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Title: Improved Genome Editing *via* Oviductal Nucleic Acids Delivery-based In Vivo Electroporation Technique for Knockout Mice Generation

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

x Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes**

3.9.1

3.11.1

3.11.2

3.11.3

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **15/09/2025**

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare.

Current Protocol Length
Number of Steps: 34
Number of Shots: 56

Introduction

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

1.1. **Ilyas Akhmarov:** The scope of our research is to simplify the generation of knockout mice by using in utero electroporation, delivering genome-editing components directly into the oviducts of pregnant females.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: Figure 1*

What are the current experimental challenges?

1.2. **Ilyas Akhmarov:** Microinjection into zygotes requires maintaining a large number of animals, including donor and recipient females and vasectomized males. Therefore, protocols that minimize animal use are preferable.

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

What advantage does your protocol offer compared to other techniques?

1.3. **Ilyas Akhmarov:** The use of the Nepa21 system for zygote electroporation provides a unique opportunity to generate targeted gene knockout mice in a single step with efficacy comparable with standard microinjection-based techniques.

1.3.1. 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.

Ethics Title Card

This research has been approved by the Animal Research Ethics Committee of Saint Petersburg State University

Protocol

2. Single-Guide RNA (sgRNA) Design and its Preparation for Transfection

Demonstrator: Oleg A. Kirillov

2.1. To begin, use computational analysis tools to identify potential single guide RNA sequences targeting the selected gene of interest **[1]**.

2.1.1. WIDE: Talent seated at a workstation with computational analysis tool visible on the screen.

2.2. Select sequences located in exons that are as close as possible to the desired mutation site **[1]**. Use a genome browser to cross-reference exon structure and prioritize sequences present in most splice isoforms **[2]**.

2.2.1. SCREEN: SCREEN 2.2.1.mkv: 00:03-00:12.

2.2.2. SCREEN: SCREEN 2.2.2.mkv

2.3. Analyze the predicted off-target activity of each guide sequence using the tool's integrated scoring system **[1]**. Choose sequences that demonstrate the lowest likelihood of off-target binding **[2-TXT]**.

2.3.1. SCREEN: SCREEN 2.3.1.mkv

2.3.2. SCREEN: SCREEN 2.3.2.mkv: **TXT: Order primers for sgRNA template PCR**

2.4. To produce a linear double-stranded DNA template, prepare the PCR master mix using the components specified in the given table **[1]**. Add the polymerase last and keep the master mix on ice to preserve enzymatic stability **[2]**.

2.4.1. LAB MEDIA: Table 1

2.4.2. Talent adding polymerase to the tube and placing it on an ice rack.

2.5. Then, run the PCR using the following thermal cycling conditions **[1]**.

2.5.1. TEXT ON A PLAIN BACKGROUND

Initial Denaturation: 95 °C for 30 s

Denaturation: 95 °C for 3 s, 30 cycles

Annealing: 55 °C for 20 s

Extension: 72 °C for 15s

Final Elongation: 72 °C for 2 min

Hold: 4 °C.

2.6. Next, prepare the in vitro transcription master mix using the given components **[1]** and incubate the reaction for 1.5 hours at 37 degrees Celsius using a thermocycler for consistent temperature control **[1]**.

2.6.1. LAB MEDIA: Table 2

2.6.2. Talent placing the tube into a thermocycler set at 37 degrees Celsius.

2.7. Then, add 2 microliters of DNase I to the reaction **[1]**. Incubate at 37 degrees Celsius for an additional 30 minutes to digest the DNA template and then inactivate the enzyme at 75 degrees Celsius for 5 minutes **[2]**.

2.7.1. Talent adding DNase I into the tube.

2.7.2. Talent placing the tube in the thermocycler to 37 degrees Celsius.

2.8. After TRIZOL *(Trizol)* purification of RNA, measure the RNA concentration using a spectrophotometer and check the A260 *(A-Two-Sixty)* to A280 *(A-Two-Eighty)* and A260 to A230 *(A-Two-Thirty)* ratios **[1-TXT]**.

2.8.1. Show spectrophotometer results with A260/A280 and A260/A230 ratios. **TXT: A260/280 ≥ 2.0; A260/230 ≥ 2.0; Store at -80 °C until use**

2.9. To prepare the ribonucleoprotein complex for transfection, dilute 1 molar Tris-HCl buffer to a concentration of 100 millimolar using nuclease-free water **[1]**. Filter the solution using a syringe filter with a 0.022-micrometer pore size, aliquot into 10-microliter portions **[2],** and store at 4 degrees Celsius until use **[3]**.

2.9.1. Talent performing the dilution of Tris-HCl buffer with nuclease-free water.

2.9.2. Talent filtering the solution using a syringe filter, aliquoting into labeled tubes.

2.9.3. Talent placing the tubes into a 4 degrees Celsius refrigerator.

2.10. Next, prepare the transfection mix using spCas9 *(S-P-Cas-Nine)* nuclease, single guide RNA, and Tris-HCl diluted with DEPC *(D-E-P-C)* water in a nuclease-free tube **[1-TXT]**.

2.10.1. Talent pipetting spCas9, sgRNA, and diluted Tris-HCl into a nuclease-free microcentrifuge tube to assemble the transfection mix. **TXT: 500 ng/µL spCas9 nuclease; 80 ng/µL sgRNA; 10 mM Tris-HCl**

2.11. Incubate the transfection mix at 37 degrees Celsius for 10 minutes to allow formation of the ribonucleoprotein complex **[1]**. Then, aliquot the transfection mix into small volumes suitable for single I-GONAD *(I-Gonad)* sessions and keep it at 4 degrees Celsius to preserve reagent activity **[2]**.

2.11.1. Talent placing the tube into a thermocycler set at 37 degrees Celsius.

2.11.2. Talent pipetting 2 microliter portions of the transfection mix into individual low-retention tubes and keeps it on ice.

3. Preparation of Female Mice for Genome Editing via Oviductal Nucleic Acid Delivery

Demonstrators: Elena I. Leonova, Ilyas I. Akhmarov

3.1. After mating CD1 female mice with single-housed CD1 male mice overnight, check each female for the presence of a copulation plug **[1]**. Transfer only those females with visible plugs into a new cage for further experimental procedures **[2]**.

3.1.1. Talent gently lifting the tail of a female mouse to check for a copulation plug the following morning.

3.1.2. Talent transferring plug-positive females into a clean, labeled cage.

3.2. Autoclave all stainless-steel surgical instruments to ensure sterility before use **[1]**.

3.2.1. Talent loading surgical tools into an autoclave tray and initiating the sterilization cycle.

3.3. Then, weigh the selected plug-positive female mice using an electronic scale before anesthetizing them **[1-TXT]**.

3.3.1. Talent placing a female mouse on a digital scale and recording the weight. **TXT: Anesthesia: Ketamine (100 mg/kg) and Xylazine (10 mg/kg)**

3.4. Monitor the anesthetized mice for loss of the toe pinch reflex to confirm adequate anesthesia **[1]**. Place the mouse on a heating pad to maintain body temperature during the procedure **[2]**. Then, apply veterinary ointment to the eyes to prevent dryness **[3]**.

3.4.1. Talent gently pinching a toe to check for anesthesia depth.

3.4.2. Talent positioning the mouse on a heating pad.

3.4.3. Talent applying ointment to the mouse's eyes.

3.5. Next, disinfect the dorsal region of the mouse using 70 percent ethanol to sanitize the surgical area **[1]**. Using fine scissors, make a 0.7-centimeter longitudinal incision in the skin of the lower back region **[2]**.

3.5.1. Talent applying 70 percent ethanol to the lower back of the mouse using a soaked cotton pad. NOTE: Use timestamps; 00:14-00:17

3.5.2. Talent making a precise skin incision in the lower back using fine scissors. NOTE: Use timestamps; 3.5.2. 00:22-00:27

3.6. Wipe the incision site again with a cotton pad soaked in 70 percent ethanol and remove any remaining hair around the wound area **[1]**.

3.6.1. Talent cleaning the incision and clearing residual hair using a soaked cotton pads. NOTE: Use timestamps; 00:30-00:33

3.7. Then, laterally spread the slightly opened tips of the scissors to gently extend the skin incision to approximately 1.3 centimeters **[1]**. After that, carefully make a 0.7-centimeter incision in the peritoneum to expose the abdominal cavity **[2]**.

3.7.1. Talent spreading the incision laterally using partially opened scissors. NOTE: Use timestamps; 0:41-0:45

3.7.2. Talent making a small, controlled peritoneal incision with scissors.

3.8. Extend the peritoneal incision to 1.1 centimeters by laterally pushing with slightly opened scissor tips **[1]**. Locate the fat pad connected to the ovary and gently pull it outward using forceps until the ovary, oviduct, and uterus are exposed **[2]**. Using a vessel clamp, secure the fat pad to stabilize the reproductive structures **[3]**.

3.8.1. Talent widening the peritoneal opening using the scissor tips.NOTE: Use timestamps; 0:55-0:56

3.8.2. Talent identifying the fat pad and pulling it outward carefully with forceps.NOTE: Use timestamps; 1:10-1:15

3.8.3. Talent placing a vessel clamp to hold the fat pad in position.NOTE: Use timestamps; 1:17-1:20

3.9. Now, place the surgical setup under a stereoscopic microscope and visualize the oviduct for precise manipulation **[1]**.

3.9.1. SCOPE: 3.11.1-3.11.3.mp4: 00:25-00:27

3.10. Next, load 2 microliters of the ribonucleoprotein injection mix into a disposable plastic capillary using a Stripper Pipettor **[1]**.

3.10.1. Talent aspirating the injection mix into the plastic capillary using the Stripper Pipettor.

3.11. Using micro-scissors, make a 0.2-centimeter incision in the oviduct wall a few millimeters upstream of the ampulla **[1]**. Insert the plastic capillary attached to the Stripper Pipettor into the oviduct incision and carefully expel the injection mix without introducing air **[2]**. Gently withdraw the plastic capillary from the oviduct to complete the injection **[3]**.

3.11.1. SCOPE: 3.11.1-3.11.3.mp4: 00:45-00:55

3.11.2. SCOPE: 3.11.1-3.11.3.mp4: 01:10-01:26

3.11.3. SCOPE: 3.11.1-3.11.3.mp4: 01:35-01:38

4. Electroporation and Suturing of the Abdominal Cavity

4.1. Turn on the electroporator by pressing the power switch on the front panel **[1]**.

4.1.1. Talent pressing the switch to power on the electroporator.

4.2. Set the electroporation parameters on the device as shown on the screen **[1]**.

4.2.1. TEXT ON A PLAIN BACKGROUND

Poring Pulse	**Transfer Pulse**
Voltage: 50 V	Voltage: 10 V
Pulse length: 5 m	Pulse length: 50 ms
Pulse interval: 50 ms	Pulse interval: 50 ms,
Number of pulses: 3	Number of pulses: 3,
Decay rate – 10%	Decay rate – 40%,
Polarity: +/-;	Polarity: +/-;
	Current limit: *in vivo*

4.3. Using tweezer-type electrodes, gently grasp the oviduct **[1]** and press the **kΩ** *(Kiloohm)* button to measure the resistance **[2-TXT]**.

4.3.1. Talent holding the oviduct with tweezer-type electrodes.

4.3.2. Talent pressing the kΩ button**. TXT: Compress electrodes to keep resistance**

350–400 Ω

4.4. Immediately after resistance measurement, press the **Start** button to begin electroporation and wait for the "end" signal to appear on the Ω button **[1]**.

4.4.1. Talent pressing the **Start** button while holding the oviduct in position.

4.5. After electroporation of the first oviduct, gently return the reproductive tract to the abdominal cavity **[1]**. Close the incision using 4-0 polyglycolic acid surgical suture **[2-TXT]**.

4.5.1. Talent positioning the reproductive tract back into the abdomen using forceps.

4.5.2. Talent suturing the incision with steady hand motions using 4-0 surgical suture.
TXT: Repeat the operation and electroporation on the second oviduct

4.6. Place the mouse in a clean cage warmed to 37 degrees Celsius for recovery and monitor continuously until the mouse fully regains consciousness **[1]**.

4.6.1. Talent placing the mouse on a heating pad inside a recovery cage and observing its breathing and movement.

5. Genotyping to Confirm Knockout Generation

Demonstrator: Ilyas I. Akhmarov

5.1. For genotyping, design 18–21 nucleotide-long primers, with a melting temperature near 60 degrees Celsius, to generate a 300–600 base pair PCR product **[1]**.

5.1.1. SCREEN: 5.1.1.mp4

5.2. To confirm the specificity of each primer, perform a BLAST search against the mouse genome database to eliminate the risk of non-specific amplification **[1]**.

5.2.1. SCREEN: 5.2.1.mp4

5.3. Next, for DNA extraction, using an ear punch, collect a small tissue sample approximately 2 millimeters in size from the mouse's ear **[1]**. Add 100 microliters of lysis buffer **[2]**. Incubate the sample at 95 degrees Celsius for 60 minutes, vortexing every 20 minutes to ensure efficient lysis **[3]**.

5.3.1. Talent performing an ear punch and placing the tissue fragment into a labeled microcentrifuge tube.

5.3.2. Talent pipetting lysis buffer into the tube containing the ear tissue.

5.3.3. Talent placing the tube into a heat block at 95 degrees Celsius and vortexing at regular interval.

5.4. After performing Sanger sequencing, analyze the results to assess genome editing outcomes **[1]**.

5.4.1. SCREEN: 5.4.1.mp4: 00:05-00:10

5.5. To use ICE *(I-C-E)* Analysis, upload both the Sanger sequencing files, enter the guide RNA sequence, and select the appropriate nuclease used in the CRISPR experiment **[1]**.

5.5.1. SCREEN: 5.5.1.mp4

5.6. Next, to use TIDE *(Tide),* upload the control and experimental sequencing files in .ab1 *(A-B-One)* format and enter the guide RNA sequence **[1]**.

5.6.1. SCREEN: 5.6.1.mp4

Results

6. Results

6.1. ICE analysis detected three distinct indel variants at the Cas9 cut site: a 1 base pair deletion in 42% of sequences **[1],** a 3 base pair deletion in 25% of sequences **[2],** and a 23 base pair deletion in 27% of sequences **[3].**

6.1.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the row labeled "-1" and the 42% contribution column next to it*.

6.1.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the row labeled "-3" and the 25% contribution column next to it.*

6.1.3. LAB MEDIA: Figure 3B. *Video editor: Highlight the row labeled "-23" and the 27% contribution column next to it*.

6.2. The model fit value was 0.94, and the knockout score was 69, indicating a strong predicted correlation and likely gene knockout **[1].**

6.2.1. LAB MEDIA: Figure 3A

6.3. Sequence chromatograms confirmed disruption at the Cas9 cut site in the edited sample **[1],** whereas the control chromatogram showed an intact sequence at the same site **[2].**

6.3.1. LAB MEDIA: Figure 3C (Top Panel). *Video editor: Zoom in on the region labeled "Cas9 cut site" .*

6.3.2. LAB MEDIA: Figure 3C (Bottom Panel). *Video editor: Highlight the smooth, uninterrupted peaks across the "Cas9 cut site*"

6.4. Out of 6 electroporated mice, only 4 produced pups with mutations, resulting in a 60% overall editing efficiency **[1].** All litters across the six electroporated mice showed reduced pup numbers, with only 3 to 4 pups per mouse **[2]**

6.4.1. LAB MEDIA: Table 4 *Video editor: Highlight last 4 rows*

6.4.2. LAB MEDIA: Table 4 *Video editor: Highlight the column "Amount of pups"*

Pronunciation Guide

1. **Trizol (TRIzol)**
   Pronunciation link: https://www.howtopronounce.com/trizol (HowToPronounce)
   IPA: /tɹaɪˈzɑl/ or /tɹaɪˈzɔl/
   Phonetic spelling: try-ZOL
2. **sgRNA**
   (Abbreviation: "S-G-R-N-A")
   You would typically say each letter: "ess-gee-ar-en-ay"
   (No single-word dictionary link, as it's an acronym in molecular biology.)
3. **DEPC (diethyl pyrocarbonate)**
   (Often pronounced as the acronym: "dee-ee-pee-see")
   — If spelled out chemically, "diethyl pyrocarbonate" would be /ˌdaɪˈiːθəl paɪroʊˈkɑrbənət/
   Phonetic: dye-ETH-uhl py-roh-CAR-boh-nate
4. **electroporation**
   Pronunciation link: https://www.merriam-webster.com/dictionary/electroporation
   IPA: /ɪˌlɛktroʊpəˈreɪʃən/
   Phonetic spelling: i-LEC-troh-po-RAY-shun
5. **polyglycolic**
   Pronunciation link: https://www.merriam-webster.com/dictionary/polyglycolic
   IPA: /ˌpɑliglaɪˈkɑlɪk/
   Phonetic spelling: pol-ee-glye-KAH-lik
6. **copulation plug**
   - *copulation*
     Pronunciation link: https://www.merriam-webster.com/dictionary/copulation
     IPA: /ˌkoʊpjʊˈleɪʃən/
     Phonetic spelling: co-pyoo-LAY-shun
   - *plug*
     Pronunciation link: https://www.merriam-webster.com/dictionary/plug
     IPA: /plʌg/
     Phonetic spelling: plug
7. **thermocycler**
   Pronunciation link: https://www.merriam-webster.com/dictionary/thermocycler (if available)
   IPA: /ˈθɜrmoʊˌsaɪlər/
   Phonetic spelling: THER-moh-sigh-ler
8. **ampulla**

Pronunciation link: https://www.merriam-webster.com/dictionary/ampulla
IPA: /æmˈpʌlə/
Phonetic spelling: am-PUL-uh

9. **base pair (bp)**
   Usually pronounced “base pair” (rhymes with “face” + “pear”)
   — “bp” spelled out: “bee-pee”

10. **ICE Analysis**
    (Acronym pronounced as “ice”)
    — Analysis: /əˈnæləsɪs/
    Phonetic: uh-NAL-uhsis

11. **TIDE**
    (Typically pronounced like the English word “tide”)
    — (acronym for Tracking of Indels by Decomposition)