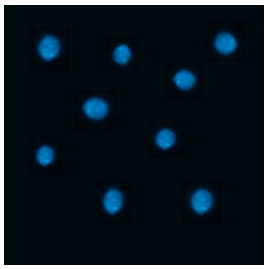
Oct 25, 2019

Isolation of single nuclei from solid tissues



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Human Cell Atlas Metho... KPMP 1 more workspace

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.ufketkw

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

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Manuscript citation:
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).

Protocol status: Working
We use this protocol and it's working

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Keywords: single nuclei, frozen tissues, genomic assays, isolation of single nuclei, solid tissues nuclei, isolation of nuclei, nucleus genomic assay, whole cell dissociation method, single cell, incomplete dissociation of solid tissue, adult human tissue atlas, human tissue atlas, nuclei, frozen tissue, discovery of molecular cell type, cell, solid tissue, rna, rna degradation, molecular cell type, tissue, overall cellular makeup, isolation

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.

Materials

MATERIALS

- DAPI **Thermo Fisher Scientific** **Catalog #**D1306
- Dounce homogenizers **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**D8938-1SET
- RNAse Inhibitor **Enzymatics** **Catalog #**Y9240L
- CellTrics Filters (30um) **Sysmex** **Catalog #**04-004-2326

STEP MATERIALS

- RNase Zap **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**R2020-250ML
- RNAlater **Thermo Fisher Scientific** **Catalog #**AM7020
- RNAse Inhibitor **Enzymatics** **Catalog #**Y9240L
- cOmplete™, Mini Protease Inhibitor Cocktail **Roche** **Catalog #**11836153001
- DAPI **Invitrogen - Thermo Fisher** **Catalog #**D3571
- RNAlater **Thermo Fisher Scientific** **Catalog #**AM7020

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Troubleshooting

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] 320 mM sucrose 5 mM CaCl2 3 mM MgAc2 0.1 mM EDTA 0.1% TritonX-100 dH20	1M 1M 1M 1M 0.5 M 10% -	0.5ml 8ml 125µl 75µl 5µl 250 µl 16ml

NEB Base Solution Composition

Note

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

DAPI **Invitrogen - Thermo Fisher Catalog #**D3571

RNAse Inhibitor **Enzymatics Catalog #**Y9240L

cOmplete™, Mini Protease Inhibitor Cocktail **Roche Catalog #**11836153001

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

RNase Zap **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**R2020-250ML

3 Transfer vial containing tissue to ice.

Note

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 **Thermo Fisher Scientific Catalog #**AM7020

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

> Note
>
> 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)

Note

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

Note

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

Equipment

new equipment	NAME
Sysmex	BRAND
04-004-2324	SKU
30 uM Celltrics Filter	SPECIFICATIONS

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

> Note
>
> 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)

Equipment

new equipment	NAME
Bio-Rad	BRAND
1450011	SKU
Cell Counting Slides for TC10™/TC20™ Cell Counter, Dual-Chamber	SPECIFICATIONS

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.

Note

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 **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)