture of the cell membrane **[2]**.

3.9.1. LAB MEDIA: 3.8_to_3.10.1: 00:12-00:22

3.9.2. LAB MEDIA: 3.8_to_3.10.1: 00:23-00:28

3.10. Apply light negative pressure to the microinjection pipette and deliver four piezo impulses while pushing the tip of the microinjection pipette toward the perivitelline space to break through the zona pellucida **[1]**.

3.10.1. LAB MEDIA: 3.10.2_to_3.15: 00:12-00:22

3.11. Penetrate the oocyte with the microinjection pipette so that the oolemma stretches to the opposite side and apply one piezo pulse to pierce the oolemma, making sure that the oolemma relaxes along the shaft of the microinjection pipette **[1]**.

3.11.1. LAB MEDIA: 3.10.2_to_3.15: 00:23-00:32

3.12. Inject the double knockout-parthogenetic ES cell with a minimal volume of medium into the ooplasm and withdraw the microinjection pipette smoothly from the oocyte **[1]**.

3.12.1. LAB MEDIA: 3.10.2_to_3.15: 00:33-00:45 *Video Editor: can speed up*

3.13. Release the injected oocyte from the holding pipette and move it on the side of the microdrop for later collection **[1]**.

3.13.1. LAB MEDIA: 3.10.2_to_3.15: 00:45-00:50

3.14. When all of the oocytes have been injected, transfer the oocytes to a pre-warmed center-well dish containing KSOM (K-S-O-M) medium **[1]** and incubate the dish at 37 degrees Celsius and 5% carbon dioxide for 1 hour before activation **[2-TXT]**.

3.14.1. Talent transferring oocytes from the microscope to a dish, with medium container visible in frame

3.14.2. Talent placing dish into incubator **TEXT: See text for constructed embryo activation details**

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see? Please list 4 to 6 individual steps.
3.8.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success? Please list 1 or 2 individual steps from the script above.
3.10., 3.11. The most difficult aspect is to set the piezo actuator for the following steps. Too strong settings will damage the oocyte, too weak settings will not have effect on the drilling process. It is important to empirically set the piezo actuator every time you change the microinjection pipette as every pipette are slightly different; use 2 or 3 oocytes for this purpose and discard them.

Results

4. Results: Representative Establishment and Application of DKO-phaESC

4.1. DNA content analysis of this representative double knockout-parthogenetic ES cell line carrying the CAG-EGFP (kag E-G-F-P) transgene **[1]** by flow cytometry illustrates the distribution of haploid **[2]** and diploid cells at the G0 (G-zero)-G1-, S-, and G2-M-phases **[3]**.

4.1.1. LAB MEDIA: Figure 2A
4.1.2. LAB MEDIA: Figure 2A *Video Editor: please emphasize blue columns of Transfected graph (under blue G1, S, G2/M squares)*
4.1.3. LAB MEDIA: Figure 2A *Video Editor: please emphasize red columns of Transfected graph (under red G1, S, G2/M squares)*

4.2. Genotyping can be performed **[1]** to confirm the absence of wildtype **[2]** alleles at the H19 (H-nineteen)- and intergenic-differentially methylated regions **[3]**.

4.2.1. LAB MEDIA: Figure 2B
4.2.2. LAB MEDIA: Figure 2B *Video Editor: please emphasize Second genotyping H19 gels*
4.2.3. LAB MEDIA: Figure 2B *Video Editor: please emphasize Second genotyping IG gels*

4.3. A CAG-EGFP transgene can be introduced into double knockout-parthogenetic ES cells **[1]** to study their contribution to semi-cloned embryos by the visualization of green fluorescence under a microscope **[2]**.

4.3.1. LAB MEDIA: Figure 3A
4.3.2. LAB MEDIA: Figure 3A *Video Editor: please emphasize green signal in EGFP figure*

4.4. Flow cytometry analysis **[1]** shows two populations corresponding to the G2-M phase arrested haploid and diploid cells after demecolcine treatment **[2]**. The absence of a 1n (one-N) haploid peak indicates that the cell cycle arrest was largely complete **[3]**.

4.4.1. LAB MEDIA: Figure 3B
4.4.2. LAB MEDIA: Figure 3B *Video Editor: please emphasize 500 and 800 peaks in bottom graph*
4.4.3. LAB MEDIA: Figure 3B *Video Editor: please emphasize lack of peak between 200-300*

4.5. After injection, EGFP expression is rarely detected in the constructed semi-cloned embryos **[1]**, as the cytoplasm of double knockout-parthogenetic ES cell disperses into the large cytoplasm of the oocyte **[2]**.

4.5.1. LAB MEDIA: Figure 3D
4.5.2. LAB MEDIA: Figure 3D *Video Editor: please emphasize arrowheads/green signal indicated by arrowheads*

4.6. Six hours after activation **[1]**, up to 3 polar bodies can be observed under the microscope - the first and second polar bodies of the oocyte and one pseudo polar body from the haploid ESC **[2]**.

4.6.1. LAB MEDIA: Figure 3E
4.6.2. LAB MEDIA: Figure 3E *Video Editor: please emphasize arrowheads/polar bodies indicated by arrowheads*

4.7. To demonstrate their developmental competence, semi-cloned embryos can be cultured to the blastocyst stage **[1]**.

4.7.1. LAB MEDIA: Figure 5A *Video Editor: please sequentially add/emphasize images from Day 1 to Day 5*

4.8. Furthermore, full-term female mice can be obtained from semi-cloned 2-cell stage embryos transferred into the oviducts of recipient females **[1]**.

4.8.1. LAB MEDIA: Figure 5B *Video Editor: please sequentially emphasize pup in left image, both pups and a mouse in middle figure, and mouse in right image*

Conclusion

NOTE: All interview statements uploaded to AWS and slated according to script.

5. Conclusion Interview Statements

5.1. **Charles Etienne-Dumeau**: During microinjection, it is important to rupture the plasma membrane of the haploid embryonic stem cells, as a failure in this step prevents chromosome segregation when the oocyte is activated **[1]**.

5.1.1. LAB MEDIA: Named talent says the statement above in an interview-style shot, looking slightly off-camera (3.10.)

5.2. **Eishi Aizawa**: Instead of parthenogenetic haESCs, androgenetic haESCs can also be used, allowing either the maternal or paternal genome to be selected as the sperm replacement, depending on the study purpose **[1]**.

5.2.1. LAB MEDIA: Named talent says the statement above in an interview-style shot, looking slightly off-camera