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Nuclei Isolation Protocol Applicable to Adult Human Kidney Tissues

Abstract: Nuclei can be readily isolated from frozen tissues with a combination of chemical and physical treatments that can circumvent the non-uniform or incomplete dissociation of solid tissues into single cells. Furthermore, isolated nuclei can circumvent RNA degradation or technical artefacts (such as stress response) that can be triggered during whole cell dissociation methods. The data generated from single-nucleus (sn)RNA-seq, including identification of cell types and gene expression related to known or novel cell types identified, will be compared with other single-cell technologies in the KPMP to further define cellular identities and ultimately inform on the adult human kidney atlas.

Materials and Reagents

1. DAPI (5mg/ml, Invitrogen D1306)
2. RNAse Inhibitor (stock 40U/µl, Enzymatics Y9240L)
3. Dounce homogenizer (Sigma-Aldrich D8938)
4. 30 or 50-µm CellTrics filters
5. RNaseZAP (Sigma-Aldrich R2020-250ML)
6. Nuclei Isolation Reagents:
   a) NEB buffer

Final Conc	Stock	Volume (25ml)
20 mM Tris [pH 8]	1M	0.5ml
320 mM sucrose	1M	8ml
5 mM $CaCl_2$	1M	125µl
3 mM $MgAc_2$	1M	75µl
0.1 mM EDTA	0.5M	5µl
0.1% TritonX-100	10%	250µl
dH20	-	16ml

   b) PBSE

Final Conc	Stock	Volume (50ml)
1x PBS	10x 1M	5ml
1 mM EGTA	0.1M	50µl
dH2O		45ml

   c) PBSE + 1% BSA

   10ml 1xPBSE
   0.1g Fatty Acid Free (FAF)-BSA (Gemini)

Nuclei isolation protocol [1,2] modified for kidney tissue

1. Prepare Reagents and Tissue:
   a) Prepare NEB containing 1:1000 dilution of DAPI and 0.1% RNAse Inhibitor (RI), chill on ice
   b) Treat dounce with RNAseZap, rinse with sterile water (if possible: UV treat 15 minutes)
   c) Transfer vial containing tissue (e.g. 7x40 µm cryosections for ~5 mm^2 total, ranging 1-20 mm^3) to ice
   d) For sections in RNAlater, wash briefly with PBSE and immediately proceed to step 2a below
2. Isolate Nuclei
   a) Add 1 mL ice cold NEB/DAPI/RI buffer to tissue segments using p1000 and pipette up and down ~10 times, transfer to dounce homogenizer
   b) Cut the end off a p1000 tip to increase bore size using a sterile scalpel, then pipette sections up and down to disperse and dissolve O.C.T., ~20 times
   c) Using regular p1000 tip, pipette ~10x to further dissociate tissues into manageable sizes (note: tissue needs to be able to pass through a p1000 tip easily before proceeding), transfer to dounce homogenizer
   d) Gently dounce tissue on ice:
      - 5 strokes with pestle A
      - ~20 strokes with pestle B (minimize bubble formation)
   e) Transfer solution to a 15 mL tube.
   f) Wash dounce with 1 mL NEB/DAPI/RI buffer and add this into the same tube
   g) Incubate on ice 10 minutes
   h) Pass supernatant through 30-µm CellTrics filter to a new 15-mL conical tube
   i) Bring up to 10 ml with PBSE
   j) Pellet nuclei: 900g, 10 min at 4°C
   k) The nuclei can be used directly, or can be stored in RNAlater for use on a later date (snRNA-seq assays only):
      - Remove supernatant and resuspend pellet in 100 µl PBSE+1%BSA
      - Add 900 µL RNAlater, incubate at 4°C 1-2 hours then store at -20°C up to 1 month
      - To remove RNAlater, centrifuge nuclei at 4000g, 10 min at 4°C. Proceed to method (e.g. snDrop-seq)

   QA/QC:

   l) Count nuclei (e.g. BioRad T20 Cell Counter)
   m) Check nuclei integrity under fluorescent microscope using DAPI channel. Nuclei should appear <u>distinct</u>, have <u>rounded borders</u> and the majority occurring as <u>singlets</u>. High clumping rates would indicate damaged nuclei and would require re-filtering using 30-µm CellTrics filter or exclusion. At least <u>50,000 nuclei</u> are needed to proceed with snDrop-seq.

References

1. Lake et al. (2016). *Science*, doi:10.1126/science.aaf1204.
2. Lake et al. (2018). *Nature Biotechnology*, doi:10.1038/nbt.4038.