E12.5 & E14.5 mouse DRG dissociation for FACS, 053114

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Reagents;

- Hibernate-E; Life Technologies, #A12476-01
- DMEM/F12; Life Technologies, #11330
- 0.05% Trypsin/0.02% EDTA; Sigma, #59417C or #T3924
- DNaseI; Sigma, #D5025
- RNase inhibitor; RNaseOUT (Life Technologies, #10777, 40units/ul) or equal
- 7-AAD; Life Technologies, #A1310 (powder, dissolve in DMSO at 1mg/ml)

Reference: modified from (Sasaki et al, J. neuro 2009).

Procedures;

1) Euthanize a pregnant mouse following your animal protocol approval and extract embryos/fetuses from the uterus.
2) Save the embryos/fetuses in a 24-well plate with 1ml of Hibernate-E medium (Life Technologies) in each well of the plate on ice.
3) Take one embryo in 10cm plastic plate with dissection medium (DMEM/F12 (Life Technologies) or Hibernate-E (30ml)) and dissect the DRGs from embryos. (T11 to S1, total 20 DRGs from each embryo; or as needed for your study)
   (*when you use DMEM/F12, the medium should be put in a CO_2 incubator (cell culture, 5%CO2 incubator at 37C is fine) for more than 2 hours to keep the pH balanced (gauge by the color, prefer orange).
4) Collect DRGs in a 1.5ml tube on ice with dissection medium in a pipette tip. Keep the tube on ice until all dissections are done.
5) Centrifuge at 500 xg for 5min at 4C and discard as much supernatant as possible by using P-200 pipette.
6) Add 500ul of 0.05% Trypsin/ 0.02% EDTA (Sigma) with 200ug/ml of DNaseI (Sigma) to the tube and briefly vortex to mix.
7) Incubate it at 37C for 10min.
8) Triturate with P-1000, then P-200 pipette till you notice complete dissociation of the specimen. Add 500ul of DMEM/F12 + 10% FCS (filtered) and mix by pipetting.
9) Centrifuge at 500 xg for 5min at 4C and discard supernatant by using a pipette. Leave around 30ul to avoid disturbing the cell pellet.
10) 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 by pipetting.
11) Centrifuge at 500 xg for 5min at 4C and discard supernatant by using a pipette.
12) 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 with only few rounds of pipetting.
13) After discarding supernatant, 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.

14) Filter the dissociated cells with 40um pore size cell strainer (BD, #352340) and collect the cells into a FACS tube (BD, #352063).
15) 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.
16) Sorted cells are collected in 750ul of TRIzol-LS (Life Technologies, #10296-010) for RNA extraction and saved in -80C until use.