

Submission ID #: 68299

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Project Page Link: <https://review.jove.com/account/file-uploader?src=20831243>

Title: Identification of Pre-Weanling 7-Day-Old Mice with Pinna Edge Biopsies

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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? **No**

- 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**

Current Protocol Length

Number of Steps: 10

Number of Shots: 22

Introduction

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

- 1.1. **Diane Chen:** The scope of this research is to discover, develop, and then verify a novel method of simultaneous identification and genotyping for pups between the ages of 7 and 14 days old.

- 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.1*

What research gap are you addressing with your protocol?

- 1.2. **Diane Chen:** This protocol demonstrates a novel method to identify and genotype mice at 7 days old, enabling earlier research access than current methods typically performed at 14 days or later.

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

What new scientific questions have your results paved the way for?

- 1.3. **Diane Chen:** The current PEB method provides 9 ID patterns, sufficient for litters of up to 9. For larger litters, new PEB combinations must be developed to expand identification options.

- 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.9*

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

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Massachusetts General Hospital

Protocol

2. Mouse Pinna Biopsy for Identification and Genotyping

Demonstrator: Diane Chen

- 2.1. To begin, gently restrain a mouse pup in a position that permits visualization and access to both ears [1]. Using the non-dominant hand, pick up the pup by the base of the tail [2] and place it on a stable surface on top of the cage bars [3].
 - 2.1.1. WIDE: Talent securing a mouse pup with ears visible and accessible.
 - 2.1.2. Talent lifting the pup by the tail base with their non-dominant hand. **NOTE: 2.1.2 and 2.1.3 were combined into 1 shot**
 - 2.1.3. Talent positioning the pup onto a flat, stable surface on top of the cage bars.
- 2.2. Use the thumb and forefinger of the dominant hand to apply gentle pressure along the pup's back until the loose skin between the ears can be pinched [1]. Then pinch the skin securely to restrict head movement, ensuring the animal breathes normally [2].
 - 2.2.1. Talent pinching the skin between the pup's ears with the dominant hand. **NOTE: 2.2.1. and 2.2.2 were combined into 1 shot**
 - 2.2.2. Shot of the animal's head held firmly but not impairing breathing.
- 2.3. If restraining with the left hand, orient the pup so that its nose points away from the handler's body, making the right ear pinna accessible [1]. To access the left ear pinna, rotate the restraining hand so that the left side of the pup faces the handler [2].
 - 2.3.1. Talent positioning the pup's nose away while restraining with left hand.
 - 2.3.2. Talent rotating their hand to expose the left ear pinna.
- 2.4. If restraining with the right hand, orient the pup's nose away from the handler to expose the left ear pinna [1]. To access the right ear pinna, supinate the hand grasping the animal until the right side of the pup faces the handler [2].
 - 2.4.1. Talent positioning the pup's nose away while restraining with right hand.
 - 2.4.2. Talent rotating their hand to expose the right ear pinna.
- 2.5. Use a total of nine patterns for the pinna edge biopsy or PEB (*P-E-B*), which includes eight biopsy patterns and one no-biopsy option [1-TXT]. ~~Record the chosen PEB pattern [2].~~
 - 2.5.1. Talent selecting a pattern from a chart or list showing nine options. **TXT: Record the chosen PEB pattern**

~~2.5.2. Talent looking at the recording of PEB pattern.~~

~~Videographer: Please capture the screen of the instrument showing the PEB pattern.~~ **NOTE: Not filmed, VO moved as on-screen text**

- 2.6. Use clean small dissection scissors or McPherson-Vannas scissors to perform each biopsy [1]. Disinfect instruments by spraying them with disinfectant [2] and wiping them clean using 70% isopropyl alcohol after every use [3].
 - 2.6.1. Talent holding recommended scissors for biopsy. **NOTE: 2.6.1, 2.6.2, 2.6.3 were combined**
 - 2.6.2. Talent cleaning the scissors with disinfectant.
 - 2.6.3. Shot of the scissors being wiped with alcohol.
- 2.7. Hold the scissors using the dominant hand [1]. To create a vertical PEB, trim the lateral edge of the ear vertically, removing approximately one-third of the pinna [2].
 - 2.7.1. Talent gripping the scissors with dominant hand.
 - 2.7.2. Talent trimming the lateral edge of the ear in a vertical direction.
- 2.8. To create a horizontal PEB, trim the dorsal edge of the pinna at a 45-degree angle, removing about one-third of the pinna [1-TXT].
 - 2.8.1. Talent trimming the dorsal edge of the ear at a 45-degree angle. **TXT: The angled cut accommodates ear growth and ensures horizontal pattern**
- 2.9. Create one of eight designated PEB patterns [1]. Use fine forceps to carefully place the excised tissue into a sample container for genotyping [2].
 - 2.9.1. LAB MEDIA: Figure 2
 - 2.9.2. Talent transferring pinna tissue into a labeled sample container.
- 2.10. If bleeding occurs at the biopsy site, apply gentle pressure using clean gauze or a cotton tip applicator while the animal remains restrained [1]. Gently return the pup to its cage with the dam [2]. Then observe the animal for 1 to 2 minutes to check for continued bleeding from the biopsy site [3].
 - 2.10.1. Talent holding gauze or applicator to the biopsy site.
 - 2.10.2. Talent placing pup back into the cage.
 - 2.10.3. Talent monitoring pup behavior and site for bleeding for up to 2 minutes.

Results

3. Results

- 3.1. Pinna biopsy patterns remained visibly identifiable up to postnatal day 63 in mice initially marked on postnatal day 7 [1].
 - 3.1.1. LAB MEDIA: Figure 3. *Video editor: Please sequentially show each row from PND 7 to PND 63*
- 3.2. The right-horizontal pinna biopsy created at a 45-degree dorsal angle appeared more horizontal by postnatal day 63 as the ear matured [1].
 - 3.2.1. LAB MEDIA: Figure 4. *Video editor: Begin with the left image showing the angled cut, then crossfade to the right image showing the same ear fully grown with the corrected angle.*
- 3.3. Both male and female mice showed a significant increase in body weight from postnatal day 7 to 63, with males weighing more than females from postnatal day 30 onwards [1].
 - 3.3.1. LAB MEDIA: Figure 5. *Video editor: Highlight the data points for PND 30 and PND 63, first on the blue (male) line and then the red (female) line.*

1. pinna

Pronunciation link:

<https://www.merriam-webster.com/dictionary/pinna> (Cambridge Dictionary, Merriam-Webster)

IPA (American): /'pɪnə/

Phonetic Spelling: pin-nuh

2. McPherson-Vannas (scissors)

Pronunciation link:

<https://www.howtopronounce.com/vannas-scissors> (How To Pronounce, How To Pronounce)

IPA (American approximate): /mæk'fɜrsən 'vænəs/ scissors

Phonetic Spelling: muhk-FUR-suhn VAN-uhs scissors

3. supinate

No confirmed link found

IPA (American): /'su:pə,neɪt/

Phonetic Spelling: soo-puh-nayt

4. biopsy

Pronunciation link:

<https://www.merriam-webster.com/dictionary/biopsy> (Avantor, [TheFreeDictionary.com](https://www.thefreedictionary.com), [Wordsmyth Kids](https://www.wordsmythkids.com)) (if clipping doesn't exist, use MW search)

IPA (American): /'baɪ,ɑpsi/

Phonetic Spelling: BY-op-see

Additional term suggestions (though more common, included for clarity):

- **genotyping**

Pronunciation link:

<https://www.merriam-webster.com/dictionary/genotype> (Wikipedia)

IPA (American): /'dʒɪnə,taɪpɪŋ/

Phonetic Spelling: JEE-nuh-ty-ping

- **dissection**

Pronunciation link:

<https://www.merriam-webster.com/dictionary/dissection> (Wikipedia, [wpiinc.com](https://www.wpiinc.com))

IPA (American): /,dɪs'sɛkʃən/

Phonetic Spelling: dis-SEK-shun
