Adult mouse DRG dissociation for FACS, 060214

Masato Hoshi, MD, PhD,
Sanjay Jain Lab, Renal Division, Department of Medicine,
Washington University School of Medicine

Reagents;

- HBSS; Mediatech, #SC45000-452
- Papain; Worthington, #3124
- DMEM/F12; Life Technologies, #11330
- 0.05% Trypsin/0.02% EDTA; Sigma, #59417C or #T3924
- DNaseI; Sigma, #D5025
- Collagenase; Sigma, #C0130
- RNase inhibitor; RNaseOUT (Life Technologies, 40units/ul) or equal
- 7-AAD; Life Technologies, #A1310 (powder, dissolve in DMSO at 1mg/ml)

Reference: modified from hu et al, J. neuro, 2002

Procedures;

1) Follow your approved animal protocol to euthanize the mouse and dissect the DRGs of interest.
2) Collect DRGs in 1ml of Hanks BSS solution (HBSS w/o Ca, Mg (Mediatech) + 10mM HEPES, filter sterilized) in 1.5ml tube on ice until all dissections are done.
3) Centrifuge at 500 xg for 5min at 4C and discard as much supernatant as possible by using a pipette.
4) Add 1ml of 0.05% Trypsin/ 0.02% EDTA (Sigma) with 200ug/ml of DNaseI (Sigma) to the tube and briefly vortex to mix. **(Alternative)** You can use 1ml of Papain solution (15units/ml of Papain in HBSS with HEPES) instead of Trypsin/EDTA/DNaseI solution (this method is routinely used for patch clamping experiments on isolated DRG sensory neurons at our institution).
5) Incubate at 37C for 20min.
6) Triturate with P-1000, then P-200 pipette to further dissociate the specimen (It may be hard to completely dissociate adult DRGs at this step).
7) Add 500ul of of DMEM/F12 + 10% FCS (filtered) and mix well by pipetting.
8) Centrifuge at 500 xg for 5min at 4C and discard supernatant by using a pipette.
9) Add 250ul of DMEM/F12 + 10% FCS (filtered), mix well by pipetting, and then add 750ul of collagenase solution (Sigma, dissolved in DMEM/F12 + 10%FCS (sterilized) at 2mg/ml, final concentration is 1.5mg/ml) with 200ug/ml of DNaseI (Sigma). Mix well by pipetting.
10) Incubate at 37C for 20min.
11) Triturate with P-1000, then P-200 pipette until you notice complete dissociation of the specimens.
12) Centrifuge at 500 xg for 5min at 4C and discard supernatant by using a pipette. Leave around 30ul to avoid disturbing the cell pellet.
13) Add 1000ul of DMEM/F12 + 10% FCS (filtered) and mix well by pipetting.
14) Perform cell dispersion by on a plate to remove debris and other unwanted cells/structures and increase the purity of neurons.
    a) Prepare four 35mm plastic dishes.

b) Put the DRG dissociates carefully in the first dish and wait for 2 min at room temperature.
c) Collect solution from the first dish very carefully to minimize disturbance to the cells that are weakly attached to the bottom of the first dish and transfer it to the second dish and wait for 2 minutes (In the mean time perform step (d) on the first dish.
d) Add 450ul of DMEM/F12 + 10% FCS (filtered) to the first dish, wash a couple of times to collect the attached cells from the first dish. Transfer the wash containing viable cells in a new 1.5ml tube.
e) Repeat c) through d) to the second dish & third dish. Check if the fourth dish has cells by microscope, if needed.
f) You will have near 1.5ml of cell suspension (the collection from the three dishes) in a new 1.5ml tube.

15) Centrifuge the new collection tube at 500 xg for 5min at 4C and discard supernatant by using a pipette. Leave around 30ul to avoid disturbing the cell pellet.
16) Add 500ul of PBS + 5% FCS (filtered) with 0.1units/ul of RNase inhibitor (Life Technologies, 40units/ul, 25ul to 10ml PBS/FCS solution) and mix well by pipetting.
17) Centrifuge at 500 xg for 5min at 4C and discard supernatant by using a pipette.
18) Repeat this washing step twice more with PBS/FCS/RNase inhibitor solution. Total three times. In the last wash, the cells get dispersed very quickly by a couple of pipetting.
19) After discarding as much supernatant as possible, add 300ul (or appropriate volume for your FACS) of PBS + 5% FCS (filtered) with 0.1units/ul of RNase inhibitor and mix well by pipetting.
20) Filter the dissociated cells with 40um pore size cell strainer (BD, #352340) and collect the cells into a FACS tube (BD, #352063).
21) Add 7-AAD at 1ug/ml concentration, if needed, and do sorting. 7-AAD is added 10min before sorting. We have had good success using MoFlo, Sony or AriaII, at 100 um nozzle size, and 30psi.
22) Sorted cells are collected in 750ul of TRIzol-LS (Life Technologies) for RNA extraction and saved in -80C until used.