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SPARC_Duke_PelotGrill_OT2-OD025340_PigVagusNerve_Collection_Histology_Microscopy

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1 *Works for me* dx.doi.org/10.17504/protocols.io.6bqhamw

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ABSTRACT

Protocol for collection, histological processing (paraffin embedding, Masson's trichrome staining), and imaging of pig vagus nerves.

DOI

dx.doi.org/10.17504/protocols.io.6bqhamw

PROTOCOL CITATION

J. Ashley Ezzell, Nicole A. Pelot, Kara A. Clissold, Warren M. Grill 2020. SPARC_Duke_PelotGrill_OT2-OD025340_PigVagusNerve_Collection_Histology_Microscopy. **protocols.io** dx.doi.org/10.17504/protocols.io.6bqhamw

KEYWORDS

Histology, Vagus nerve, Masson’s trichrome, Pig vagus nerve, Nerve morphology

CREATED

Aug 09, 2019

LAST MODIFIED

Jun 30, 2020

PROTOCOL INTEGER ID

26704

MATERIALS TEXT

- 4% paraformaldehyde
- Mordant & tissue dye
- Ethanol
- Clearite
- Paraffin
- Xylene
- Bouin's fixative (mordant)
- Weigert's iron hematoxylin solution
- Biebrich scarlet-acid fuchsin solution
- Phosphomolybdic-phosphotungstic acid solution
- Aniline blue solution
- Acetic acid

Citation: J. Ashley Ezzell, Nicole A. Pelot, Kara A. Clissold, Warren M. Grill (06/30/2020). SPARC_Duke_PelotGrill_OT2-OD025340_PigVagusNerve_Collection_Histology_Microscopy. https://dx.doi.org/10.17504/protocols.io.6bqhamw

- Microscope with color camera

DISCLAIMER:

Collect pig vagus nerve samples.

1 We collected fresh vagus nerve samples from Yorkshire pigs after they were euthanized following medical training courses at Duke University.

2 We collected samples of the carotid sheath in the neck bilaterally. We collected 2 cm samples at the level midway along the rostocaudal line from the rostral end of the sternum to the line connecting the left and right angles of the mandible (typically ~12 to 14 cm apart). We measured from the "valley" of the common carotid bifurcation to the center of each sample.

3 We collected ~2 cm samples of the anterior and posterior subdiaphragmatic vagus nerve trunks along the esophagus approximately halfway between the diaphragmatic esophageal hiatus and the gastroesophageal junction.

4 We dyed the rostral end of each sample green to maintain orientation during processing.

5 We placed each sample between two histology sponges in a mega-sized histology cassette. We placed the cassettes in a tub with 4% paraformaldehyde in a 4^{o}C refrigerator.

Perform histological processing.

6 We rinsed each sample with deionized water.

7 We processed each sample on the long cycle in the Leica ASP300S Tissue Processor for ~10 hours: 70, 80, 95, 95, 100, 100,100% ethanol for 30, 35, 40, 40, 40, 40, 40 min, respectively; Clearite for 50min, three times; paraffin wax for 50 min, three times.

8 We cut each sample in half transversely and embedded the halves together cut side down in paraffin in order to obtain transverse sections starting from the center of the nerve and moving outward.

9 We collected 5 µm sections, placing two serial sections per microscope slide for fifteen slides.

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10 The slides were air dried overnight at room temperature, then baked at 37^{O}C overnight.

11 Of the 15 slides per sample, we stained slides 2 and 14 with Masson's trichrome as follows.

12 The slides were baked at 60^{O}C for 1.5 hours and then cooled overnight.

13 We deparaffinized the slides and hydrated them to distilled water: xylene (2x 6 min), 100% ethanol (5 min), 95% ethanol (4 min), 70% ethanol (3 min), dH2O (2x 1 min).

14 We placed the slides in Bouin's fixative (mordant) at room temperature overnight.

15 We washed the slides in running tap water until the yellow color disappeared (~10 minutes).

16 We rinsed the slides in distilled water.

17 We placed the slides in Weigert's Iron Hematoxylin solution for 10 min.

18 We washed the slides in running tap water for 10min.

19 We placed the slides in Biebrich Scarlet-Acid Fuchsin solution for 5 min.

20 We washed the slides in running tap water for 2 min.

21 We placed the slides in Phosphomolybdic-Phosphotungstic Acid solution for 10 min.

22 We transferred the slides directly to Aniline Blue solution for 3 min.

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23 We differentiated the counterstain (A. blue) in 1% Acetic Acid solution for 1 min.

24 We dehydrated, cleared, and coverslipped the slides: 95% ethanol (1 min), 100% ethanol (4 min), xylene (5 min).

Perform microscopy.

25 Each sample was imaged at 10x using a Nikon Ti2 microscope with a Photometrics Prime 95B-25MM camera (Nikon Instruments Inc.). We selected the best of four slices for each sample based on the quality of the slice (no tearing or fraying).

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