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A Standard Operating Protocol for the Processing of Human Trigeminal Ganglia Tissue for Different Experiments

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PRECISION Human Pain ...

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We use this protocol and it's working

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Abstract

Human tissues from consent donors are precious but limited materials for biomedical research. It is thus important to carefully think about how to process human tissues to efficiently use them for different experimental purposes. New studies interrogating single-cell transcriptomics of human trigeminal and dorsal root ganglia (TG and DRG, respectively) greatly expand our understanding of the neuronal pathways that mediate somatosensory perception. Results from these studies also have the great potential to inform much-needed efficient therapies against chronic pain disorders, such as migraine. Here, we describe a detailed standard operating protocol (SOP) for processing human TG tissues, including freshly frozen embedding, cryosectioning, evaluation of tissue quality, and distributing human TG sections for their subsequent use in genomic, transcriptomic, and epigenetic experiments. Similar design and procedures can be readily employed for handling of other human tissues. Our protocol will enable the use of precious human tissues in independent but complementary experiments and facilitate integration and comparison of multiple modality datasets.

Guidelines

Consortia: The members of the Penn High Precision Pain Center (HPPC) are Anne Marshall, Åsa Rydmark Kersley, Caitlin Cronin, Christine Zay, Dmitry Usoskin, Dongming Liang, Dorota Persson, Ebenezer Simpson, Eric Kaiser, Eric Zager, Ewa Jarocka, Faiz Kassim, Frida Larsson Torri, Hakan Olausson, Hanying Yan, Hao Wu, Huasheng Yu, Johan Nikesjö, Juan Inclan-Rico, Julie Leu, Katarina Laurell, Leah Coghlan, Lotta Medling, Maria Bograkou, Mingyao Li, Oumie Thorell, Patrik Ernfors, Roland Baur, Saad Nagi, Wenqin Luo, Ying Li, and Zarina Ali.

Materials

	A	B	C
	Table 1. RESOURCES TABLE		
	REAGENT OR RESOURCE	SOURCE	IDENTIFIER
	Reagents		
	Embedding mold	ThermoFisher	Cat# 1841
	Tissue-Tek O.C.T. Compound	Sakura finetek USA inc	Cat# 4583
	Microtome blades	C.L. Sturkey Inc.	Cat# DT315S50
	Superfrost plus glass microscope slides	Globe Scientific	Cat# 1358Y
	PEN membrane (2µm) coated glass slides, RNAse free (LCM slides)	Leica	Cat# 11505189
	DMEM - high glucose 4.5 gm/L	Invitrogen	Cat# 11965084
	50mL tube	Fisher Scientific	Cat# 05-539-6
	1.7mL tube	Denville Scientific	Cat# C2170
	Ethanol 200 proof	Decon Labs	Cat# 2701
	Isopropanol	Fisher Scientific	Cat# BP2618-4
	Hematoxylin solution	Sigma Aldrich	Cat# GHS116
	Eosin-Y Alcoholic solution 0.25%	StatLab	Cat# SL98-16
	Bluing Buffer	Dako	Cat# CS702
	Tris base	ThermoFisher	Cat# BP152-500
	Nuclease-free water	Invitrogen	Cat# AM9937
	Acetic Acid	Millipore Sigma	Cat# A6283
	Cytoseal 60	Electron Microscopy Sciences	Cat# 18006
	Commercial Assays		
	Xenium Standalone Custom Gene Panel (4 rxns)	10X Genomics	Product Code 1000649

	A	B	C
	RNeasy Mini Kit	Qiagen	Cat # 74104
	Qubit™ RNA High Sensitivity (HS) Assay Kit	ThermoFisher	Cat # 32852
	Instruments and equipment		
	Cryostat	RWD	Model Minux FS800
	Qubit™ Flex Fluorometer	ThermoFisher	Cat# Q33327
	APERIO VERSA 8	Leica	N/A
	4200 Tapestation System	Agilent	G2991BA

Before start

Always wear appropriate personal protective equipment (PPE), including a lab coat, safety glasses, facial mask, and gloves. All human tissues are BSL-II reagents.

Thoroughly clean the lab bench with 70% ethanol, followed by an RNAse decontamination solution like RNaseZap.

Ensure that the cryostat is kept at approximately -20ºC.

Processing, section, and storage of human TG samples.

1 Fresh human TG tissue is collected in a 50 mL tube containing approximately 10 mL of DMEM. The tube is placed on ice for transport. Clean up the tissue under a dissection microscope to trim off the excessive non-neuronal tissues. If the tissue has been archived and frozen, proceed directly to OCT embedding (**Fig. 1**).

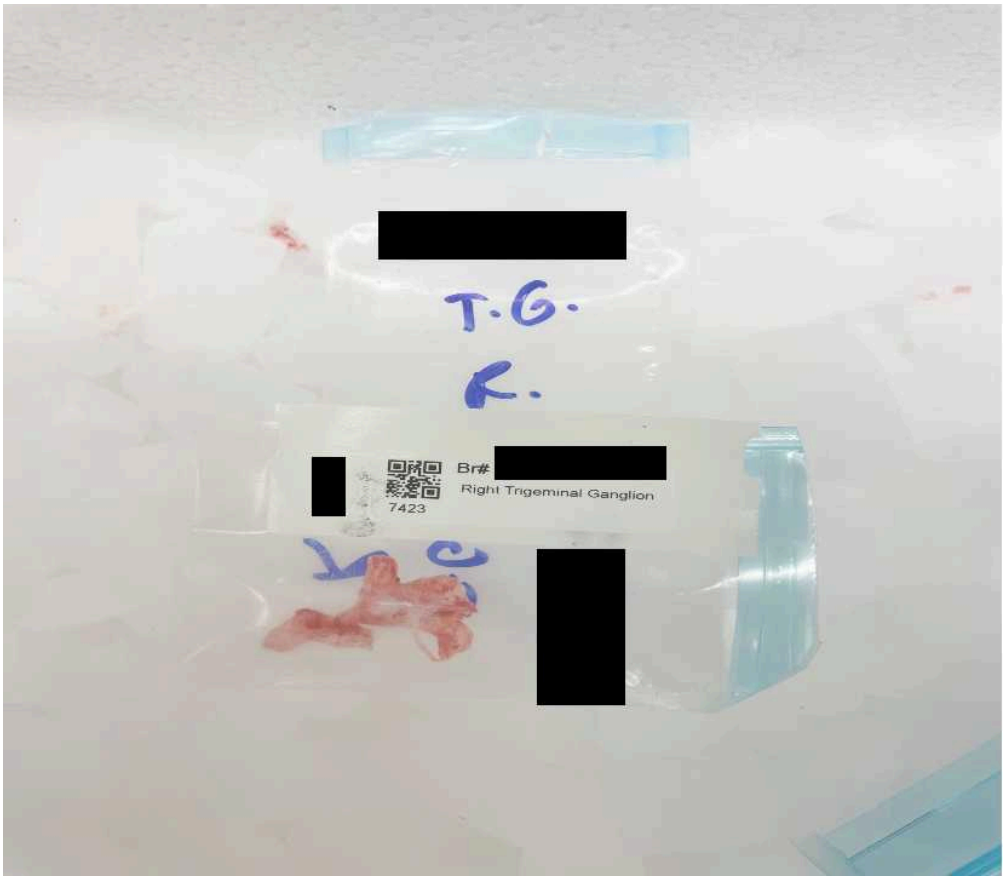

Fig. 1. An example of a piece of archived frozen human TG tissue.

2 Record information from the donor, take a photograph of the tissue while measuring its size with a ruler.

3 Fill an embedding mold with OCT medium and submerge human TG, ensuring OCT completely covers the tissue, take a picture for gross morphology to discern the central and peripheral V1, V2, and V3 branches. Mark the mold accordingly (**Fig. 2**).

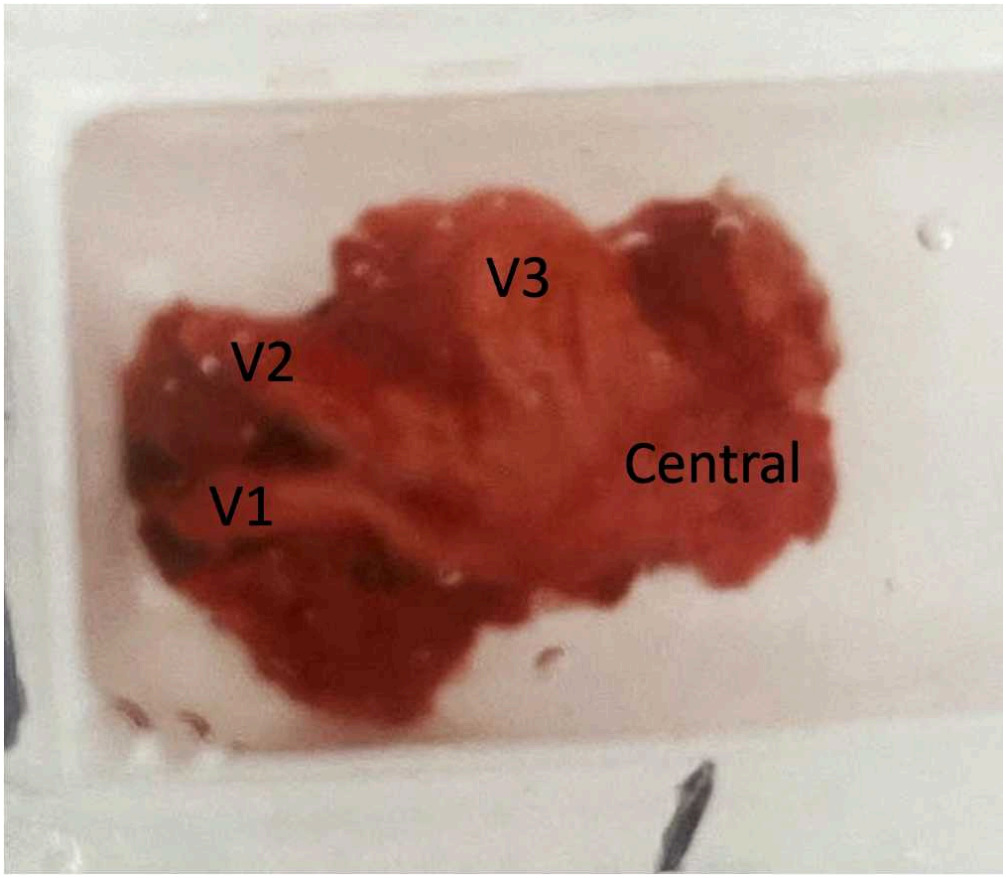

Fig. 2. Embedding a human TG in OCT medium. The central and peripheral (V1-V3) branches are labeled.

4 Place the embedding mold in a dry ice ethanol bath, allowing the tissue to freeze for 5-10 minutes, until the OCT is completely frozen. Store the tissue block in -80 freezer or proceed to step 5.

5 Place the tissue block into the cryostat, allowing it to acclimate for ~15 minutes. Trim the tissue in 20µm sections. Every 100µm, collect a section using a superfrost glass slide and check it under the microscope until the neuronal cell bodies are visible.

6 Collect two sections of 100µm to be used for single-nuclei isolation in 1.7 mL microcentrifuge tubes. Place the tube in dry ice and store at -80ºC until use for single nuclei experiments.

7 Collect one 20µm section in a 1.7 mL Eppendorf tube for RNA extraction to determine RNA concentration and RIN (RNA integrity number). RNA concentration and RIN are two important parameters for evaluating human tissue quality. A low RNA concentration and RIN (less than 5) may indicate RNA degradation in the tissue and poor tissue quality.

8 Collect one 20µm section in a superfrost plus glass slide for H&E staining and histological scoring (the third parameter for evaluating human tissue quality. Please see

the histological scoring protocol published by the group of Drs Stephanie Irene Shiers and Ted Price **dx.doi.org/10.17504/protocols.io.kqdg32qr1v25/v1).**

9 Collect ten 20µm sections in laser capture microdissection (LCM) slides.

10 Repeat steps 7-9 two more times to collect a total of three sections for RNA extraction, three sections for histological score, and twenty sections for LCM.

11 Collect one section, where the three TG branches are visible, using 10x Xenium collection slides for 10x Xenium experiments following the manufacturer's protocol.

12 Collect one section, where the three TG branches are visible, using a superfrost plus glass slide, for 10x Visium experiments following the manufacturer's protocol.

13 Collect three more 100µm sections for single-nuclei isolation as in step 6.

14 Store sections and any remaining tissue at -80ºC until further use.

15 Process the sections collected for RNA extraction using the Qiagen RNeasy Mini kit following the manufacturer's protocol.

16 RNA concentration is measured with Qubit™ RNA High Sensitivity (HS) Assay Kit, and RIN value is measured using the Agilent 4200 Tapestation System.

Hematoxylin and Eosin Staining.

17 Remove slide(s) from the -80°C freezer or the -20°C cryostat and transfer to the lab bench over dry ice.

18 Place slides in a 37°C incubator for 1 minute to thaw, and then immediately transfer the slides to a dish containing 10% formalin for fixation, 15 minutes at room temperature in a fume hood.

19 Dehydrate sections in ethanol by placing the slides in 50% ethanol for 1 minute, 70% ethanol for 1 minute, 100% ethanol for 1 minute, and another 100% ethanol for 1 minute.

20 Air dry slides briefly. Ethanol should evaporate within a few minutes.

21 Cover sections on each slide with 500 µL of isopropanol and incubate for 1 minute at room temperature.

22 Discard the reagent by holding the slide at an angle with the bottom edge touching a laboratory wipe.

23 Air dry the slide for 2-3 minutes.

24 Cover sections on each slide with 1 mL of Hematoxylin and incubate for 7 minutes at room temperature.

25 During the incubation, prepare the eosin mix, which consists of 100 µL of Eosin-Y solution and 900 µL Tris-Acetic Acid Buffer (0.45M, pH 6.0). 1mL of eosin mix is needed per slide.

25.1 Tris Acetic Acid Buffer (0.45M, pH 6.0) can be prepared as a stock and stored at room temperature. To prepare, dissolve 11g Tris base in 100mL nuclease-free water. Adjust pH to 6.0 using 100% Acetic Acid. Bring volume to 200mL with nuclease-free water.

26 Discard the reagent by holding the slide at an angle with the bottom edge touching a laboratory wipe.

27 Use a squirt bottle containing Milli-Q water and dispense a stream of water across the slide while holding it to remove any residual hematoxylin from the slide and sections.

28 Remove the excess water from the slide by holding the slide at an angle with the bottom edge touching a laboratory wipe.

29 Cover sections on each slide with 1 mL of Bluing Buffer and incubate for 2 minutes at room temperature.

30 Discard the reagent by holding the slide at an angle with the bottom edge touching a laboratory wipe.

31 Use a squirt bottle containing Milli-Q water and dispense a stream of water across the slide while holding it to remove any residual Bluing Buffer from the slide and sections.

32 Remove the excess water from the slide by holding the slide at an angle with the bottom edge touching a laboratory wipe.

33 Cover sections on each slide with 1 mL of pre-made eosin mix (prepared in step 25) and incubate for 1 minute at room temperature.

34 Discard the reagent by holding the slide at an angle with the bottom edge touching a laboratory wipe.

35 Use a squirt bottle containing Milli-Q water and dispense a stream of water across the slide while holding it to remove any residual eosin mix from the slide and sections.

36 Remove the excess water from the slide by holding the slide at an angle with the bottom edge touching a laboratory wipe.

37 Air dry the slides overnight. Then, apply Cytoseal 60 mounting medium and a coverslip.

38 Image slides on APERIO VERSA 8 slide scanner at 10X magnification (numerical aperture 0.75) using the Brightfield imaging feature. Raw image files (SVS) files are saved to a protected data server. An example is shown in **Fig. 3**.

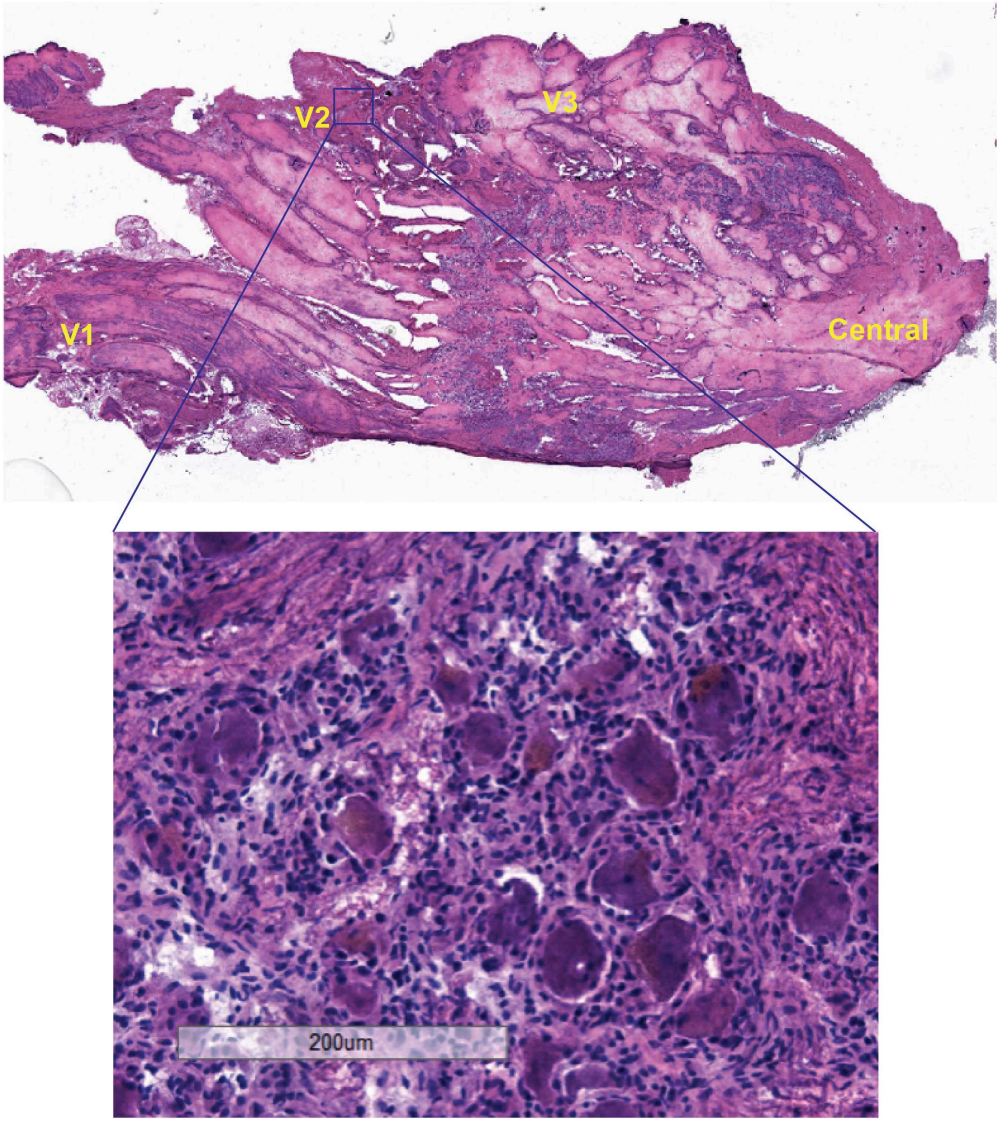

Fig 3. An example of a human TG section stained with H&E, with a section of the tissue magnified.

39 Determine histological score using the guide in **dx.doi.org/10.17504/protocols.io.kqdg32qr1v25/v1**.

Decontamination and waste disposal procedures.

40 All human tissues are BSL-II reagents. Reusable dissection tools and materials in contact with human tissues must be decontaminated with a 10% sodium hypochlorite solution, rinsed and cleaned, and autoclaved before reuse. Cryostat machine must be thoroughly decontaminated and wiped with a 10% sodium hypochlorite solution and then with 70% ethanol. Liquid waste from human tissues is collected in a 1 L glass bottle initially

> containing 100 mL of 10N NaOH. Once filled, liquid waste is discarded through the EHRS chemical waste stream. Sharp and non-sharp disposable materials in contact with human tissues must be immersed in a 20% sodium hypochlorite solution overnight and then properly discarded through the EHRS biohazard waste stream.

Acknowledgements

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