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## **Title: Isolation of Alveolar Type II Epithelial Cells from Neonatal, Juvenile, and Adult Murine Lungs Adaptable to Infectious Experimental Settings**

### **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? **No**

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

**Videographer's note:** Please note, that the screencapture shots 3.7.1 & 3.7.2 has been filmed with the camera. There will be no video upload by the scientists, only screenshots.

**3. Filming location:** Will the filming need to take place in multiple locations? **Yes, 1 floor apart (within same building)**

**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: 20

Number of Shots: 35

# Introduction

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

## INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

- 1.1. **Pia Grimpe:** Our research aims to identify roles of alveolar type II epithelial cells for lung development, infection and inflammation.
  - 1.1.1. 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?~~

- 1.2. **Pia Grimpe:** As a novelty, our protocol enables AECII isolation from neonatal and juvenile mice - including contagious samples.
  - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

## CONCLUSION:

~~What significant findings have you established in your field?~~

- 1.3. **Pia Grimpe:** Our lab has previously identified crucial immunoregulatory roles and trained immunity of AECII during lung inflammation and infection.
  - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What are the current experimental challenges?~~

- 1.4. **Pia Grimpe:** In general, obtaining high AECII yields and high purity at the same time is an experimental challenge.
  - 1.4.1. 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?~~

- 1.5. **Pia Grimpe:** This protocol paves the way for future research on the roles of AECII during respiratory infection and postnatal lung development.

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.***

**Ethics Title Card**

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the Helmholtz-Centre for Infection Research

# Protocol

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## 2. Isolation and Enzymatic Digestion of Murine Lung Tissue for AECII Preparation

**Demonstrator:** Pia Grimpe and Julia D. Boehme

- 2.1. To begin, obtain an euthanised juvenile mouse [1]. ~~Position the mouse, open the abdominal side with a scissor and exsanguinate mouse [2].~~ After exsanguination, carefully remove the diaphragm to expose the heart and lungs [3].

2.1.1. WIDE: Talent placing an exsanguinated mouse on the worktable.

~~2.1.2. Added shot: Shot of the mouse being positioned, the abdominal side being opened and the mouse being exsanguinated.~~

**NOTE: Please do not show the blood shedding or blood draining of the mouse**

2.1.3. Talent carefully removing the diaphragm to reveal the heart and lungs.

**TXT: Do not injure lungs to prevent efficiency of enzyme-mediated digestion**

- 2.2. Open the ribcage using scissors [1]. ~~Insert a 26-gauge cannula into the right ventricle of the heart [2].~~

2.2.1. Talent cutting open the ribcage with scissors to expose the thoracic cavity.

~~2.2.2. Talent inserting the cannula into the right ventricle.~~

**NOTE: Shot deleted by Authors**

- 2.3. For enzymatic digestion for adult and juvenile mice, expose the trachea by removing the salivary glands and surrounding muscle [1].

2.3.1. Talent removing salivary glands and muscle to expose the trachea.

- 2.4. Insert a 22-gauge indwelling cannula into the trachea [1], remove the needle [2], and advance the plastic catheter toward the lungs [3].

2.4.1. Talent inserting the cannula into the trachea.

2.4.2. Talent removing the needle from the cannula.

2.4.3. Talent advancing the catheter toward the lungs.

- 2.5. Secure a piece of yarn around the trachea and catheter to hold it in place [1]. Instill dispase through the catheter to prepare the lung tissue [2-TXT].

2.5.1. Talent tying yarn around the trachea and catheter.

2.5.2. Talent injecting dispase through the tracheal catheter. **TXT: Adult mice: 2 mL dispase; Juvenile: 500 µL dispase**

- 2.6. Exchange the syringe [1] and instill low-melt agarose at 1 percent weight per volume and 45 degrees Celsius to maintain lung structure [2-TXT].

- 2.6.1. Talent exchanging syringes.
- 2.6.2. Talent injecting low-melt agarose. **TXT: Adult mice: 500 µL low-melt agarose; Juvenile: 200 µL low-melt agarose**
- 2.7. Now, cover the lung with laboratory tissue paper [1] and add ice for 2 minutes to allow the agarose to solidify [2].
  - 2.7.1. Talent covering lungs with tissue paper.
  - 2.7.2. Talent placing ice over the tissue-covered lungs.
- 2.8. Remove the ice, tissue paper, syringe, and catheter [1]. Then tie the yarn to close the trachea and retain the enzyme in the lower airways [2].
  - 2.8.1. Talent removing all materials from the lung.
  - 2.8.2. Shot of yarn being tightened around the trachea.
- 2.9. Remove the heart and thymus [1]. Cut the trachea above the yarn [2] and remove the intact lung from the chest [3].
  - 2.9.1. Talent excising the heart and thymus.
  - 2.9.2. Talent cutting the trachea above the ligature.
  - 2.9.3. Talent lifting the intact lung from the thoracic cavity.
- 2.10. Transfer the lung into a 15-milliliter tube containing pre-warmed dispase and incubate [1-TXT].
  - 2.10.1. Talent placing lung into a tube with dispase. **TXT: Adult: 2 mL dispase; Juvenile: 1.5 mL dispase; Incubation: RT, 45 mins**
- 2.11. Incubate the neonatal lung tissue in dispase at room temperature for 45 minutes [1].
  - 2.11.1. Talent placing the tube on the bench for incubation.

### **3. Preparation of Lung Tissue and Flow Cytometric Sorting of Alveolar Type II Cells**

**Demonstrator:** Pia Grimpe

- 3.1. Remove the lung from dispase [1] and dissect the lung lobes from the bronchial stem [2].
  - 3.1.1. Talent removing lung from dispase solution.
  - 3.1.2. Talent dissecting lung lobes from the bronchial stem.  
**AUTHOR'S NOTE: Wrong Clapperboard! Use 3.2.1.1**
- 3.2. Place the lung lobes into a Petri dish containing 7 milliliters of DMEM (*D-M-E-M*)

supplemented with HEPES (*He-Pees*) and 100 microliters DNase (*D-N-Ase*) [1].

3.2.1. Talent transferring lung lobes into the prepared Petri dish with 7 mL DMEM and 100  $\mu$ L DNase.

**AUTHOR'S NOTE: Wrong Clapperboard! Use the true 3.2.1.1**

3.3. Using forceps, disintegrate the lung tissue before staining for fluorescence-activated cell sorting [1].

3.3.1. Shot of forceps mechanically dissociating lung tissue.

3.4. To sort alveolar epithelial type II (*two*) cells, install a 100-micrometer nozzle on the fluorescence-activated cell sorting device [1].

3.4.1. Talent preparing the sorting instrument with a 100-micrometer nozzle.

3.5. Filter the cells through a 50-micrometer cell strainer before sorting [1] and rinse the strainer with supplemented DMEM to prevent cell loss [2-TXT].

3.5.1. Talent filtering the cell suspension through a strainer.

3.5.2. Talent rinsing the strainer with medium. **TXT: For adult mice use 300  $\mu$ L/lung; For juveniles, use 200  $\mu$ L/lung; For neonates, use 100  $\mu$ L/lung**

3.6. Resuspend the cells by vortexing immediately before sorting [1].

3.6.1. Talent vortexing the cell suspension.

3.7. Gate for alveolar epithelial type II cells by selecting events with high side scatter, negative lineage fluorochromes, and positive CD326 signal [1]. Exclude doublets or aggregates using forward scatter height versus area and side scatter height versus area [2].

**Videographer's note: Please note, that the screencapture shots 3.7.1 & 3.7.2 has been filmed with the camera. There will be no video upload by the scientists, only screenshots.**

3.7.1. SCREEN: Display the flow cytometry gating strategy highlighting side scatter, lineage exclusion, and CD326 positivity.

3.7.2. SCREEN: Show doublet discrimination plots during gating.

3.8. Collect the sorted cells into tubes containing supplemented DMEM [1] and centrifuge for further analysis or culture [2].

3.8.1. Talent collecting sorted cells into collection tubes.

3.8.2. Talent placing tubes into the centrifuge.

# Results

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## 4. Results

- 4.1. Flow sorting generated pure alveolar epithelial type II cell populations [1], with post-sort analysis validating almost 100 percent specificity for established cellular markers [2].
  - 4.1.1. LAB MEDIA: Figure 2A.
  - 4.1.2. LAB MEDIA: Figure 2 B *Video editor: Highlight the post-sort plots*
- 4.2. Quantification of post-sort samples showed yields of approximately 0.15 million viable alveolar epithelial type II cells from neonatal lungs [1] and up to 1 million cells from adult lungs per whole lung preparation [2].
  - 4.2.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the neonatal bar*
  - 4.2.2. LAB MEDIA: Figure 3A. *Video editor: Highlight the adult bar*
- 4.3. Isolated alveolar epithelial type II cells maintained typical cellular morphology after isolation [1]. Cellular integrity of isolated alveolar epithelial type II cells was preserved throughout the isolation process [2].
  - 4.3.1. LAB MEDIA: Figure 3B.
  - 4.3.2. LAB MEDIA: Figure 3C.
- 4.4. Isolated alveolar epithelial type II cells supported *ex vivo* infection with influenza A virus expressing the mCherry reporter protein, demonstrating viral replication over time [1].
  - 4.4.1. LAB MEDIA: Figure 4. *Video editor: Highlight the IAV m-Cherry images*
- 4.5. High-quality RNA was successfully extracted from paraformaldehyde-fixed alveolar epithelial type II cells across different processing time points [1].
  - 4.5.1. LAB MEDIA: Table 1. *Video editor: Highlight the rows corresponding to PFA-fixed samples*
- 4.6. The quality of extracted RNA showed a direct relationship with the duration of thermal treatment at 80 degrees Celsius during extraction [1].
  - 4.6.1. LAB MEDIA: Figure 5. *Video editor: sequentially show the plots corresponding to 5 min (80 °C), 10 min (80°C) and 15 min (80°C)*
- 4.7. Quantitative reverse transcription PCR analysis confirmed successful amplification of ribosomal protein S9 and surfactant protein C transcripts from paraformaldehyde-fixed and fresh alveolar epithelial type II cell samples [1].

4.7.1. LAB MEDIA: Figure 6. *Video editor: Sequentially highlight curves corresponding to Rps9 and Sftpc*

Pronunciation Guide:

1. Alveolar  
Pronunciation link: <https://dictionary.cambridge.org/us/pronunciation/english/alveolar>  
IPA: /æl'vi:.ə.lə/  
Phonetic Spelling: al·VEE·uh·lur
2. Epithelial  
Pronunciation link: <https://dictionary.cambridge.org/us/pronunciation/english/epithelial>  
IPA: /,ep.i'θi:.li.əl/  
Phonetic Spelling: eh·puh·THEE·lee·uhl
3. Neonatal  
Pronunciation link: <https://dictionary.cambridge.org/us/pronunciation/english/neonatal>  
IPA: /,ni:.oʊ'nei.tə l/  
Phonetic Spelling: nee·oh·NAY·tuhl
4. Murine  
Pronunciation link: <https://dictionary.cambridge.org/us/pronunciation/english/murine>  
IPA: /'mjʊr.aɪn/  
Phonetic Spelling: MYUR·ine
5. Diaphragm  
Pronunciation link: <https://dictionary.cambridge.org/us/pronunciation/english/diaphragm>  
IPA: /'daɪ.ə.fræm/  
Phonetic Spelling: DYE·uh·fram
6. Trachea  
Pronunciation link: <https://dictionary.cambridge.org/us/pronunciation/english/trachea>  
IPA: /'treɪ.ki.ə/  
Phonetic Spelling: TRAY·kee·uh
7. Ventricle  
Pronunciation link: <https://dictionary.cambridge.org/us/pronunciation/english/ventricle>  
IPA: /'ven.trɪ.kəl/  
Phonetic Spelling: VEN·tri·kuhl
8. Cannula  
Pronunciation link: <https://dictionary.cambridge.org/us/pronunciation/english/cannula>  
IPA: /'kæn.jə.lə/  
Phonetic Spelling: KAN·yuh·luh
9. Dispace  
Pronunciation link: <https://www.howtopronounce.com/dispace>  
IPA: /dɪ.sp'eɪs/  
Phonetic Spelling: di·SPAYSS
10. HEPES  
Pronunciation link: <https://www.howtopronounce.com/hepes>

IPA: /h'i:ps/

Phonetic Spelling: heaps

11. DNase

Pronunciation link: <https://www.howtopronounce.com/dnase>

IPA: /d'i:. ne.ɪs/

Phonetic Spelling: DEE·nayss

12. Fluorescence

Pronunciation link:

<https://dictionary.cambridge.org/us/pronunciation/english/fluorescence>

IPA: /flʊ:'res.ə ns/

Phonetic Spelling: floor·ESS·uhns

13. Cytometry

Pronunciation link: <https://www.howtopronounce.com/cytometry>

IPA: /saɪ.. 'tɔ:.məti/

Phonetic Spelling: sye·TAH·muh·tree

14. Centrifuge

Pronunciation link:

<https://dictionary.cambridge.org/us/pronunciation/english/centrifuge>

IPA: /'sen.trə.fju:dʒ/

Phonetic Spelling: SEN·truh·fyooj

15. Surfactant

Pronunciation link:

<https://dictionary.cambridge.org/us/pronunciation/english/surfactant>

IPA: /sɜ:'fæk.tənt/

Phonetic Spelling: sur·FAK·tuhnt

16. Influenza

Pronunciation link:

<https://dictionary.cambridge.org/us/pronunciation/english/influenza>

IPA: /,ɪn.flu'en.zə/

Phonetic Spelling: in·floo·EN·zuh

17. Exsanguinated

Pronunciation link: <https://www.howtopronounce.com/exsanguinated>

IPA: /ɛkss'æ.ŋgwɪne.ɪrɪd/

Phonetic Spelling: ekss·ANG·gwih·nay·tuhd

18. Paraformaldehyde

Pronunciation link: <https://www.howtopronounce.com/paraformaldehyde>

IPA: /pæ.r.ə.fo:.ɪm'.æl.dɪhaɪd/

Phonetic Spelling: pa·ruh·for·mal·dee·hyde

19. mCherry (Mcherry)

Pronunciation link: <https://www.howtopronounce.com/mcherry>

IPA: /mə.kh'ɛɪ/

Phonetic Spelling: muh·KHEH·ree