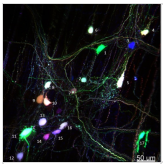

Dec 08, 2020

Multicolor adeno-associate virus labeling and 3D digital tracing of enteric plexus in mouse proximal colon

Lixin Wang[1], Collin Challis[2], Honghui Liang[1], Songlin Li[1], Charless Fowlkes[3], Aidan Sullivan[4], Kumar SR[2], Yvette Taché[1]

[1]University of California, Los Angeles; [2]California Institute of Technology; [3]University of California, Irvine; [4]MBF

1 Works for me dx.doi.org/10.17504/protocols.io.bqavmse6

SPARC
Tech. support email: info@neuinfo.org

Manmeet Bains

ABSTRACT

Using a multicolor adeno-associate virus system to label the colonic enteric nervous system for digital tracing of individual neurons and nerve fibers in microcircuits in three-dimensions (3D). The methods include viral vectors retro-orbital injection in mice, preparation of colon tissues, microscopy and 3D digital tracing.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Wang L, Challis C, Li C, Fowlkes CC, Kumar SR, Yuan P-Q Taché Y. Multicolor sparse viral labeling and 3D digital tracing of enteric plexus in mouse proximal colon using a novel adeno-associated virus capsid. Neurogastroentero. Motil. 2020 Oct 23;e14014. doi: 10.1111/nmo.14014.

DOI

dx.doi.org/10.17504/protocols.io.bqavmse6

PROTOCOL CITATION

Lixin Wang, Collin Challis, Honghui Liang, Songlin Li, Charless Fowlkes, Aidan Sullivan, Kumar SR, Yvette Taché 2020. Multicolor adeno-associate virus labeling and 3D digital tracing of enteric plexus in mouse proximal colon. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bqavmse6

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Wang L, Challis C, Li C, Fowlkes CC, Kumar SR, Yuan P-Q Taché Y. Multicolor sparse viral labeling and 3D digital tracing of enteric plexus in mouse proximal colon using a novel adeno-associated virus capsid. Neurogastroentero. Motil. 2020 Oct 23;e14014. doi: 10.1111/nmo.14014.

KEYWORDS

Viral tracing, enteric neuron, colon, 3D digital tracing, mouse

CREATED

Dec 01, 2020

LAST MODIFIED

Dec 08, 2020

PROTOCOL INTEGER ID

45109

MATERIALS TEXT

a. Animals: Mice C57BL/6J (Jackson Laboratories, Sacramento, CA), male and female, age range of 6-10 weeks old.
b. A tunable two-component multicolor four-vector system (AAV-PHP.S:hSyn-tTA:TRE-XFP)
c. isoflurane
d. oxygen
e. induction chamber
f. Sylgard™ 184 silicone elastomer (Electron Microscopy Science, Hatfield, PA)
g. saline
h. cold 4% paraformaldehyde in 0.1 phosphate buffer
i. 0.01 M phosphate buffered saline
j. sodium azide
k. Refractive Index Matching Solution:

Histodenz (Sigma D2158)	40 g	88%
Tween-20	30 µl	0.1%
Sodium azide	3 mg	0.01%
0.02 M phosphate buffer (pH7.5)	30 ml	

l. microscopic glass slides
m. iSpacer (SunJin Lab Co., Hsinchun City, Taiwan, www.sunjinlab.com).
n. *Software*:Neurolucida (NL) 360 (version 2020.1.1.), NL Explorer (version 2019.2.1.), Imaris 9.2 for Neuroscientist and a customized version of neuTube (6).

ABSTRACT

Using a multicolor adeno-associate virus system to label the colonic enteric nervous system for digital tracing of individual neurons and nerve fibers in microcircuits in three-dimensions (3D). The methods include viral vectors retro-orbital injection in mice, preparation of colon tissues, microscopy and 3D digital tracing.

Viral constructs

1 Production of a tunable two-component multicolor four-vector system (AAV-PHP.S:hSyn-tTA:TRE-XFP) is described in details in a previous publication: Challis RC, Ravindra Kumar S, Chan KY, Challis C, Beadle K, Jang MJ, et al. Systemic AAV vectors for widespread and targeted gene delivery in rodents. Nat Protoc. 2019;14(2):379-414. DOI: 10.1038/s41596-018-0097-3

AAV injection

2 The multicolor AAV mix was injected unilaterally (60 µl/mouse) into the retro-orbital sinus of male and female mice aged 7-10 weeks.

3 The master mix was vortexed for 1–2 s before use.

4 Mice were placed in the induction chamber in a fume hood and anesthetized initially with 4.5% isoflurane in oxygen and 2.5% during the injection.

5 The vectors were loaded without air bubbles in a BD Veo Insulin Syringe with BD Ultra-Fine 6 mm x 31G needle (324911, Becton, Dickinson and Company, Franklin Lakes, NJ).

6 The anesthetized mouse was removed from the induction chamber, and placed in a prone position with the nose cone to maintain anesthesia.

Citation: Lixin Wang, Collin Challis, Honghui Liang, Songlin Li, Charless Fowlkes, Aidan Sullivan, Kumar SR, Yvette TachÃÂ© (12/08/2020). Multicolor adeno-associate virus labeling and 3D digital tracing of enteric plexus in mouse proximal colon. https://dx.doi.org/10.17504/protocols.io.bqavmse6

7 The needle was inserted, bevel down, at a 30–45° angle into the medial can thus and through the conjunctival membrane to the retro-orbital sinus.

8 The vectors were injected slowly into the retro-orbital sinus and the needle was removed gently.

9 Mice survived 3 weeks before tissue collection.

Tissue preparation

10 Mice were euthanized by an overdose of 5% isoflurane in oxygen.

11 After thoracotomy, perfusion cannula (18G) was inserted into the aorta via the left ventricle.

12 Saline was perfused for ~1 min.

13 The gastrointestinal (GI) tract from the lower esophagus to rectum was removed.

14 The whole colon from ileocecal junction to the end of distal colon at the level of pelvic brim where runs the iliac artery, as well as the lower esophagus, gastric corpus, gastric antrum, 1 cm of jejunum and 1 cm of ileum at 3-5 cm and 2-3 cm from the ileocecal junction respectively and rectum were flat-pinned on a Sylgard™ 184 silicone elastomer (Electron Microscopy Science, Hatfield, PA).

15 Perfusion of mouse with 40 ml of cold 4% paraformaldehyde in 0.1 phosphate buffer (pH 7.3) at 2 ml/min.

16 The pelvic, celiac and dorsal root (L6) ganglia were dissected out.

17 Post-fixation of the ganglia in 4% paraformaldehyde at 4°C overnight.

18 The GI tissues and ganglia were rinsed in 0.01 M phosphate-buffer saline (PBS) for 1 day.

19 The colon of some mice was prepared to maintain the layers from submucosa to serosa by scratching of the mucosa,

Citation: Lixin Wang, Collin Challis, Honghui Liang, Songlin Li, Charless Fowlkes, Aidan Sullivan, Kumar SR, Yvette TachÃÂ© (12/08/2020). Multicolor adeno-associate virus labeling and 3D digital tracing of enteric plexus in mouse proximal colon. https://dx.doi.org/10.17504/protocols.io.bqavmse6

because the mucosa is not cleared well when embedded in Refractive Index Matching Solution (RIMS, recipe below), and it interferes with the focus of laser beams during image acquisition in the whole colon wall.

20 Tissues were cleared by immersion in a custom-made imaging media, RIMS (5) with a reflective index of 1.46], 2 h at room temperature for ganglia and overnight at 4°C for GI tissues.

21 The ganglia after 2 h and GI tissues after overnight immersion in RIMS were embedded on microscopic glass slides in fresh RIMS sealed by iSpacer (SunJin Lab Co., Hsinchun City, Taiwan, www.sunjinlab.com). The colon samples were placed onto the slides with serosa on top.

Digital segmentation and neuronal tracing in confocal image

22 Microscopic image post-processing: presented as original confocal microscopic images visualized in Imaris 9.2 and 9.5 with adjusted brightness, but no deconvolution, noise ratio cutoff and contrast adjusting. Images presented in the figures of the article had a 20% increase in brightness just for visualizing effect.

23 Digital neuronal tracing: NL360 and Imaris 9.2: Adaptive thresholds were used in tracing algorithms, not isosurfacing, using semi-automated, namely user-guided mode. Somas were detected by sensitivity based on contrast between foreground and background. Nerve fibers were traced by points, no tortuosity limits. Wrong routes of traces in fiber-dense regions or fibers with similar hues were manually traced and corrected.

24 All the programs used in this study traced soma and neurites in separate modes in the confocal microscopic images. Varicosities were not spotted because none of the software we used had the ability. When the individual structures were traced, the original images were adjusted by whiteness and/or contrast to the detectable thresholds. For the white fluorescence (“hue bleaching”) by intensive AAV labeling, tracing was performed in one or two colors instead of all channels. Whereas for faint elements, brightness/contrast was increased. Manual tracing was used when there was interfering by close-by labeling or the fluorescence was under automated detectable levels. The digital traces were confirmed by magnification, rotation, partial projection or dicer of layers. Axons were identified by varicosities or thin fiber with the same diameter.

Citation: Lixin Wang, Collin Challis, Honghui Liang, Songlin Li, Charless Fowlkes, Aidan Sullivan, Kumar SR, Yvette TachÃÂ© (12/08/2020). Multicolor adeno-associate virus labeling and 3D digital tracing of enteric plexus in mouse proximal colon. https://dx.doi.org/10.17504/protocols.io.bqavmse6