

DAUGHERTY LAB
Saha Cardiovascular Research Center
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Protocols

Harvesting and Sectioning the Ascending Aorta

Harvesting the ascending aorta

Materials:

1. Insulin syringe
2. Ketamine/xylazine cocktail (90 mg/kg, 10 mg/kg, respectively)
3. Saline perfusion supplies: saline, 10 ml syringe, 23G needle, extension tubing, and absorbent dissection pad
4. Black plastic sheet (black trash bag, cut into 1.5 cm x 1.5 cm)
5. Instruments: scissors, forceps, hemostat, and razorblade
6. OCT compound and tissue base molds

Equipment:

1. Turn on dissection scope lamp and ensure that the field of the dissection scope is well lit and in focus.
2. Open Nikon AR software.
3. Check cryostat temperature setting (optimal -20 °C for aorta).

Methods:

1. Inject mice with 30 units of ketamine/xylazine cocktail i.p.
2. Open the chest cavity and expose the heart and aorta.
3. Nick the right atrium and inject 6 to 8 ml saline into the left ventricle of the heart. Once thoroughly perfused, the liver and kidney become a light brown color.
4. To expose the ascending aorta and its branches, gently remove the lungs, thymus, esophagus, trachea, and adipose tissues.
5. Gently tease the aorta and arteries from connective tissues.
6. If a gross specimen photograph is needed, place a black thin plastic sheet behind the heart and ascending aorta and take a photograph using the Nikon AR software (see Figure 1).

Note: 1.0x lens is recommended for taking a photograph because of focus depth. Surrounding area would be blurred under higher magnification lenses.



Figure 1.

7. Cut the descending aorta at the heart level and dissect away from the backbone.
8. Cut around the heart and aortic branches to free it from all connective tissue. This frees the heart and aorta from the body.
9. Separate the heart from the aorta by holding the heart with the forceps and using a razorblade. The cut line is made perpendicular to the ascending aorta (Figure 2).

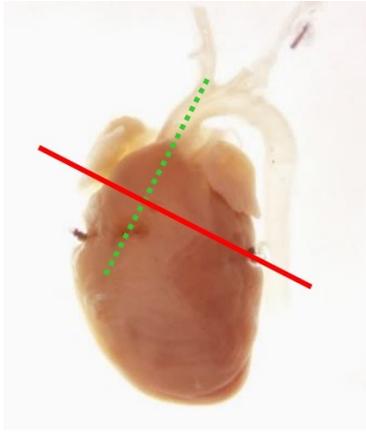


Figure 2.
 Red line: cut line
 Green line: the axis of ascending aorta

10. Put the aortic tissue into a OCT mold (Figure 3)
 - Cross sectioning
 - Place the upper portion of the sectioned heart, containing the ascending aorta in a mold and cover with OCT compound. The ascending aorta needs to be positioned so that it is perpendicular to the bottom surface of the tissue mold.
 - Sagittal sectioning
 - Trim the heart tissue from the ascending aorta as much as possible. Place the anterior portion of the trimmed aorta in a mold and cover with OCT compound so that it is parallel to the bottom surface of the tissue mold.



Cross sectioning



Sagittal sectioning

Figure 3

11. Label the mold with the study name, mouse #, and date or code as appropriate.
12. Freeze the OCT embedded aortic root. Wrap each mold in parafilm and store at -20 °C.

Sectioning the heart and aorta

Materials:

1. Instruments: forcep, razorblade, and microtome blade
2. OCT compound
3. Microscope slides: label slides with study name, sample #, slide #
4. Slide box

Equipment:

1. Check cryostat temperature setting (optimal -20 °C for aorta). If necessary, change the microtome blade and allow several minutes for the blade to cool.

Methods:

Cross sectioning

1. Trim off the excess OCT using a razorblade.
Note: Leave the descending aorta. The descending aorta will be used to orient the ascending aorta.
2. Place a small amount of OCT compound in the center of a cutting disk.
3. Mount the frozen tissue block onto the cryostat disk with the ventricular tissue facing upward.
4. The ventricular tissue is sectioned and discarded until the aortic sinus is reached. This is identified by checking under the microscope.
5. Once the aortic root has been located, begin collecting sections using sequentially labeled slides (see Figure 4, 5).

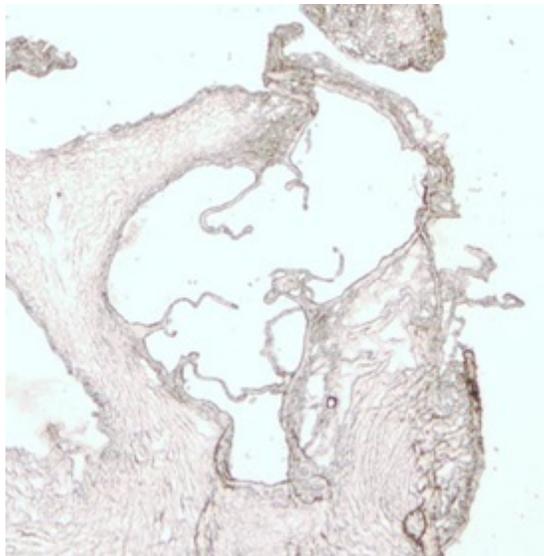


Figure 4. Aortic root

6. 10 μ m serial sections are collected, 9 sections per slide (see Figure 5).

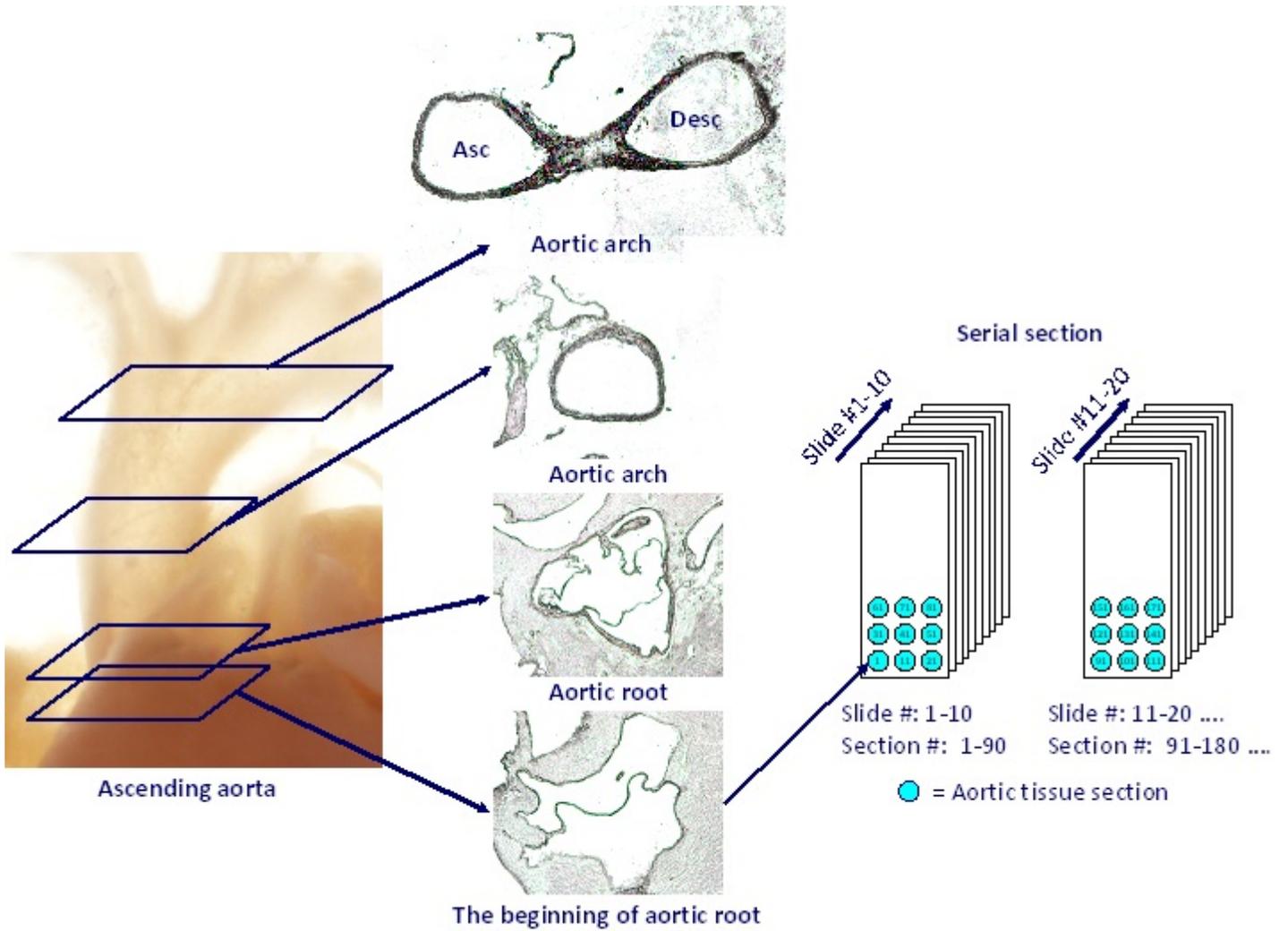


Figure 5.

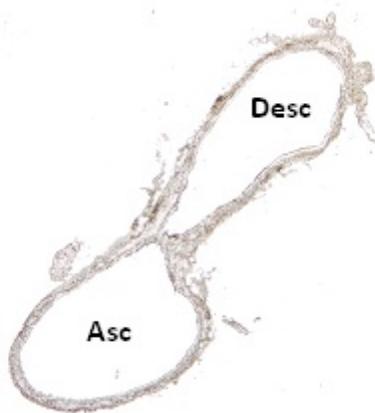


Figure 6.

Stop collecting sections once the ascending and descending aorta meet. Collection does not need to continue when the ascending and descending aorta form the shape of an eight (see Figure 5, 6).

8. Label slide box with study name, mouse #, tissues, date and initials on side and top of box.
9. Place the slides into a slide box and store the slide box in the appropriate freezer.
10. Clean the cryostat by emptying waste into the trash and rinsing the waste collection tray with ethanol.

Sagittal Sectioning

1. Trim off the excess OCT using a razorblade.
2. Place a small amount of OCT compound in the center of a cutting disk.
3. Mount the frozen tissue blocks on the cryostat disk with the left side of ascending aorta facing outward.



4. The left side of aortic tissue is sectioned and discarded until the aortic forms a thin broken "u". This is identified by checking under the microscope (Figure 7)

Figure 7

5. Once the aorta has been reached, start collecting sections using the previously labeled slides (see Figure 8).
6. 10 μ m serial sections are collected, 4 - 9 sections per slide.

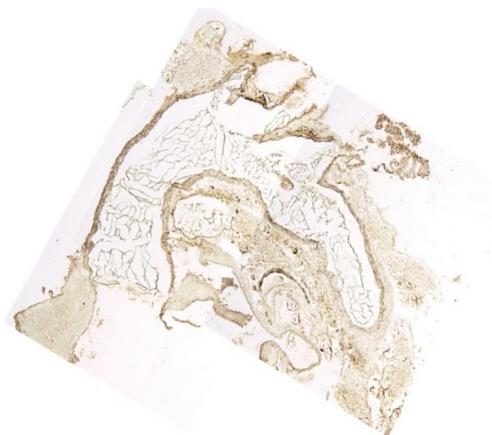


Figure 8.

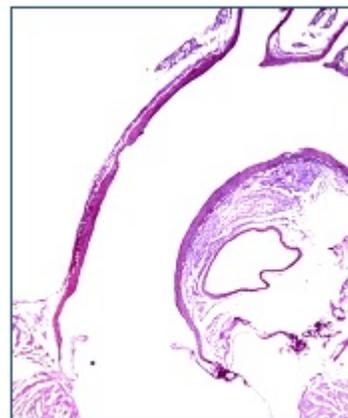


Figure 9. Example - HE staining
(Rateri DL, et al. Am J Pathol. 2014)

7. Stop collecting sections after the aorta has been sliced through completely (Figure 10).



Figure 10.
This image is representative of the end point of collection, much of the aorta has been sliced through and only a small segment remains. It is not necessary to slice through the whole aorta so long as the final sections show a result similar to this image.

8. Label slide box with study name, mouse #, tissues, date and initials on side and top of box.
9. Place the slides into a slide box and store the slide box in the appropriate freezer.
10. Clean the cryostat by emptying waste into the trash and rinsing the waste collection tray with ethanol.

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Verified: Alan Daugherty on 4/3/17

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