JIN LAB
Cell dissociation for 10X scRNAseq v3

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NOTES

This protocol is designed for dissociation of prenatal and postnatal (<P10) mouse brains. The key to dissociation is to move fast and keep samples cold to preserve high-quality neurons for scRNAseq. From live animals to loading chip should be within 3 hours for optimal cell health.

METHODS

Preparation

1. Coat collection tube with PBS + 10% BSA the day before.
2. Prepare dissociation kit: Worthington Papain Dissociation System (Worthington DBA, cat. no. LK003150).
    a. Add 5 ml of EBSS (vial 1) to a papain vial (vial 2). Place vial 2 in a 37°C water bath for ten minutes or until the papain is completely dissolved and the solution appears clear.
    b. Add 500 µls of EBSS (vial 1) to a DNase vial (vial 3). Pipette mix gently -- DNase is sensitive to shear denaturation.
    c. Check if Ovomucoid protease inhibitor with bovine serum albumin (vial 4) is reconstituted. If not, add 32ml of EBSS (vial 1) to it and mix gently.
3. Prepare dissection buffer (keep cold).

	Volume
Hibernate medium (Fisher Scientific, NC0442869)	50 ml
B27 supplement (50X plus retinoic acid)	1 ml
Trehalose (Sigma-Aldrich T9531-100G)*	1 ml

*Trehalose stock (store in -20C):

Add DPBS to 10g Trehalose for a final volume 20ml. Shake well to dissolve and aliquot to 1ml.

4. Prepare sorting buffer (keep cold).

	Volume
Dissection buffer	10 ml
Heat-inactivated FBS (ThermoFisher, # 16000069)	0.5 ml
Dyes needed for sorting	According to manual for dye

5. Cool down FACS machine to 4C (at least 30 min before start).

Dissection on ice ~30min

1. Anesthetize the mouse with isoflurane or ice and then sacrifice by cervical dislocation.
2. Cut open the skin and skull through the midline.
3. When the brain is still in the head, use a PBS-immersed Kimwipe to wipe off blood vessels and meninges.
4. Microdissect the cortex (remove meninges and other brain regions) in dissection buffer under a dissection scope.
5. Rinse the cortex pieces with dissection buffer.

Whole Cell Dissociation ~40min

6. Transfer the tissue into a 6cm dish with 5 ml of papain solution (vial 2) and add 250 µl of DNase solution (vial 3) (under the TC hood).
7. Cut the tissue into small pieces with a blade.
8. Incubate for 30 minutes on a cell culture incubator rocker.
   Incubation time varies on age of animals, older tissue needs longer time. 30min is good for animals < P7.
9. Trituate the tissue on the dish with a 10ml pipette, mix 10x in the middle of incubation (at ~15min, pause timer).
10. Add 2.7 ml of EBSS, 3 ml of reconstituted albumin-ovomucoid inhibitor solution (vial 4), plus 250 µl DNAse (vial 3), mix but avoid bubbles.
11. Collect digested tissues into the 15 ml tube.
12. Triturate the mixture with a 10 ml pipette (20 times up and down). Allow any pieces of undissociated tissue remaining after trituration to settle to the bottom of the tube.
13. Carefully transfer the cloudy cell suspension to a 15 ml tube. Be careful to avoid including any pieces of undissociated tissue.
14. Use a P1000 to triturate the rest (~1ml of cells), let it settle down.
15. Carefully transfer the cloudy cell suspension to the 15 ml tube with supernatant.
16. Centrifuge at 300g for 5 minutes at 4C.
17. Resuspend with 5ml of dissection buffer (to remove debris). Centrifuge at 200g for 7 minutes at 4C.
    If mice are >P7 another wash and spin down will be helpful.
18. Resuspend in 1 ml sorting buffer.

FACS ~1 h

19. Rinse the filter with sorting buffer and filter cells at FACS core.
    Dilute sample with sorting buffer if needed (e.g. sorting efficiency is very low).
20. FACS sorting
    1. Set gate for cells first, then dead cells, then fluorescent protein markers.
    2. Use at least 500ul medium to collect cells (dissection buffer or PBS+0.04% BSA).
21. Set the centrifuge (including the bucket) to 4C in advance for the next step.

Post FACS ~10 min

22. Spin down in a swing bucket 4C centrifuge, 500g 5min.
23. Aspirate supernatant immediately after spinning down.

    Remember the orientation of tubes when centrifuging
24. Resuspend in <35uL/channel (for 10X Genomics) PBS+0.04%BSA. Proceed with 10x Genomics GEM chip loading and cDNA synthesis.