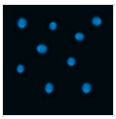

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Isolation of single nuclei from solid tissues

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1 Works for me dx.doi.org/10.17504/protocols.io.ufketkw

KPMP Human BioMolecular Atlas Program (HuBMAP) Method Development Community

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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. The isolation of nuclei can also circumvent RNA degradation or any introduction of technical artefacts (such as stress responses) that could be triggered during whole cell dissociation methods. Data generated from single-nucleus genomic assays permits discovery of molecular cell types that can be used to define the overall cellular makeup of a tissue or organ, and ultimately will inform upon adult human tissue atlases.

EXTERNAL LINK

http://genome-tech.ucsd.edu/ZhangLab/

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Lake, B.B. et al. A single-nucleus RNA-sequencing pipeline to decipher the molecular anatomy and pathophysiology of human kidneys. Nature Communications 10, 2832 (2019).

MATERIALS

NAME	CATALOG #	VENDOR
DAPI	D1306	Thermo Fisher Scientific
Dounce homogenizers	D8938-1SET	Sigma
RNAse Inhibitor	Y9240L	Enzymatics
CellTrics Filters (30um)	04-004-2326	Sysmex

STEPS MATERIALS

NAME	CATALOG #	VENDOR
DAPI	D3571	Invitrogen - Thermo Fisher
RNAse Inhibitor	Y9240L	Enzymatics
cOmplete™, Mini Protease Inhibitor Cocktail	11836153001	Roche
RNase Zap	R2020-250ML	Sigma Aldrich
RNAlater	AM7020	Thermo Fisher Scientific
RNAlater	AM7020	Thermo Fisher Scientific

Prepare Reagents and Tissue

1 Prepare NEB-complete (NEB containing 5 ug/ml DAPI and 0.04 U/ul RNAse Inhibitor)

chill on ice

Final Concentration	Stock	Volume (25 ml)
20 mM Tris [pH 8]	1M	0.5ml
320 mM sucrose	1M	8ml
5 mM CaCl2	1M	125µl
3 mM MgAc2	1M	75µl
0.1 mM EDTA	0.5M	5µl
0.1% TritonX-100	10%	250µl
dH20	-	16ml

NEB Base Solution Composition

For chromatin accessibility assays, include 1:100 dilution of cOmplete™ Protease Inhibitor Cocktail (stock one tablet in 0.5 ml H_2O)

DAPI
by Invitrogen - Thermo Fisher
Catalog #: D3571

RNAse Inhibitor
by Enzymatics
Catalog #: Y9240L

cOmplete™, Mini Protease Inhibitor Cocktail
by Roche
Catalog #: 11836153001

2 Treat dounce with RNAseZap, rinse with sterile water (if possible: UV treat 00:15:00)

RNase Zap
by Sigma Aldrich
Catalog #: R2020-250ML

3 Transfer vial containing tissue to ice.

For solid tissues (e.g. adult human kidney), 40 µm cryosections can be used with the number of sections dependendent on desired yield and the size and type of tissue. For kidney ~6 cubic mm will give ~150-200K nuclei.

4 For sections stored in a stabilizing solution (e.g. RNAlater), wash briefly with PBS and immediately proceed to Step 5 below

RNAlater
by Thermo Fisher Scientific
Catalog #: AM7020

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

PBSE Composition

Isolate Nuclei

5 Add 1 ml ice cold NEB buffer to tissue segments

6 Cut end off of a p1000 tip to increase bore size using a sterile scalpel, then pipette sections up and down to disperse and dissolve OCT, ~20 times

7 Using a regular p1000 tip, pipette ~10x to further dissociate tissues into manageable sizes.

tissue needs to be passable through a p1000 tip easily before proceeding

Then transfer to dounce homogenizer

8 Gently dounce tissue on ice:

5 strokes with pestle A

~20 strokes with pestle B (minimize bubble formation)

Increase number of pestle A strokes if the tissue appears too granular before proceeding with pestle B. Number of pestle B strokes used here is dependent on tissue toughness:
soft tissues use ~10-15 strokes
hard tissues use ~15-20 strokes

Avoid making bubbles

9 Transfer solution to a **15 ml** tube

10 Wash dounce with **1 ml** NEB-complete buffer and add this into the same tube

11 Incubate on ice **00:10:00**

12 Pass supernatant through 30 uM CellTrics filter to a new **15 ml** conical tube

Sysmex 04-004-2324
30 uM Celltrics Filter

13 Bring up to **10 ml** with PBSE

14 Pellet nuclei: **900 g**
00:10:00 at **4 °C**

snRNA-Seq methods: nuclei can be stored in RNAlater

15 Remove supernatant and resuspend pellet in **100 µl** - **1000 µl** PBS + 0.1% RNAse Inhibitor

Resuspension buffer and volume is dependent on downstream assays and nuclei concentration requirements. 1% BSA can be included here

16 **QA/QC:** Count nuclei (e.g. BioRad T20 Cell Counter)

Bio-Rad 1450011
Cell Counting Slides for TC10™/TC20™ Cell Counter, Dual-Chamber

17 **QA/QC:** Check nuclei integrity under fluorescent microscope using DAPI channel. Nuclei should appear distinct, have rounded borders and the majority occurring as singlets.

High clumping rates would indicate damaged nuclei and would require re-filtering using 30-µm CellTrics filter or exclusion from downstream analyses.

At least **50,000 nuclei** are needed to proceed with snDrop-seq

At least **10,000 nuclei** are needed to proceed with 10X 3' RNA v3

Isolate Nuclei

18 To use nuclei directly for single nucleus assays, proceed to method

To use nuclei on a later date, proceed to Step 19

snRNA-Seq methods: nuclei can be stored in RNAlater

19 Add **900 µl** RNAlater to **100 µl** nuclei in PBS, incubate at **4 °C** for **01:00:00** to **02:00:00**

then transfer to **-20 °C** for 1-2 months

RNAlater
by Thermo Fisher Scientific
Catalog #: AM7020

20 To remove RNAlater, centrifuge nuclei at 4000g, **00:10:00** at **4 °C** .

Remove solution and resuspend in associated nuclei resuspension buffer (assay dependent)