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## Patch-seq of cultured human dorsal root ganglion neurons

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PRECISION Human Pain ...



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**Protocol status:** Working

**We use this protocol and it's working**

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## Abstract

This protocol describes the process of Patch-seq of cultured human dorsal root ganglion neurons for combinatorial electrophysiological and molecular profiling of human sensory neurons.

## Materials

Sample collection tube (0.5 ml with o-ring seal screw, Pipette.com, T334-2SPR)

Centrifugal filter unit (0.22 um, Millipore, UFC30GV0S)

Nuclease-free gel loading tip (Fisher Scientific, 05-408-151)

1000 ul Nuclease-free pipet tips (Thermo Scientific, AM12660)

200 ul Nuclease-free pipet tips (Thermo Scientific, AM12650)

20 ul Nuclease-free pipet tips (Thermo Scientific, AM12645)

10 ul Nuclease-free pipet tips (Thermo Scientific, AM12635)

Takara RNase Inhibitor (Takara Bio, 634891)

RT-PCR grade water (Invitrogen, 9935G)

Nuclease-free water (Thermo Scientific, 10977023)

10X Lysis Buffer (Takara Bio, 634891)

Patch-seq internal solution:

- Adenosine 5'-triphosphate disodium salt hydrate (Millipore Sigma, A2383-1G)
- Guanosine 5'-triphosphate sodium salt hydrate (Millipore Sigma, G8877-10MG)
- Phosphocreatine disodium salt hydrate (Millipore Sigma, P7936-10MG)
- Biocytin (optional, hellobio, HB5035)
- HEPES (Sigma-Aldrich, H4034-500G)
- Ethylene glycol-bis(2-amino-ethylether)-N,N,N',N'-tetraacetic acid (Sigma-Aldrich, E4378-25G)
- Potassium gluconate (Sigma-Aldrich, P1847-100G)
- Potassium chloride (Sigma-Aldrich, P9333-5006)

Thin-walled borosilicate glass (Sutter, BF150-110-10HP)

Glycogen (Thermo Scientific, R0551)

Rnase inhibitor (Sigma-Aldrich, 3335402001)

Patch-seq library preparation:

SMART-Seq v4 Ultra Low Input RNA Kit (Takara, 634894)

Nextera XT DNA Library Preparation Kit (Illumina, FC-131-1096)

Illumina DNA/RNA UD Indexes Sets A-D (Illumina, 20091654, 20091656, 20091658, 20091660)

## Before start

- **For human tissue procurement and culture of human dorsal root ganlion cells, see our published protocol:**  
<https://doi.org/10.1038/nprot.2016.111>

- **Make Patch-seq internal solution:**

1. Wipe out the surface with RNase Zap.
2. Rinse pH meter with nuclease-free water.
3. Clean weigh bowl and spatular with RNase Zap and nuclease-free water.
4. Make the internal solution stock:

|  | A                          | B                 | C          | D           | E           | F      |
|--|----------------------------|-------------------|------------|-------------|-------------|--------|
|  |                            | Recipe conc. (mM) | MW (g/mol) | Weight (mg) | Stock conc. | Volume |
|  | K-gluconate                | 110               | 234.25     | 1288.38     |             |        |
|  | KCl                        | 4                 | 74.55      | 14.91       |             |        |
|  | HEPES                      | 10                | 238.3      | 119.15      |             |        |
|  | ATP-Na2 (-20 C)            | 1                 | 551.1      | 27.56       |             |        |
|  | GTP-Na (-20 C)             | 0.3               | 546.17     | 8.19        |             |        |
|  | Na-phosphocreatine (-20 C) | 10                | 255.08     | 127.54      |             |        |
|  | EGTA                       | 0.2               |            |             | 100 mM      | 100 ul |
|  | Glycogen                   | 20 ug/ml          |            |             | 20 mg/ml    | 50 ul  |
|  | Nuclease-free water        |                   |            |             |             | 40 ml  |

5. Adjust pH of the internal solution stock to 7.3 with 10N KOH.
6. Measure and adjust osmolarity to ~250 by adding nuclease-free water.
7. Filter the stock and make 10 ml aliquots into 15 ml conical tubes, and store the internal solution stocks at -80C.

8. Make the working internal solution:

|  | A | B                 | C          | D           | E           | F      |
|--|---|-------------------|------------|-------------|-------------|--------|
|  |   | Recipe conc. (mM) | MW (g/mol) | Weight (mg) | Stock conc. | Volume |
|  |   |                   |            |             |             |        |

|  | A                       | B         | C      | D    | E       | F       |
|--|-------------------------|-----------|--------|------|---------|---------|
|  |                         |           |        |      |         | /weight |
|  | Internal solution stock |           |        |      |         | 10 ml   |
|  | RNaseIn                 | 0.25 u/ul |        |      | 40 u/ul | 62.5 ul |
|  | Biocytin (optional)     | 3mg/ml    | powder | 13.7 | 41mg    | 41 mg   |

9. Transfer 3 ml to a new tube. Measure osmolarity and pH.

Note: Best osmolarity should range between 285-300, and pH between 7.2-7.4.

10. Using the remaining working internal solution, make 50 ul aliquots in PCR tubes. Store at -80C.

■ **To minimize contamination, make aliquots of the following reagents in nuclease-free tubes:**

1. Nuclease-free water for rinsing the electrode, 1 ml aliquots, store at -20C
2. RT-PCR grade water for sample preparation, 150 ul aliquots, store at -20C
3. 10X lysis buffer, 19 ul aliquots, store at 4C

■ **Before recording, check that the 50 MΩ feedback resistor is used for current clamp. This will allow you to inject higher currents needed for suprathreshold stimulation in hDRG.**

1. Click the yellow wrench on the Multiclamp 700B command window
2. Go to the "Gains" tab and click 50 MΩ option in the "Current clamp, Feedback Resistor" window (see right).
3. **Note:** Waveform tab in "edit protocol" will now be in nA increments. Make sure you switch this back before recording from mouse tissue!
4. Set primary and secondary output gains in the "Multiclamp 700B" window both to 1
5. Make sure telegraphed inputs are "IN 0" for channel #0 and "IN 1" for channel #1 – this will help with automated analysis across users.



Options ✕

General | Gains | Auto | Audio | Quick Select | Advanced | About |

☒ Channel 1 ☐ Channel 2

Voltage Clamp

| Feedback Resistor                               | Experiment Type | Range        |
|-------------------------------------------------|-----------------|--------------|
| <input type="radio"/> 50 M $\Omega$             | Whole Cell      | 1 - 200 nA   |
| <input checked="" type="radio"/> 500 M $\Omega$ | Whole Cell      | 0.1 - 20 nA  |
| <input type="radio"/> 5 G $\Omega$              | Patch           | 10 - 2000 pA |
| <input type="radio"/> 50 G $\Omega$             | Patch           | 0.2 - 200 pA |

External Command Sensitivity

☐ OFF

☒ 20 mV/V

☐ 100 mV/V

Current Clamp

| Feedback Resistor                              | Experiment Type | Max. Current |
|------------------------------------------------|-----------------|--------------|
| <input checked="" type="radio"/> 50 M $\Omega$ | Whole Cell      | 200 nA       |
| <input type="radio"/> 500 M $\Omega$           | Whole Cell      | 20 nA        |
| <input type="radio"/> 5 G $\Omega$             | Whole Cell      | 2 nA         |

External Command Sensitivity

☐ OFF

☒ 4 nA/V

☐ 20 nA/V

Close Help

## Patch-seq preparation

- 1 Clean up working area with 70% ethanol followed by RNase away.
- 2 Collect the following items:
  1. A bucket of ice.
  2. Patch pipettes (1-3 MOhms).
  3. Sample collection tube.
  4. Centrifugal filter (patch-seq filters only).
  5. Nuclease-free gel loading tip.
  6. Nuclease-free pipet tips.
- 3 Prepare the following reagents and store them on ice:
  1. Collect one aliquot of 1 ml nuclease-free water from -20C for rinsing the electrode.
  2. Collect one aliquot of 150 ul RT-PCR grade water from -20C (sufficient for ~15 cells).
  3. Collect one aliquot of 19 ul 10X lysis buffer from 4C.
  4. Collect Takara RNase Inhibitor from -20C.
  5. Prepare 20 ul of 10X Reaction Buffer (sufficient for ~15 cells) by mixing up 19 ul of 10X Lysis Buffer and 1 ul of Takara RNase Inhibitor.
  6. Collect one aliquot of internal solution from -80C.
  7. Load the internal solution into a nuclease-free centrifugal filter and centrifuge at max speed for 1 min.
- 4 For each aliquot of internal solution to be used, prepare one negative control sample in a sample collection tube:
  - 4.1 Mix 2 ul of filtered internal solution, 8 ul of RT-PCR grade water, and 1 ul of 10X Reaction Buffer.
  - 4.2 Label the tube with your initials, IS (internal solution), and experimental date (e.g JY-IS, 220804) with permanent markers.
  - 4.3 Store the negative control sample in the -20C sample collection box.

## Recording

- 5 Wear gloves sprayed with RNase away while handling pipettes and extracted samples.

- 6 Rinse the electrode with nuclease-free water before and between recording experiments.
- 7 Using the nuclease-free gel loading tip, transfer 2 ul of internal solution to the recording pipette. Use a new tip for each recording to minimize contamination.
- 8 Write down pipette tip resistance upon entering the bath then follow protocols below:
  1. Seal resistance must be  $>1\text{G}\Omega$
  2. After breaking in, series resistance ( $R_s$ ) in the V-clamp 1 tab must be stable and below  $20\text{ M}\Omega$  before continuing. Apply a very small amount of negative pressure to remove additional membrane, which should result in larger transients and lower and more stable  $R_s$  values.
  3. Before switching to current clamp, note the "Cp Fast" value from the Voltage-Clamp 1 tab. Reduce this by 10% and write this down, then enter that value in the "Pipette Capacitance Neutralization" in the Current-Clamp 1 tab.
  4. After switching to current clamp, check the "bridge balance" box, use "auto" function, then manually adjust as needed. Write down resistance value in the spreadsheet.
- 9 Before patching or after sealing but before breaking in, save an image of the DRG with the same sample ID number (for quantifying soma diameter)
- 10 Record resting membrane potential (record within 2 minutes of achieving whole-cell mode):
  - 10.1 Set current to 0 by clicking  $I=0$  in Multiclamp command window
  - 10.2 Run "hDRG\_RMP" for 1 sweep (10 seconds)
  - 10.3 Go back to injecting current to keep  $V_m \sim -60\text{ mV}$  for remaining protocols
- 11 Record 1 second rheostep to determine threshold,  $R_{in}$ , and sag:
  - 11.1 Run "hDRG\_rheo\_1s" until cell fires APs to 3 consecutive sweeps
    - $-0.25 \Delta 0.05\text{ nA}$ :  $-250\text{ pA}$  step that goes up in  $50\text{ pA}$  increments
  - 11.2 Note sweep number of first AP and write down rheobase (need for Step 12)
    - Rheobase (nA) = (sweep number - 6)  $\times$  0.05



12 Record suprathreshold response to 1-4x rheobase:

12.1 Open hDRG\_rheo\_4x protocol and go to "edit protocol"

12.2 In the waveform tab, enter rheobase from step 2 (in nA) in "First level (nA)" tab on the left and the same value in the "Delta level (nA)" tab. For example, if the cell fired to 0.5 nA above, enter 0.5 for both (see below).

| Epoch Description       | A    | B    | C    | D    | E    | F    | G    | H    | I    | J    | K    | L    | M    | N    | O    | P    |
|-------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Type                    | Step | Step | Step | Off  | Off  | Off  | Off  | Off  | Off  | Off  | Off  | Off  | Off  | Off  | Off  | Off  |
| Sample rate             | Fast | Fast | Fast | Fast | Fast | Fast | Fast | Fast | Fast | Fast | Fast | Fast | Fast | Fast | Fast | Fast |
| First level (nA)        | 0    | 0.5  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| Delta level (nA)        | 0    | 0.5  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| First duration (ms)     | 200  | 1000 | 200  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| Delta duration (ms)     | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| Digital bit pattern (#) | 1111 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 00   |
| Digital bit pattern #   | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 00   |
| Train rate (Hz)         | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| Pulse width (ms)        | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |

13 Record 1 second ramp to determine threshold to ramping stimulus:

13.1 Run "hDRG\_ramp\_1s" until cell fires APs to 3 consecutive sweeps

- $0\Delta 0.05$  nA: Ramp stimulus goes up in 50 pA increments

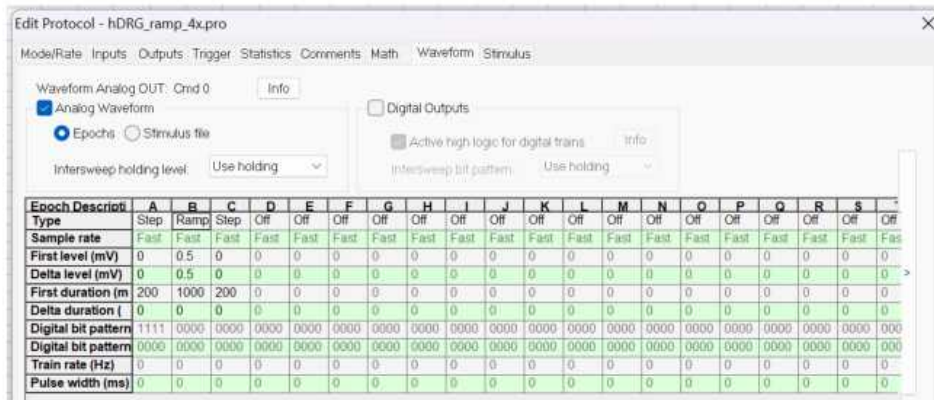
13.2 Note sweep number of first AP and write down ramp threshold (need for Step 14)

- Threshold (nA) = (sweep number - 1) x 0.05

14 Record suprathreshold response to 1-4x ramp threshold:

14.1 Open hDRG\_ramp\_4x protocol and go to “edit protocol”

14.2 In the waveform tab, enter ramp threshold from step 4 (in nA) in “First level (nA)” tab on the left and the same value in the “Delta level (nA)” tab. For example, if the cell fired to 0.5 nA above, enter 0.5 for both (see below).



(No explicit legend provided for this figure on the page.)

14.3 Run for 4 sweeps

15 Record AP waveform to short current step to determine kinetics

15.1 Run “hDRG\_shorthreo” until cell fires APs to 3 consecutive sweeps

- 0Δ0.1 nA: 5 ms depolarizing pulse in 100 pA increments

16 Extract cytoplasm with negative pressure and deposit contents in tube with lysis buffer  
\*ON ICE\* per patch-seq protocol (see details below)

17 Rinse wire with nuclease free water and repeat

## Cytosol extraction and processing

18 Extract the cytosol from the recorded cell by applying negative pressures.

- 18.1 Return to v-clamp mode to monitor holding current (I<sub>h</sub>) with seal test – write down starting value  
Using a 10 ml syringe, pull plunger back by 3-6 ml (write down value)
- 18.2 Close valve and maintain negative pressure for 3-5 minutes (start timer)
  - If I<sub>h</sub> drops to below -500 pA the seal may be compromised. Proceed immediately to the next step and write down time
- 18.3 Extraction should be confirmed by shrinking of cell size under the microscope.
- 18.4 After extracting write down I<sub>h</sub> value. Maintain negative pressure, then begin to slowly withdraw pipette in a diagonal direction (speed of 9 until completely out of frame and detached from cell, then change speed to 5)
- 19 Once the extraction is done, slowly retract the recording pipette.
- 20 Record whether the whole cell is lifted during pipette retraction.
- 21 Hook the recording pipette to an expulsive device (like a 10 ml syringe)
- 22 Put the tip of the recording pipet into a collection tube. Crack the pipet by pushing the tip against the bottom of the tube.
- 23 Once the tip is cracked open, release the internal solution to a 0.2 ml PCR tube by apply positive pressures.
- 24 Add 8 ul of RT-PCR grade water and 1 ul of 10X Reaction Buffer to the sample. Mix by gently tapping the tube a few times.
- 25 Tightly screw the cap to prevent evaporation.
- 26 Quick spin to collect sample at the bottom of the tube.
- 27 On the side of the tube, label with your initials, sample number, and experimental date (e.g JY-1, 220804) with permanent markers.

- 28 Store the sample in the -20C sample collection box, located in the freezer at the hallway of ephys rooms.
- 29 Repeat the recording and cytosol extraction steps for each Patch-seq sample.
- 30 Once all recording experiments are done for the day, transfer all samples to the -80C for storage.
- 31 Add the sample information to the sample spreadsheet.

## Patch-seq library preparation

- 32 On the day of library preparation, collect Patch-seq cytosol samples from -80C and store on dry ice.
- 33 Record all sample information.
- 34 Prepare three positive control samples using 50 pg of total RNA provided in the XXX kit, and three negative control samples using nuclease-free water as the template.
- 35 Prepare the cDNA library by following the user manual of SMART-Seq v4 Ultra Low Input RNA Kit.
- 36 After cDNA library construction, take 2 ul of the library and measure the cDNA concentration using TapeStation.
- 37 Identify high-quality samples containing sufficient cDNA for sequencing.
- 38 Proceed to sequencing library preparation by following the user manual of Nextera XT DNA Library Preparation Kit.
- 39 After sequencing library construction, take 2 ul of the library and measure the concentration using TapeStation.
- 40 Sequence the samples targeting at least 200k reads per sample.



## Protocol references

Human sensory neurons exhibit cell-type-specific, pain-associated differences in intrinsic excitability and expression of *SCN9A* and *SCN10A*. Jiwon Yi, Lite Yang, Allie J. Widman, Alexa Toliver, Zachariah Bertels, John Smith Del Rosario, Richard A. Slivicki, Maria Payne, Adam J. Dourson, Jun-Nan Li, Rakesh Kumar, Prashant Gupta, Juliet M. Mwirigi, Alexander Chameessian, John Lemen, Bryan A. Copits, Robert W. Gereau IV. bioRxiv 2025.03.25.645367; doi: <https://doi.org/10.1101/2025.03.25.645367>

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