Oct 30, 2019

Tache_Yuan_OT2OD024899_CLARITYAnd3DImagingOfColonicENSintheMouseAndPig_1_2019-Mouse_Protocol (Annotation Copy)

Pu-Qing Yuan[1], Lixin Wang[1], Yvette Taché[1]

[1]University of California, Los Angeles

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

Gabrielle Pine

1 The male adult C57BL/6J mice (6-8 weeks, 25.4-28.6g, n=4) were used for CLARITY by PARS (perfusion-assisted agent release in situ) technique, a method for whole-body clearing and immunolabeling. The mice were perfused transcardially with 10 ml of ice cold PBS followed by 20 mL of the ice cold hydrogel solution at a slow rate of about 1 mL/min. Colonic samples (about 2-3 cm long) were removed from the proximal (~0.5 cm from the cecum) and distal colon (~1 cm from the anus). The colon was opened along the mesenteric border, 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 1-2 weeks. Immunofluorescence of PGP 9.5 (1:1000, ab108986, Abcam) and double labeling of neurofilament 200 (NF) with neuronal nitric oxide synthase (nNOS) (1:500, sc-648, Santa Cruz), S100β (1:500, ab52642, Abcam) with Hu C/D (1:200, A-21271, Life Technologies) were performed. Samples were incubated in primary antibody solution at room temperature (RT) with shaking for 2 days and washed with PBS at RT with shaking for 1 day followed by incubation in secondary antibodies at RT with shaking for 1 day. Samples were immerse samples in RIMS at RT for 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.