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Tache_Yuan_OT2OD024899_CLARITYAnd3DImagingOfColonicENSintheMouseAndPig_1_2019-Pig_Protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.4r9gv96

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1 The male young adult (6-7 months old, 25-30kg) and castrated Yucatan minipigs (n=2) were used for CLARITY by PACT (passive clarity technique), a method for whole-body clearing and immunolabeling. Colonic samples (~5 cm long) were removed from the proximal (~10 cm from the ceco-colic junction), transverse (~10 cm from the end of the proximal, specifically about 10 cm from the end of the centrifugal spiral colon) and distal colon (~20 cm from the ano-rectum). The colon was opened along the mesenteric border and the colon contents were rinsed off by washes using ice-cold PBS, then colon tissues were pinned flat in Sylgard-coated dish and incubated in the ice cold hydrogel solution overnight for hydrogel-tissue hybridization. On the second day, the flat samples were cut into small pieces in 1.5x1.5 cm, keep the samples at 4°C for more 2 days in the hydrogel solution. Then the samples (6-8 pieces) were moved into 50 ml conical tubes with 15 mL of fresh hydrogel solution. Place the conical tubes on a rack in the desiccation chamber for degassing followed by a hydrogel polymerization in a temperature-controlled 37°C water bath. The sample were removed in a new 50 ml conical tube with 40 mL of clearing solution to wash out the excess hydrogel monomers from inside the tissue at 37°C overnight on a shaker/rotator plate until clearing was achieved in 3-4 weeks. Immunofluorescence of PGP 9.5 (1:1000, ab108986, Abcam) and double labeling of Neurofilement 200 (NF) (1:1000, AB5539, Millipore) with PGP 9.5 (1:1000, ab108986, Abcam) were performed. Samples were incubated in primary antibody solution at RT with shaking for 1 days and 4°C for 4 days, and then washed with PBS at 37°C with shaking for 1 day followed by incubation in secondary antibody solution at RT°C with shaking for 2 day. Samples were immerse samples in RIMS at RT 3 hours and 4°C overnight and them mounted with RIMS in a sealed watertight well prepared with iSpacers (SunJin Lab, Hsinchu City, Taiwan). Images acquired with ZEISS LSM710 confocal and SP8 DIVE multi photon microscope were reconstructed into 3D images and videos using Imaris 9.1 for neuroscientists.