

1. Preoperative preparation

1.1. Anesthetize the rat with isoflurane (3–5% at a flow rate of 1–2 L/min) for induction. Once the animal loses its righting reflex, reduce to 1.5–2.5% for maintenance of a surgical plane of anesthesia. Monitor the rat's responses closely. Proceed only when the pedal reflex is completely absent.

1.2. Using a blunt-tip needle, slowly administer Evans blue (EB) solution (approximately 60–100 $\mu\text{L/kg}$, 5%)⁵ into the nasal mucosa of a unilateral nasal cavity approximately 5 min before the start of the next step. Position the rat in lateral recumbency with the EB-injected side down to facilitate gravity-assisted drainage of the dye toward the deep cervical lymph nodes, while preventing EB from entering the contralateral nostril or respiratory tract.

1.3. Shave the hair on the rat's neck to ensure a clear surgical field. Apply Vaseline to both eyes to prevent dryness or corneal injury during surgery.

2. Surgical incision

2.1. Disinfect the midline of the rat's neck three times, alternating between iodine tincture and 70% isopropyl alcohol, to ensure a sterile surgical field. **File name:** 69201_Video 1.MOV **Timestamps:** 01:25 – 02:25

2.2. Make a midline incision approximately 3 cm in length using a scalpel. **File name:** 69201_Video 1.MOV **Timestamps:** 04:40 – 05:40

2.3. NOTE: Take care not to damage the submaxillary gland (SMG) and blood vessels during dissection. **File name:** 69201_Video 1.MOV **Timestamps:** 04:40 – 05:40

2.4. Retract the skin to expose the sternothyroid muscle (STM), sternocleidomastoid muscle (SCM), and the omohyoid muscle (OHM). **File name:** 69201_Video 1.MOV **Timestamps:** 12:30 – 13:30

3. Preparation of the PFV

3.1. Identify the PFV, a tributary of the external jugular vein (EJV), along the lateral margin of the SCM. **File name:** 69201_Video 1.MOV **Timestamps:** 14:52 – 15:52

3.2. At the bifurcation between the PFV and the EJV, occlude the PFV using a microvascular clip. **File name:** 69201_Video 1.MOV **Timestamps:** 16:52 – 17:52

3.3. Continue to bluntly dissect the PFV to obtain sufficient length, and clamp it at the distal end. Ensure the PFV segment remains as loose as possible to allow for subsequent traction during anastomosis with the dCLN. **File name:** 69201_Video 1.MOV **Timestamps:** 19:00 – 20:00

3.4. Create a longitudinal incision parallel to the vessel axis using microvascular scissors. **File name:** 69201_Video 1.MOV **Timestamps:** 20:47 – 21:47

3.5. NOTE: Make sure the PFV incision is longitudinal, as the transverse incision can be enlarged and ruptured by traction. **File name:** 69201_Video 1.MOV **Timestamps:** 20:47 – 21:47

3.1. Irrigate the isolated vascular segment thoroughly with heparinized saline (0.1% sodium heparin in 0.9% NaCl) until the blood erythrocytes are completely cleared. **File name:** 69201_Video 1.MOV **Timestamps:** 21:47 – 22:47

4. Lymph node-vein anastomosis

4.1. Explore and fully expose dcLN and its afferent lymphatic vessel (ALV) in the middle of the STM and SCM. **File name:** 69201_Video 1.MOV **Timestamps:** 27:00 – 28:00

4.2. Dissect the dcLN gradually using a blunt technique. Carefully incise the fascia between the dcLN and surrounding tissues, avoiding damage to both the dcLN and its ALV. **File name:** 69201_Video 1.MOV **Timestamps:** 28:00 – 28:47

4.3. NOTE: Ensure that PFV and dcLN are close to each other to minimize tension after anastomosis. **File name:** 69201_Video 1.MOV **Timestamps:** 28:00 – 28:47

4.4. Create an opening at the distal end of the dcLN by partially resecting the lymph node tissue. Ensure that the width of the opening is smaller than or similar to the diameter of the PFV, so that the PFV blood flow will not be significantly affected. **File name:** 69201_Video 1.MOV **Timestamps:** 28:47 – 29:47

4.5. Using a 12-0 nylon suture, anastomose the proximal end of the PFV incision to the proximal end of the dcLN incision. **File name:** 69201_Video 1.MOV **Timestamps:** 31:54 – 32:54

4.6. Continue to expand the incision length of the PFV to the distal end so that it is slightly larger than the length of the dcLN incision. **File name:** 69201_Video 1.MOV **Timestamps:** 37:22 – 37:40

4.7. Pass the needle sequentially through the outer edge of the dcLN transection and the inner edge of the PFV incision, thereby pulling the dcLN transection into the lumen of PFV. **File name:** 69201_Video 1.MOV **Timestamps:** 38:56 – 39:56

4.8. Perform a continuous suture from the inferior to the superior edge of the dcLN transection. Irrigate the lumen with heparin sodium solution once more just prior to completing the final suture to prevent thrombus formation within the PFV lumen.

Maintain appropriate tension on the suture during the continuous closure to ensure a secure closure and prevent subsequent leakage. **File name:** 69201_Video 1.MOV
Timestamps: 45:40 – 46:40

4.1 Place interrupted sutures wherever the continuous anastomosis is loose.

5. Lymphatic vessel-vein anastomosis

5.1 Transect the ALV and preserve the surrounding tissue to serve as a suture anchor point. **File name:** 69201_Video 2.MOV **Timestamps:** 13:32 – 14:32

5.2 Using a 12-0 suture needle, insert it about 2 mm proximal to the PFV incision and advance it to exit through the opening of the PFV incision. **File name:** 69201_Video 2.MOV **Timestamps:** 21:13 – 22:13

5.3 Enter the tissue near the stump of ALV with the suture needle to catch the ALV. **File name:** 69201_Video 2.MOV **Timestamps:** 24:18 – 25:18

5.4 Pass the needle into the PFV incision and out through its proximal edge, then tie the suture to fixation the ALV stump within the lumen of PFV. **File name:** 69201_Video 2.MOV **Timestamps:** 26:05 – 27:05

5.5 NOTE: Completely insert the ALV stump into the lumen of PFV , orienting it toward the proximal direction to maintain physiological drainage. **File name:** 69201_Video 2.MOV **Timestamps:** 26:05 – 27:05

5.5 Suture the surrounding tissue near the stump of ALV to the inner edge of the PFV incision to provide external fixation. Irrigate the lumen with heparin sodium solution once more just prior to completing the final suture to prevent thrombus formation within the PFV lumen. **File name:** 69201_Video 2.MOV **Timestamps:** 32:07 – 33:07

6. Suture and postoperative care

6.1 Perform continuous suturing for the subcutaneous fascia and interrupted suturing for the skin.

6.2 Disinfect the surgical area with iodine tincture. Then apply erythromycin ointment uniformly around the incision to prevent infection.

6.3 Postoperatively, provide analgesia by subcutaneous injection of meloxicam (1–2 mg/kg) once daily for up to 72 hours, in accordance with institutional animal care guidelines. Inject into the loose subcutaneous tissue over the neck or flank.

7. Validation of anastomosis

7.1 Identify and dissect the anterior facial vein (AFV), a tributary of the EJV, lateral to the PFV.

7.2 Verify the success of the anastomosis by observing significantly more intense staining in the PFV compared to the adjacent AFV.

7.3 Quantify color changes objectively using the standardized CIE LAB color space. Define and extract Regions of Interest (ROIs) in Fiji to obtain the mean CIE L*a*b* values, then calculate the hue angle (h°) in degrees using the formula:

$$h^\circ = \text{atan2}(b, a) \times (180/\pi)^{6,7}.$$