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Sectioning of Mouse Proximal Thoracic Aorta

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1 Works for me dx.doi.org/10.17504/protocols.io.be9mjh46

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ABSTRACT

This protocol is used for generation of ascending aortic serial sections for histology. This protocol generates a set of 10 slides covering ~1mm of ascending aortic tissue. Inclusion of the descending aorta provides orientation and a non-TAA control from the same animal.

EXTERNAL LINK

<http://cvrc.med.uky.edu/lab-protocols>

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Chen JZ, Sawada H, Moorlegghen JJ, Weiland M, Daugherty A, Sheppard MB. Aortic Strain Correlates with Elastin Fragmentation in Fibrillin-1 Hypomorphic Mice. *Circ Rep.* 2019;1(5):199–205. doi:10.1253/circrep.CR-18-0012

ATTACHMENTS

Sectioning of Mouse
Proximal Thoracic
Aorta.pdf

MATERIALS TEXT

1. Dissecting scissors, forceps, scalpel blade, and Parafilm
2. Tissue molds 15x15 mm (Fisher Scientific Cat # 41-741) and microcentrifuge tube with screw top.
3. OCT compound (Fisher Scientific Cat # 14-373-65)
4. Probe-on-Plus microscope slides (Fisher Scientific Cat # 22-230-900)

SAFETY WARNINGS

Be careful when using the cryostat, blade is sharp.

BEFORE STARTING

1. OCT will become rubbery and difficult to cut if frozen for long periods. If you plan to cut the tissues within a few weeks, place aortic root tissues in tissue molds covered with OCT compound, wrapped tightly in parafilm, and store at -20°C. Otherwise, store aortic roots covered by OCT compound in microcentrifuge tube with screw top at -20°C for long-term storage. Label tissue molds or microcentrifuge tubes with the study name, date, and mouse ID or code as appropriate.
2. After anesthesia, cut open the thoracic cavity and draw blood from the right ventricle. Cut open the right atrium. Perfuse with saline from the apical side of the left ventricle to remove blood from the systemic circulation. A good sign for successful removal of blood from the systemic circulation is visualization of the liver turning from red to pale.

Collection of thoracic aorta 15m

15m

1 Euthanize mouse with ketamine : xylazine cocktail

Open thoracic cavity and expose heart

2

3 Nick right atrium with sharp scissors

4 Flush vasculature with cold saline (>5 mL) introduced slowly into the left ventricle

5 Dissect away the thymus, lungs, trachea, and esophagus

6 Inject optimal cutting temperature compound (OCT; ~0.5 mL) slowly into the left ventricle to keep patent, inject until the lumen of the descending aorta expands

7 Dissect the heart and thoracic aorta by ligating the left/right subclavians and common carotids near the thoracic outlet and ligating the aorta above the diaphragm

8 Dissect the descending aorta from the spine, taking care not to nick the aorta

Preparing tissue block

9 Put a thin layer of OCT in mold

10 Separate the heart from the aorta by holding the heart with the forceps and cutting with either scissors or a scalpel blade, as close to the heart as possible without causing damage.

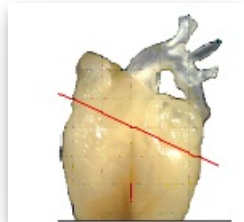


Figure 1: Approximate line to cut ventricle

11 Cut away approximately 60 - 70% of the ventricles (the red line shown in the **Fig 1**)

12 Mount the heart, ascending aorta, and descending aorta with the heart facing the left and descending aorta on the right. With permanent marker, mark where the aorta lays in the mold

- 13 Cover tissue with OCT. Place in cryostat with peltier on for 🕒 00:15:00 for OCT to solidify.

Preparing the tissue block and cryostat for cutting

- 14 Set temperature to 🌡️ -20 °C
- 15 Set chuck advance to ➡️ 10 μm
- 16 Make sure stage is level
- 17 Make sure sectioning starts with new region of blade or new blade
- 18 Make sure anti roll plate is not chipped
- 19 Label 10 slides / sample / region. For example: Study name, mouse ID and group, region collected (ascending or root) and number of slide (1-10)
- 20 Remove block from mold
- 21 Trim excess solid OCT from tissue, keeping intact the descending aorta
- 22 Cut the heart at the mid ventricle level
- 23 Put small drop of OCT onto mount
- 24 Mount trimmed tissue block with the aortic arch facing up onto holder
- 25 Place in peltier and wait 10 minutes for interface to solidify

26



The method requires cutting sequential sections in the correct orientation and without tearing, curling, or sticking incorrectly to the slide. Therefore, do not cut aortas collected from your experiments until you are sure you can reliably cut sections sequentially.

Place holder onto cryostat chuck with the heart facing down and the descending aorta on top.

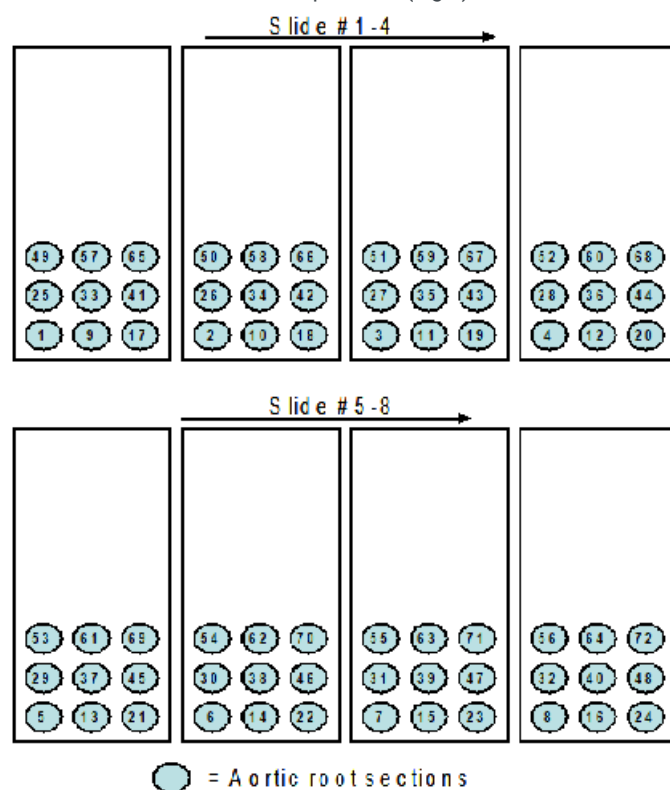
27 Start sectioning, checking every 10 cycles until tissue can be seen.

28 Start collecting serial sections when arch divides into ascending aorta and descending aorta. Ascending aorta should be towards you and descending aorta should be away.



This should produce sections with both the ascending aorta and descending aorta. Ascending aortas should be towards the top of the slide, descending aortas should be towards the bottom. Verify orientation of sections by counting the number of elastic laminae seen: Ascending = 8-10 lamina, Descending = 8-6 lamina.

29 Collect at least 9 serial sections per slide. (Fig 2)





Collect first section produced on bottom left corner of slide 1. Collect second section on bottom left corner of slide 2 and subsequent section in this mode. Collect eleventh section to the right of first section on slide 1. Collect serial sections by holding labeled slide downward with the label facing you. The ascending aorta should be on towards the top of the slide and the descending on bottom of slide

- 30 If section rolls or flips - use antistatic brush to unroll section
- 31 Stop collecting at the level of the aortic root - where cusps of valve leaflets are seen. Serial sections are collected at 10 μm /section, and 8 to 10 slides/aorta until the aortic wall disappears or is not intact anymore. Usually at least 9 serial sections/slide can be collected.

Clean up cryostat

- 32 Lock chuck wheel
- 33 Remove block from chuck and mount - save or discard excess tissue
- 34 Brush clean all surfaces with 70% ethanol and brush
- 35 Discard trimmings
- 36 Turn off light
- 37 Close cryostat window

Storage

- 38 Slides should be stored in a appropriately labeled slide box (both the top and one of the short edges of the box) at -20°C .