

Project Page Link: <https://review.jove.com/account/file-uploader?src=21564768>

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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.

☒ Correct

☐ Incorrect

2. Interview statements: Which interview statement filming option is the most appropriate for your group? **Please select one.**

☒ Interviewees self-record interview statements. JoVE can provide support for this option.

☐ Interview Statements are read by JoVE's voiceover talent.

3. Proposed interview filming date: Please indicate the proposed date that your group will self-film interviews: **04/30/2026**

Current Protocol Length

Number of Steps: 16

Number of Shots: 53

Introduction

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. **Cheng Qi:** We study transplant immunology using a refined mouse cervical heart transplant model with end-to-side anastomosis.

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. **Zipei Wang:** Compared to traditional cuff method, our end-to-side anastomosis avoids foreign material, preserves brain perfusion, and improves long-term graft survival.

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. **Cheng Qi:** This protocol is particularly suitable for genetically or drug-induced fragile mice and secondary or combined transplantation.

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. **Zipei Wang:** Manual suture of tiny cervical vessels is technically challenging; this method suits surgeons with prior abdominal heart transplant experience.

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

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

1.5. **Cheng Qi**: Future studies can explore tolerance mechanisms, secondary transplantation, combined organ transplantation models, and novel immunosuppressive strategies.

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

Ethics Title Card

This research has been approved by the Animal Ethics Committee of Tongji Hospital at Huazhong University of Science and Technology

Protocol

NOTE: LAB MEDIA timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator (**Kathyayani Sridharan**) for queries regarding lab media.

Note: The narrations and steps are updated as per the LAB MEDIA footage and timestamps provided by the authors. Few additional timestamps from the footage were added at requisite steps for visual and narrative clarity.

2. Donor Mouse Heart Harvest

Demonstrator: Huang Xia

- 2.1. To begin, expose the peritoneal cavity of the anesthetized donor mouse covered with a sterile surgical gauze [1-TXT].
 - 2.1.1. LAB MEDIA: 70704-screenshot-1.mp4. 00:03 – 00: 07
- 2.2. Use a sterile cotton swab moistened with saline to gently retract the small intestine to the left [1] and expose the abdominal aorta and the inferior vena cava [2]. Inject 0.3 milliliters of heparinized saline into the inferior vena cava [3-TXT] and wait for one minute to allow systemic heparinization [4]. Use scissors to transect the abdominal aorta and inferior vena cava to exsanguinate the donor [5].
 - 2.2.1. LAB MEDIA: 70704-screenshot-1.mp4. 00:12 – 00: 18
 - 2.2.2. LAB MEDIA: 70704-screenshot-1.mp4. 00:27 – 00: 34
 - 2.2.3. LAB MEDIA: 70704-screenshot-1.mp4. 00:55 – 01: 02 **TXT: Heparinized saline: 300 IU/mL**
 - 2.2.4. LAB MEDIA: 70704-screenshot-1.mp4. 01: 42 – 01: 48
 - 2.2.5. LAB MEDIA: 70704-screenshot-1.mp4. 01:49 – 02: 06
- 2.3. Use scissors to incise the diaphragm along with the anterior chest wall to create a U-shaped incision through the rib cage and expose the thoracic cavity [1]. Inject two milliliters of cold saline at four degrees Celsius into the heart through the intrathoracic inferior vena cava to flush residual blood [2]. Cover the heart with crushed ice and gauze soaked in ice-cold saline and keep the gauze moist with additional ice-cold saline to maintain hypothermia [3].
 - 2.3.1. LAB MEDIA: 70704-screenshot-1. 02:28 – 03: 12. *Video Editor: If needed, speed up the video for this.*
 - 2.3.2. LAB MEDIA: 70704-screenshot-1. 03:59 – 04: 22
 - 2.3.3. LAB MEDIA: 70704-screenshot-1. 04:25 – 04: 51
- 2.4. Identify and isolate the intrathoracic inferior vena cava [1]. Ligate it using a 6-0 (six-oh) silk suture [2]. Using micro scissors, transect the vessel distal to the ligation [3].

- 2.4.1. LAB MEDIA: 70704-screenshot-1. 04:57 – 05: 13
- 2.4.2. LAB MEDIA: 70704-screenshot-1. 05:29 – 05: 46
- 2.4.3. LAB MEDIA: 70704-screenshot-1. 05:47 – 05: 52
- 2.5. Identify and remove the thymus to expose the aortic arch and pulmonary artery [1]. Identify and isolate the superior vena cava [2]. Ligate it using a 6-0 silk suture and transect distal to the ligation [3]. Next, identify and isolate the ascending aorta [4]. Transect the aorta proximal to the innominate artery to maximize vessel length [5].
 - 2.5.1. LAB MEDIA: 70704-screenshot-1. 06:26 – 06: 45
 - 2.5.2. LAB MEDIA: 70704-screenshot-1. 07:15 – 07: 22
 - 2.5.3. LAB MEDIA: 70704-screenshot-1. 07:27 – 07: 53
 - 2.5.4. LAB MEDIA: 70704-screenshot-1. 08:17 – 08: 31
 - 2.5.5. LAB MEDIA: 70704-screenshot-1. 08:45 – 08: 55
- 2.6. Identify and isolate the pulmonary artery [1]. Transect it just proximal to its bifurcation [2]. Ligate all vessels posterior to the heart including the pulmonary veins with a 6-0 silk suture [3].
 - 2.6.1. LAB MEDIA: 70704-screenshot-1. 09:14 – 09: 26
 - 2.6.2. LAB MEDIA: 70704-screenshot-1. 09:39 – 09: 50
 - 2.6.3. LAB MEDIA: 70704-screenshot-1. 10:05 – 10: 30
- 2.7. Use micro scissors to carefully excise the heart [1]. Use microscissors to carefully remove the attached lung tissue and any excess surrounding tissue [2]. Gently transfer the heart into cold saline at four degrees Celsius placed on ice for storage until transplantation [3].
 - 2.7.1. LAB MEDIA: 70704-screenshot-1. 10:32 – 10: 43
 - 2.7.2. LAB MEDIA: 70704-screenshot-1. 10:47 – 11: 03
 - 2.7.3. LAB MEDIA: 70704-screenshot-1. 11:28 – 11: 42

3. Recipient End-to-side Anastomosis and Reperfusion

- 3.1. Through the longitudinal neck incision on the anesthetized recipient mouse, carefully excise the right submandibular gland using forceps and a microcautery pen [1]. Next, mobilize the external jugular vein by cauterizing its branches [2]. Transect the right sternocleidomastoid muscle to create a sufficient pocket for donor heart implantation [3]. Isolate the common carotid artery while avoiding damage to the vagus nerve [4].
 - 3.1.1. LAB MEDIA: 70704-screenshot-2(a). 00:43 – 00:48; 01:08 – 01:17; 01:39 – 01: 56

- 3.1.2. LAB MEDIA: 70704-screenshot-2(a). 03:21 – 03:25; 03:35– 03: 39; 04:29– 04: 36; 06:30– 06: 40
- 3.1.3. LAB MEDIA: 70704-screenshot-2(a). 06:51 – 07:00; 07:16 – 07:21; 07:58 – 08: 02
- 3.1.4. LAB MEDIA: 70704-screenshot-2(b). 2:28 – 2:31; 2:46 – 2:48

- 3.2. Use two microvascular clamps to first clamp the cranial ends of the vessels [1], then the caudal ends to simultaneously occlude the external jugular vein and common carotid artery for anastomosis and reduced tracheal compression [2].
 - 3.2.1. LAB MEDIA: 70704-screenshot-2(b). 3:52– 4: 12
 - 3.2.2. LAB MEDIA: 70704-screenshot-2(b). 4:32– 4: 37

- 3.3. After rotating the mouse ninety degrees clockwise [1], use an 11-0 (*eleven-oh*) suture to pass the needle through the vessel wall and lift it at a distance roughly equal to the donor vessel's diameter [2]. Use micro scissors to excise a small portion of the vessel wall to create an anastomotic window [3]. Flush the lumen with heparinized saline to remove residual blood clots [4].
 - 3.3.1. LAB MEDIA: 70704-screenshot-2(b). 5:16– 5:19
 - 3.3.2. LAB MEDIA: 70704-screenshot-2(b). 5:57– 6: 20
 - 3.3.3. LAB MEDIA: 70704-screenshot-2(b). 6:22– 6: 29
 - 3.3.4. LAB MEDIA: 70704-screenshot-2(b). 6:35– 6: 48

- 3.4. Rotate the mouse again [1] to place the donor heart in the cervical pocket [2]. Anchor the donor ascending aorta to the recipient common carotid artery using two 11-0 sutures at proximal and distal ends [3]. Position the heart on the right side and cover it with wet sterile gauze [4]. Using the distal suture, perform a continuous running suture on the posterior walls in a distal-to-proximal direction with six to eight stitches [5].
 - 3.4.1. LAB MEDIA: 70704-screenshot-2(b). 6:51– 6:53
 - 3.4.2. LAB MEDIA: 70704-screenshot-2(b). 7:15– 7: 17
 - 3.4.3. LAB MEDIA: 70704-screenshot-3. 00:54– 01: 02; 02:10– 02: 20
 - 3.4.4. LAB MEDIA: 70704-screenshot-3. 02:21– 02: 29
 - 3.4.5. LAB MEDIA: 70704-screenshot-3. 03:20 – 03:28; 04:45 – 04:57; 05:27 – 05:42; 05:45– 05: 48

- 3.5. Rotate the mouse one hundred and eighty degrees [1]. Reflect the donor heart to the left and cover it with wet sterile gauze [2]. Flush the lumen with saline [3]. Perform the anterior wall anastomosis in a proximal-to-distal direction with six to eight stitches [4].

- 3.5.1. LAB MEDIA: 70704-screenshot-3. 05:56– 05: 58
- 3.5.2. LAB MEDIA: 70704-screenshot-3. 06:36– 06: 43
- 3.5.3. LAB MEDIA: 70704-screenshot-4(a). 01:07– 01: 10
- 3.5.4. LAB MEDIA: 70704-screenshot-4(a). 02:27– 02: 35

- 3.6. Make a longitudinal incision on the external jugular vein matching the diameter of the donor pulmonary artery [1]. Flush the lumen with heparinized saline to thoroughly remove residual blood [2]. Anchor the donor pulmonary artery to the recipient external jugular vein using two 11-0 sutures at proximal and distal ends [3]. Using the proximal suture, continuously suture the posterior walls in a proximal-to-distal direction with five to seven stitches [4].
 - 3.6.1. LAB MEDIA: 70704-screenshot-4(a). 03:48– 03: 53
 - 3.6.2. LAB MEDIA: 70704-screenshot-4(a). 04:08– 04: 12
 - 3.6.3. LAB MEDIA: 70704-screenshot-4(a). 05:56– 06: 07; 08:16– 08: 30
 - 3.6.4. LAB MEDIA: 70704-screenshot-4(a). 09:50– 10: 05

- 3.7. Continue suturing the anterior walls from outside in a distal-to-proximal direction and after flushing the lumen with saline, tie at the proximal apex [1]. After covering the sutures with sterile cotton, rotate the mouse [2] and release the distal clamp first followed by the proximal clamp to initiate reperfusion [3]. Observe the cardiac graft turning red and regaining normal rhythm and contraction within one to two minutes [4].
 - 3.7.1. LAB MEDIA: 70704-screenshot-4(b). 0:14– 0: 28; 1:01–1:12
 - 3.7.2. LAB MEDIA: 70704-screenshot-4(b). 2:37– 2:39; 2:45– 2:49
 - 3.7.3. LAB MEDIA: 70704-screenshot-4(b). 2:57– 3: 05
 - 3.7.4. LAB MEDIA: 70704-screenshot-4(b). 7:50– 7: 59

- 3.8. Confirm haemostasis and position the cardiac graft within the subcutaneous pocket [1]. Close the skin incision using 5-0 (*five-oh*) sutures [2].
 - 3.8.1. LAB MEDIA: 70704-screenshot-4(b). 8:20– 8:25; 8:44– 8: 46
 - 3.8.2. LAB MEDIA: 70704-screenshot-5. 02:02– 02: 11

Results

4. Results

- 4.1. Survival analysis of ten syngeneic and ten allogeneic recipients showed that recipients of syngeneic grafts survived for over hundred days with normal contraction rhythm and strength [1]. In contrast, recipients with allogeneic grafts showed typical rejection within six to eight days post-transplantation [2].
 - 4.1.1. LAB MEDIA: Figure 2: *Video Editor: Please emphasize the blue line in the graph and X-axis simultaneously.*
 - 4.1.2. LAB MEDIA: Figure 2: *Video Editor: Please emphasize the orange line in the graph and X-axis simultaneously.*
- 4.2. Histological evaluation revealed that syngeneic grafts maintained intact myocardial architecture without inflammation or graft vasculopathy at post-operative days seven [1] and hundred [2]. Conversely, allogeneic grafts at post-operative day seven exhibited severe acute cellular rejection, marked by massive lymphocyte infiltration, cardiomyocyte edema, and necrosis [3].
 - 4.2.1. LAB MEDIA: Figure 3A
 - 4.2.2. LAB MEDIA: Figure 3B
 - 4.2.3. LAB MEDIA: Figure 3C

Pronunciation Guide:

1. Peritoneal
Pronunciation link: <https://www.merriam-webster.com/dictionary/peritoneal>
IPA: /ˌper.ə.təˈniː.əl/
Phonetic Spelling: peh·ruh·tuh·nee·uhl
2. Anesthetized
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetized>
IPA: /əˈnes.θəˈtaɪzd/
Phonetic Spelling: uh·ness·thuh·tyzd
3. Inferior
Pronunciation link: <https://www.merriam-webster.com/dictionary/inferior>
IPA: /ɪnˈfɪr.i.ər/
Phonetic Spelling: in·feer·ee·uhr
4. Vena cava
Pronunciation link: <https://www.merriam-webster.com/dictionary/vena%20cava>

IPA: /ˌviː.nə 'keɪ.və/

Phonetic Spelling: vee·nuh kay·vuh

5. Heparinized

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

IPA: /hɪ'per.ə.naɪzd/

Phonetic Spelling: hih·pehr·uh·nyzd

6. Systemic

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

IPA: /sɪ'ste.mɪk/

Phonetic Spelling: sih·steh·mik

7. Exsanguinate

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

IPA: /ek'sæŋ.gwəˌneɪt/

Phonetic Spelling: ek·sang·gwuh·nayt

8. Diaphragm

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

IPA: /'daɪ.əˌfræm/

Phonetic Spelling: dye·uh·fram

9. Thoracic

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

IPA: /θə'ræs.ɪk/

Phonetic Spelling: thuh·rass·ik

10. Intrathoracic

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

IPA: /ˌɪn.trə.θə'ræs.ɪk/

Phonetic Spelling: in·truh·thuh·rass·ik

11. Hypothermia

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

IPA: /ˌhaɪ.pə'θɜː.mi.ə/

Phonetic Spelling: hy·puh·thur·mee·uh

12. Ligate

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

IPA: /'laɪˌgeɪt/

Phonetic Spelling: lye·gayt

13. Transect

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

IPA: /træn'sekt/

Phonetic Spelling: tran·sekt

14. Thymus

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

IPA: /'θaɪ.məs/

Phonetic Spelling: thy·muhs

15. Pulmonary

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

IPA: /'pʊl.mə.ner.i/

Phonetic Spelling: puul·muh·nair·ee

16. Bifurcation

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

IPA: /ˌbaɪ.fəˈkeɪ.jən/

Phonetic Spelling: by·fur·kay·shuhn

17. Anastomosis

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

IPA: /əˌnæs.təˈmoʊ.sɪs/

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

18. Reperfusion

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

IPA: /ˌriː.pəˈfjuː.ʒən/

Phonetic Spelling: ree·pur·fyoo·zhuhn

19. Submandibular

Pronunciation link: <https://www.merriam-webster.com/medical/submandibular>

IPA: /ˌsʌb.mænˈdɪb.jə.lə/

Phonetic Spelling: sub·man·dib·yuh·luhr

20. Sternocleidomastoid

Pronunciation link: <https://www.merriam-webster.com/medical/sternocleidomastoid>

IPA: /ˌstɜː.noʊˌklaɪ.doʊˈmæs.tɔɪd/

Phonetic Spelling: stur·noh·kly·doh·mass·toyd

21. Carotid

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

IPA: /kəˈrɑː.tɪd/

Phonetic Spelling: kuh·raa·tid

22. Vagus

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

IPA: /ˈveɪ.gəs/

Phonetic Spelling: vay·guhs

