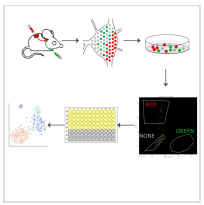

Jul 06, 2022

Ganglia dissociation and single-cell sorting

In 1 collection

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ABSTRACT

Protocol to dissociate freshly harvested stellate ganglia into single neurons and to sort them based on fluorescence

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COLLECTIONS

Single cell RNA sequencing of retrogradely labeled mouse stellate ganglion neuron

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PARENT PROTOCOLS

Part of collection

Single cell RNA sequencing of retrogradely labeled mouse stellate ganglion neuron

MATERIALS TEXT

A	B	C
Instrument	**Provider**	**Cat. no/SKU**
40 µm Cell Strainer	Corning	352340

A	B	C
Reagent	**Provider**	**Cat. no/SKU**
Earle's Balnced Salt Solution	Thermofisher Scientific	14155063
Papain	Worthington Biochemical	LK003178
Collagenase/Dispase	Roche	11097113001
D-trehalose	Sigma-Aldrich	90208
AP-V	Tocris	0105
kynurenic acid	Sigma-Aldrich	K-3375
DNAse	Worthington Biochemical	LK003170
Fetal Bovine Serum, certified, heat inactivated	Fisher Scientific	10-082-147
DMEM	Fisher Scientific	11995073
Bovine Serum Albumin	Sigma	A2153-50G
RNAse-Free Water	FIsherScientific	BP5611
Sytox blue	Thermofisher Scientific	S34857

BEFORE STARTING

We modified the dissociation methods from protocols previously published (Saxena et al., 2012, https://doi.org/10.2144/0000113878; Campbell et al., 2017, https://doi.org/10.1038/nn.4495)

Cleaning the tissue

1 Place a freshly harvested Stellate Ganglion (SG) into an ice-chilled Earle's Balanced Salt Solution (EBSS) that was equilibrated to 95% CO2/ 5% O2 for 1 hour

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2 Carefully remove fat and connective tissue from the SG, and then transfer the SG to a new dish containing cold equilibrated EBSS

Digestion

3 Cut the SG into 3-4 pieces using a small spring scissor, and placed all pieces gently into a low-bind 1.7 ml microcentrifuge tube containing 1,667 µl pre-heated (37°C) digestion solution for 1.5 h with constant agitation
Digestion solution is composed of:
- 1034 µl Papain solution in EBSS
- 200 µl Collagenase/Dispase solution (20 mg/ml in EBSS)
- 167 µl D-trehalose solution (50% in RNase-free water)
- 3 µl AP-V solution (25mM in EBSS)
- 13 µl kynurenic acid solution (100mM in EBSS)
- 250 µl DNAse (vial D2 in EBSS)

4 During the digestion, prepare a digestion-stop solution:
- 1,050 µl 50% D-trehalose solution
- 11 µl 25 mM AP-V solution
- 44 µl of the 100 mM kynurenic acid,
- 250 µl of the DNAse solution
- 250 µl fetal bovine serum (FBS)

5 Prepare medium solution containing
- 1,050 µl 50% trehalose solution
- 8 µl the 25 mM AP-V
- 19ul 100 mM kynurenic acid
- 107 µl FBS in 9,450 µl D-MEM/F12

6 After the digestion, transfer half of the digestion solution with the tissue to a fresh low-bind microcentrifuge tube, and fill each tube with the digestion-stop solution

Dissociation

7 Invert the tubes a few times gently and centrifuge them at 300g at 4C for 5 min

8 Discard the supernatant and gently resuspend the pellet with 500 µl of the digestion-stop solution described above

9 We combined the contents into a single tube and triturated the SG carefully with fire-polished

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glass Pasteur pipettes that were pre-coated with 0.5% BSA in RNase-free water for at least 1h at room temperature

10 We progressively decreased the diameter of the pipettes from 300-400 µm to 150 µm during the trituration process

11 The contents were then divided again into two tubes and washed with 1 ml of the medium solution

12 We inverted the tube gently 10 times and centrifuged it at 300 g for 5 min

13 The supernatant was discarded and the pellets were gently re-suspended with 200 µl of the medium solution

Staining and preparation for sorting

14 The suspension was filtered using a 40 µm cell strainer and collected in a 15-ml plastic tube. We used Sytox blue (1:1000) to stain dead cells

15 Keep the cell suspensions on ice until sorting

Sorting

16 Sort single cells into 96-well plates, We used FACSAria sorter with a square cuvette with a 130-µm nozzle at 12 PSI.
We sorted based on the the signal from Alexa 488, Alexa 555, or the lack of any signal

17 Centrifugate the plates at 200g for 2 min and immediately freeze them on dry ice

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