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**Title: Cuff-Free Suture-Based Cervical Model for Secondary Heterotopic Heart Transplantation to Evaluate Donor-Specific Immune Tolerance in Mice**

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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**

If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit.

If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye.**

**Carl Zeiss Stemi 2000 Eyepiece 455043-0000**

*Videographer: Please capture all SCOPE shots with a SCOPE kit*

**SCOPE: 2.6.1, 2.7.1, 2.7.3, 2.8.1, 2.8.2, 2.9.2, 3.5.1, 3.5.2, 3.6.1, 3.7.1, 3.8.1, 3.9.3, 3.10.2, 3.12.1, 3.12.2, 3.13.1, 3.13.2**

- 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. **Yes**

### Current Protocol Length

Number of Steps: 26

Number of Shots: 57

# Introduction

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

**Authors:** Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**.

## INTRODUCTION:

~~**REQUIRED:** Summarize your research focus and the main questions it aims to answer.~~

1.1. **Minxue Liao:** Our research develops murine heart transplant models to study donor-specific immune tolerance using a cuff-free cervical secondary graft.

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

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

1.2. **Minxue Liao:** We address the need for a reproducible cuff-free cervical technique for reliable secondary heart grafting in tolerance experiments.

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

## CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

1.3. **Minxue Liao:** Our cuff-free sleeve anastomosis avoids the need for intraluminal stents, works in small vessels, and supports reliable secondary tolerance testing.

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

~~Describe the most important consideration or challenge when performing this protocol.~~

1.4. **Minxue Liao:** Key challenges include small-caliber vessel handling, minimizing thrombosis, and achieving consistent technical success across operators.

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

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.5. **Minxue Liao**: This method standardizes secondary challenge experiments, improving rigor and reproducibility for mechanistic studies of donor-specific transplant tolerance.

- 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 Massachusetts General Hospital

# Protocol

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## 2. Harvesting a Donor Heart from a Mouse for Transplantation

**Demonstrator:** Kaifeng Liu

2.1. To begin, place an anesthetized donor mouse in the supine position on the surgical surface [1]. Secure the limbs with tape to prevent movement [2].

2.1.1. WIDE: Talent positioning the donor mouse in the supine position on the surgical surface.

2.1.2. Talent securing the mouse limbs with tape.

2.2. Remove the hair from the surgical area [1] and disinfect the skin 3 times using 70 percent isopropyl alcohol swabs [2].

2.2.1. Talent removing hair from the abdominal and thoracic region by using shaver and body cream.

2.2.2. Talent disinfecting the skin repeatedly with isopropyl alcohol swabs.

2.3. Use fine scissors to perform a midline laparotomy followed by a midline sternotomy and expose the abdominal and thoracic cavity [1]. With moistened gauze, gently mobilize the intestines to the left side [2]. Then place a saline-soaked gauze beneath the intestines to maintain hydration [3].

**AUTHOR'S NOTE: 2.3.1-2.3.2 have been combined**

2.3.1. Talent using fine scissors to perform a midline laparotomy and sternotomy.

2.3.2. Talent gently moving the intestines to the left with moistened gauze.

2.3.3. Talent placing saline-soaked gauze beneath the intestines.

2.4. To begin perfusion preparation, load 0.2 to 0.3 milliliters of cold heparinized saline solution into a 0.5 milliliter syringe [1-TXT]. gently cannulate the inferior vena cava using a 26-to-30-gauge needle or catheter [2]. Then slowly perfuse 0.2 to 0.3 milliliters of cold heparinized saline over 30 seconds [3-TXT].

2.4.1. Talent loading cold heparinized saline solution into a 0.5 mL syringe. **TXT: Heparinized saline: 125 IU/mL**

**AUTHOR'S NOTE: Shot deleted**

2.4.2. Talent inserting a needle or catheter into the inferior vena cava.

- 2.4.3. Talent slowly perfusing cold heparinized saline. **TXT: Wait 1 - 2 min for optimum heparinization**
- 2.5. Now perform a bilateral thoracotomy to expose the heart with maximum visualization [1]. Separate the anterior thoracic cage from the dorsal side [2] and reflect it upward [3], securing it with a needle or clamp [4].
- AUTHOR'S NOTE: 2.5.1-2.5.4 have been combined**
- 2.5.1. Talent performing a bilateral thoracotomy.
- 2.5.2. Talent separating the anterior thoracic cage.
- 2.5.3. Talent reflecting the thoracic cage upward.
- 2.5.4. Talent removing the thoracic cage.
- 2.6. Using blunt dissection, dissect the connective tissue between the ascending aorta and the pulmonary trunk [1].
- 2.6.1. SCOPE: Talent bluntly dissecting tissue between the ascending aorta and pulmonary trunk.  
*Videographer: Please capture all SCOPE shots with a SCOPE kit*
- 2.7. Ligate the inferior and superior venae cavae close to the heart using 8-0 (*Eight-oh*) nylon sutures [1]. Transect the ligated vessels distal to the ligatures [2]. Dissect the pulmonary trunk as distally as possible [3].
- 2.7.1. SCOPE: Talent placing sutures around the venae cavae.
- 2.7.2. SCOPE: Talent transecting the ligated vessels.
- 2.7.3. SCOPE: Talent bluntly dissecting the pulmonary trunk.
- 2.8. Next, ligate the left and right pulmonary veins together using a 6-0 nylon suture [1]. Transect the ligated vessels distal to the ligature [2].
- 2.8.1. SCOPE: Talent ligating the pulmonary veins together.
- 2.8.2. SCOPE: Talent transecting the pulmonary veins.
- 2.9. Excise the heart while preserving adequate length of the ascending aorta by transecting it at the origin of the brachiocephalic artery [1]. Preserve the anterior wall of the pulmonary trunk as long as possible [2]. Then trim the ascending aorta and pulmonary trunk to ensure a secure anastomosis [3].
- AUTHOR'S NOTE: 2.9.1-2.5.3 have been combined**
- 2.9.1. SCOPE: Talent excising the donor heart.
- 2.9.2. SCOPE: Shot of preserved pulmonary trunk wall.
- 2.9.3. Talent trimming the vessels.
- 2.10. Place the heart into a 60-millimeter cell culture dish filled with chilled normal saline [1].

Gently re-perfuse the heart with 1 milliliter of cold heparinized saline through the ascending aorta or pulmonary trunk [2]. Trim the vessels on the chilled plate as needed [3].

2.10.1. Talent placing the heart into a chilled dish.

2.10.2. Talent gently flushing the heart with saline.

2.10.3. Talent trimming vessels on the chilled plate.

2.11. Place the heart in cold Ringer's lactate solution on ice until implantation [1].

2.11.1. Talent transferring the heart into cold Ringer's lactate on ice.

### **3. Cervical Heterotopic Heart Transplantation in a Mouse Recipient**

**Demonstrator:** Kaifeng Liu

3.1. To begin recipient preparation, inject 0.5 milliliters of warm normal saline subcutaneously into the dorsal region of an anesthetized mouse [1-TXT].

3.1.1. WIDE: Talent injecting warm saline subcutaneously. **TXT: Place the recipient on a heat pad to maintain body temperature**

3.2. ~~Place the recipient on a heating pad to maintain body temperature [1].~~ Shave the entire cervical region [2].

~~3.2.1. Talent placing the mouse on a heating pad.~~

**NOTE: Shot moved as on-screen text at author's suggestion**

3.2.2. Talent shaving the cervical region.

3.3. Position the mouse supine with the tail facing the operator and secure the head, limbs, and tail with tape [1-TXT]. ~~Apply ophthalmic lubricant to each eye [2].~~ Then disinfect the cervical skin 3 times with isopropyl alcohol swabs [3].

3.3.1. Talent positioning and taping the mouse. **TXT: Apply ophthalmic lubricant to each eye**

~~3.3.2. Talent applying eye lubricant.~~

**NOTE: Shot moved as on-screen text at author's suggestion**

3.3.3. Talent disinfecting the cervical skin.

3.4. Now make a longitudinal skin incision in a reversed T shape over the neck from the sternum to the right mandibular angle [1].

3.4.1. Talent making a reversed T-shaped cervical incision.

3.5. Using blunt dissection, mobilize the right external jugular vein from the right medial clavicle to its major confluence [1]. Cauterize and transect the small branches of the

vein [2].

**AUTHOR'S NOTE: 3.5.1-3.5.2 have been combined**

3.5.1. SCOPE: Talent bluntly isolating the external jugular vein.

3.5.2. SCOPE: Talent cauterizing and transecting vein branches.

3.6. Next isolate the right lobe of the thyroid gland [1] and transect it by cauterization [2]. Transect the sternomastoid muscle to expose the right carotid artery [3].

**AUTHOR'S NOTE: 3.6.1-3.6.2 have been combined**

3.6.1. SCOPE: Talent isolating the thyroid gland.

3.6.2. SCOPE: Talent cauterizing the thyroid lobe.

3.6.3. Talent cauterizing the sternomastoid muscle.

3.7. Using blunt dissection, mobilize the right carotid artery as far as possible above the carotid bifurcation [1].

3.7.1. SCOPE: Talent bluntly dissecting the carotid artery.

3.8. Apply a small vascular clamp to the proximal portion of the carotid artery [1]. Then place a 10-0 nylon ligature around the distal portion at the carotid bifurcation [2].

3.8.1. SCOPE: Talent applying a vascular clamp to the carotid artery.

3.8.2. Talent placing a distal ligature.

~~3.9. Apply another 10-0 nylon ligature to the proximal external jugular vein [1]. Allow the vein to distend and control bleeding from branch sites [2]. Now apply a small vascular clamp at the confluence of the retromandibular and posterior auricular veins [3].~~

~~3.9.1. Talent ligating the external jugular vein.~~

~~3.9.2. Close up of vein distension and hemostasis.~~

~~3.9.3. SCOPE: Talent applying a vascular clamp at the venous confluence.~~

**AUTHOR'S NOTE: 3.9 was not filmed**

3.10. Transect the external jugular vein and carotid artery between the ligature and clamp using fine scissors [1]. Then flush the vessel openings with normal saline and keep the field moist [2].

**AUTHOR'S NOTE: 3.10.1-3.10.2 have been combined**

3.10.1. Talent transecting the vessels with fine scissors.

3.10.2. SCOPE: Talent flushing vessel openings with saline.

3.11. Place the donor heart graft upside down in the cervical region with the pulmonary trunk oriented laterally and the ascending aorta oriented medially [1]. Cover the graft with moistened gauze while leaving the pulmonary trunk and ascending aorta exposed [2].

**AUTHOR'S NOTE: 3.11.1-3.11.2 have been combined**

3.11.1. Talent positioning the donor heart in the cervical pocket.

3.11.2. Talent covering the graft with moist gauze.

3.12. Anastomose the donor ascending aorta to the recipient carotid artery using 11–0 nylon sutures with a sleeve technique [1-TXT]. Now, anastomose the donor pulmonary trunk to the recipient external jugular vein [2].

3.12.1. SCOPE: Talent performing aortic anastomosis. **TXT: Avoid excessive tension**

3.12.2. SCOPE: Talent performing pulmonary trunk anastomosis.

**AUTHOR'S NOTE: 3.13-3.15 was not filmed**

~~3.13. Remove the distal external jugular vein clamp [1] and then remove the proximal carotid artery clamp to restore blood flow [2]. Apply warm normal saline to the graft to help restore contractility [3].~~

~~3.13.1. SCOPE: Talent removing the venous clamp.~~

~~3.13.2. SCOPE: Talent removing the arterial clamp.~~

~~3.13.3. Talent applying warm saline to the graft.~~

~~3.14. Gently compress the anastomotic sites with cotton tips to achieve hemostasis [1]. Then position the graft securely within the cervical pocket without twisting or compressing surrounding structures [2].~~

~~3.14.1. Talent applying gentle pressure with cotton tips.~~

~~3.14.2. Talent adjusting graft position in the cervical pocket.~~

~~3.15. Close the cervical incision using 6–0 absorbable sutures with a simple interrupted stitch [1]. Leave the surgical site open to air [2].~~

~~3.15.1. Talent suturing the cervical incision.~~

~~3.15.2. Final shot of the uncovered surgical site.~~

## Results

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### 4. Results

- 4.1. After release of vascular clamps, the graft artery filled with blood, produced a palpable pulse [1], and the graft became visibly red within 10 seconds [1].
  - 4.1.1. LAB MEDIA: Figure 4D. *Video editor: Highlight the red filling of the graft artery in the third image of the panel.*
  - 4.1.2. LAB MEDIA: Figure 4E
- 4.2. Kaplan-Meier survival curves demonstrated prolonged graft survival in tolerant mice compared with rapid rejection of third-party grafts [1].
  - 4.2.1. LAB MEDIA: Figure 5B. *Video editor: Highlight the green survival curve and the orange curve*
- 4.3. Gross examination at endpoint showed visible differences between healthy and necrotic grafts [1].
  - 4.3.1. LAB MEDIA: Figure 5C. *Video editor: Highlight the two grafts labeled "3rd Party" and "Donor,"*
- 4.4. Histopathology showed preserved myocardial architecture in syngeneic and tolerant grafts [1], but interstitial hemorrhage, necrosis, and infiltrates in rejected third-party grafts [2].
  - 4.4.1. LAB MEDIA: Figure 5D. *Video editor: Highlight the tissue section labeled "Syngeneic B6" and "Donor (BALB/c)"*
  - 4.4.2. LAB MEDIA: Figure 5D. *Video editor: Highlight the tissue section labeled "3rd party (C3H)"*

Pronunciation Guide:

❑ Heterotopic

Pronunciation link: <https://www.merriam-webster.com/dictionary/heterotopic>

IPA: /ˌhet-ə-rə-ˈtă-pik/

Phonetic Spelling: het·uh·ruh·taa·pik

❑ Cervical

Pronunciation link: <https://www.merriam-webster.com/dictionary/cervical>

IPA: /ˈsər-vi-kəl/

Phonetic Spelling: sur·vuh·kuhl

❑ Anastomosis

Pronunciation link: <https://www.merriam-webster.com/dictionary/anastomosis>

IPA: /ə-ˌnas-tə-ˈmō-səs/

Phonetic Spelling: uh·nas·tuh·moh·suhs

❑ Thoracotomy

Pronunciation link: <https://www.merriam-webster.com/dictionary/thoracotomy>

IPA: /ˌthôr-ə-ˈkā-tə-mē/

Phonetic Spelling: thor·uh·kaa·tuh·mee

❑ Sternotomy

Pronunciation link: <https://www.merriam-webster.com/dictionary/sternotomy>

IPA: /stər-ˈnä-tə-mē/

Phonetic Spelling: stur·naa·tuh·mee

❑ Laparotomy

Pronunciation link: <https://www.merriam-webster.com/dictionary/laparotomy>

IPA: /ˌlə-pə-ˈră-tə-mē/

Phonetic Spelling: la·puh·raa·tuh·mee

❑ Heparinized

Pronunciation link: <https://www.merriam-webster.com/dictionary/heparinized>

IPA: /ˈhep-ə-rə-ˌnīzd/

Phonetic Spelling: hep·uh·ruh·nyzd

❑ Cannulate

Pronunciation link: <https://www.merriam-webster.com/dictionary/cannulate>

IPA: /ˈkan-yə-ˌlāt/

Phonetic Spelling: kan·yuh·layt

❑ Brachiocephalic

Pronunciation link: <https://www.merriam-webster.com/dictionary/brachiocephalic>

IPA: /ˌbrā-kē-ō-sə-ˈfa-lik/

Phonetic Spelling: bray·kee·oh·suh·fal·ik

❑ Heterotopic

Pronunciation link: <https://www.merriam-webster.com/dictionary/heterotopic>

IPA: /ˌhet-ə-rə-ˈtă-pik/

Phonetic Spelling: het·uh·ruh·taa·pik

🔍 Syngeneic

Pronunciation link: <https://www.merriam-webster.com/dictionary/syngeneic>

IPA: /ˌsɪn-jə-ˈnē-ɪk/

Phonetic Spelling: sin·juh·nee·ik

🔍 Histopathology

Pronunciation link: <https://www.merriam-webster.com/dictionary/histopathology>

IPA: /ˌhɪs-tō-pə-ˈthä-lə-jē/

Phonetic Spelling: his·toh·puh·thal·uh·jee

🔍 Myocardial

Pronunciation link: <https://www.merriam-webster.com/dictionary/myocardial>

IPA: /ˌmɪ-ə-ˈkär-dē-əl/

Phonetic Spelling: my·uh·kar·dee·uhl

🔍 Interstitial

Pronunciation link: <https://www.merriam-webster.com/dictionary/interstitial>

IPA: /ˌɪn-tər-ˈsti-shəl/

Phonetic Spelling: in·tur·stish·uhl

🔍 Necrosis

Pronunciation link: <https://www.merriam-webster.com/dictionary/necrosis>

IPA: /nə-ˈkrō-səs/

Phonetic Spelling: nuh·kroh·suhs

🔍 Retromandibular

Pronunciation link: No confirmed link found

IPA: /ˌretroʊ mənˈdɪbjələr/

Phonetic Spelling: reh·troh·man·dib·yuh·lur