Contents:

Single-Cell RNA-seq Protocol:

Dissection of Samples:

Dissections are performed based upon Bayer and Altman atlas of 2nd trimester development, photodocumentation of dissections as appropriate

Dissociation:

Dissociations are performed with papain using the Worthington Papain Dissociation System as per manufacturer's protocol. Briefly, sample is submerged in oxygenated papain with added DNAse (10X) and incubated at 37 deg C until enzymatic dissociation is observed (cloudy solution). This is usually 30 minutes to an hour, and every 15-20 minutes the sample is shaken to mix the sample and the papain.

The dissociated sample is manually triturated with a glass Pasteur pipette 10-20X, until most chunks are broken up. The sample is spun, resuspended in PBS + 0.05% BSA and filtered with polystyrene tube and cell strainer cap. The cells are counted, and if enough cells are present, cells are diluted to 1,700 cells/uL. 15.7 uL of this mixture and 18.1 uL of water is used to load the 10X.

10X Capture:

10X capture and library preparation with the above mixture is performed per manufacture instructions for the v2 3 prime Chromium kit.

Sequencing:

Sequencing is performed on the Novaseq S2 as per manufacturer recommendations.

Single-nuclei RNA-sequencing

Reagents preparation:

First, prepare stock solutions (see below) using DEPC-treated water. You can buy nuclease-free Tris-HCl solution.

Then, prepare Lysis and Sucrose solutions.

One centrifuge spin is 2.5 hours and fits 6 samples.

Book the centrifuge in advance (need Beckman Optima L-90K Ultracentrifuge that goes to 100,000 g).

Lysis buffer (50 mL):

Substance	**Stock Concentration**	**Amount**	**Final concentration**
Sucrose	-	5.47g	0.32M
$CaCl_2$	1M	250uL	3mM
$Mg(Ac)_2$	1M	150uL	3mM
EDTA	0.5M	10uL	0.1mM
Tris-HCl	1M	500uL	10mM
DTT	3M	17uL	1mM
Triton X100	100%	50uL	0.1%

Bring to 50mL with DEPC-treated water.

Sucrose solution (50 mL):

Substance	**Stock Concentration**	**Amount**	**Final concentration**
Sucrose	-	30.78g	1.8M
$Mg(Ac)_2$	1M	150uL	3mM
DTT	3M	17uL	1mM
Tris-HCl	1M	500uL	10mM

Bring to 50mL with DEPC-treated water. Make sure sucrose is dissolved (need to heat ~70 C and mix well).

Keep solutions at 4 C.

Snap-frozen brain tissue processing

1) Mount snap-frozen tissue to section on cryostat (do not embed completely in Tissue-Tek, just mount).

2) First, make thin (16-18 um) sections and mount on slides for histology/IHC/in situ. Also, make note of sample anatomy (if sectioning cortex make sure you are making coronal sections from pial surface to white matter and capturing all neuronal layers).

3) Switch to "trim" (100 um) to make thick sections and collect for RNA extraction and nuclei isolation. Weigh two empty Eppendorf tubes and let them cool on dry ice. Make 2-3 think sections (sections will curl so use a brush) and collect in the tube. Weigh again until you collect 20 mg of tissue. This is for total RNA isolation to measure RIN.

4) Repeat the procedure but this time collect 40 mg for nuclei isolation.

Note: Depending on the sample and your goals sometimes you want to trim off extra white matter to collect mostly grey matter. You can use a scalpel blade to trim extra tissue off the sections.

5) Store sectioned tissue at -80.

6) Use 20 mg of tissue to extract total RNA and run Bioanalyzer to measure RIN.

7) For single-nuclei RNA-seq, only use samples with RIN>6.5.

Nuclei isolation:

1) Clean bench and pipettes with RNAse Zap.

2) Use sectioned or finely chopped tissue (20-40 mg).

3) Put lysis and sucrose solutions on ice.

4) Use glass dounce homogenizer (Thomas Scientific; Catalog # 3431D76; size A). Put douncer on ice, pipette 1mL of lysis in the douncer. Transfer tissue either using spatula or P1000 pipette with cut tip and additional lysis buffer. Bring total volume of lysis buffer in the douncer to 5mL.

5) Dounce tissue on ice with 10 strokes or until no chunks of tissue are visible.

6) Transfer homogenized tissue in lysis buffer into a labelled thick wall ultracentrifuge tube on ice (Beckman Coulter; 355631).

7) Carefully pipette 9 mL of Sucrose solution to the bottom of the tube containing Lysis buffer. Be careful not to introduce bubbles. You should see two clearly separated phases: sucrose on the bottom and cloudy homogenate on top.

8) When you are done with all samples weigh them and bring to the same weight by adding Lysis buffer.

9) Load the samples to SW28 rotor (needs to be swing bucket). If using less than 6 samples still put empty centrifuge tubes. Set speed to 24,400 RPM (107,163.6 RCF) for 2.5 hours at 4 C.

10) After the spin, transfer samples on ice and carefully remove the supernatant using a P200 tip cut at an angle and vacuum. Make sure not to touch the bottom (stick to the wall and tilt the tube), but remove all the liquid. Carefully pipette 200uL of DEPC water-based PBS on the bottom. Wait 20 min on ice and then resuspend (do not resuspend right away!). Do not centrifuge the nuclei suspension, use right away.

11) Filter twice using Miltenyi Pre-separation filters (30um). (130-041-407)

12) Count using manual hemocytometer (no Trypan Blue). Best to have nuclei suspension at 2,000 nuclei/uL (2 million/mL). For 10X, I usually go for 3,000-4,000 nuclei captured.

13) Use 14 cycles for both cDNA amplification and PCR during 10X prep.