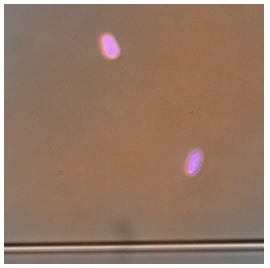
Sep 14, 2020 Version 2

Nuclei isolation from human intestinal biopsic tissue for single-cell genomic applications V.2



In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.bma2k2ge

Ran RZ Zhou[1], Oni Basu[1]

[1]University of Chicago

Human Cell Atlas Metho... Helmsley project_Basu lab

Ran RZ Zhou
University of Chicago

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.bma2k2ge

Protocol Citation: Ran RZ Zhou, Oni Basu 2020. Nuclei isolation from human intestinal biopsic tissue for single-cell genomic applications. **protocols.io https://dx.doi.org/10.17504/protocols.io.bma2k2ge**Version created by **Ran RZ Zhou**

Protocol status: In development
We are still developing and optimizing this protocol

Created: September 11, 2020

Last Modified: September 14, 2020

Protocol Integer ID: 42042

Keywords: gut, intestine, human, nuclei, single cell, nuclei isolation from human intestinal biopsic tissue, nuclei from human intestinal biopsy sample, human intestinal biopsy sample, human intestinal biopsic tissue, nuclei isolation, cell genomic applications this protocol, cell genomic application, single cell application, atac seq, rna, genomic application,

Disclaimer

The lysis buffer is formulated from the recipe in:
Drokhlyansky E, Smillie CS, Van Wittenberghe N, et al. The Human and Mouse Enteric Nervous System at Single-Cell Resolution [published online ahead of print, 2020 Aug 21].*Cell*. 2020;S0092-8674(20)30994-6. doi:10.1016/j.cell.2020.08.003

Abstract

This protocol provides an efficient method to isolate nuclei from human intestinal biopsy samples for single cell applications (RNA-seq or ATAC-seq).

Guidelines

The human intestinal tissue were obtained with patient consent and approval by the Institutional Review Board at the University of Chicago (IRB Number: 15573A). All the samples are processed for research use only.

Materials

MATERIALS

- 5M Sodium Chloride, 1000ml **Promega** **Catalog #**V4221
- BSA **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**#A8806
- RiboLock RNase Inhibitor (40 U/µL) **Thermo Fisher** **Catalog #**EO0381
- 0.5M EDTA **Fisher Scientific** **Catalog #**2482-500
- 10 x PBS no calsium no magnesiusm **Fisher Scientific** **Catalog #**BP399500
- UltraPure™ DNase/RNase-Free Distilled Water **Thermofisher** **Catalog #**10977023
- Red blood cell lysis buffer 10x **Miltenyi Biotec** **Catalog #**130-094-183
- Tween 20 **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**P7949
- 1M Tris-HCl pH 7.5 **Thermo Fisher Scientific** **Catalog #**15567027
- 1M CaCl2 **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**21115
- 1M MgCl2 **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**63069

Lysis buffer 10 ml (make fresh)
5 ml 2x ST buffer
300 ul 1% Tween-20
50 ul 2%BSA
10 ul RNAse Inhibitor stock
4.64 ml UltraPure water

2x ST buffer 10 ml (Store at 4 Celsius up to 1 month)
292 mM NaCl
20 mM Tris-HCl pH 7.5
2 mM CaCl2
42 mM MgCl2
Bring up to volume with UltraPure water

RBC lysis buffer 10 ml
1 ml Red blood cells lysis buffer 10x
9 ml ultra pure water

2% BSA 10 ml (Store at 4 Celsius up to 1 month)
0.2 g BSA
10 ml UltraPure water

1% Tween-20 10 ml (Store up to 1 month)
1ml 10% Tween-20
9 ml UltraPure water

Nuclei suspension buffer 10 ml (make fresh)
10 ul RNAse Inhibitor stock
50 ul 2% BSA
9.94 ml 1x PBS

1x PBS 500 ml (filter through 0.2 uM filter top)
50 ml 10x PBS
450 ml UltraPure water

Troubleshooting

Sample preparation

1 Rinse fresh samples in ice-cold PBS twice.

Note

Biopsy tissuse can be store up to 3 days in liquid nitrogen/at - 80 Celsius following the steps below:
Tissues are rinsed in ice-cold PBS twice
Flash freeze the tissue in 1.7 ml Eppendorf tube in liquid nitrogen
Store frozen tissue in liquid nitrogen (preferred) or at -80C
Start from step 2 if working with frozen tissues.

Tissue lysis

2 Mince the tissue, with 200 ul lysis buffer added, in a 1.7 ml Eppendorf tube by Iris Scissors on ice x 1 mins.

3 Add 1-1.5 ml ice-cold lysis buffer to the tube and incubate on ice x 5 mins. Invert the tube 3 times in the middle of the incubation to mix.

4 Wet a 40 micron cell strainer with 1 ml lysis buffer.

5 Filter the lysis through the strainer. Wash the strainer by 3 ml lysis buffer and 4 ml nuclei suspension buffer (NSB). Keep the flow through as this is where your nuclei are.

Nuclei collection

6 Spin down the flow-through, at 600 g x 5 mins, at 4 celsius in a 15 ml conical tube.

600 x g, 4°C, 00:05:00

7 Suspend nuclei in 100 ul NSB using gentle pipetting.

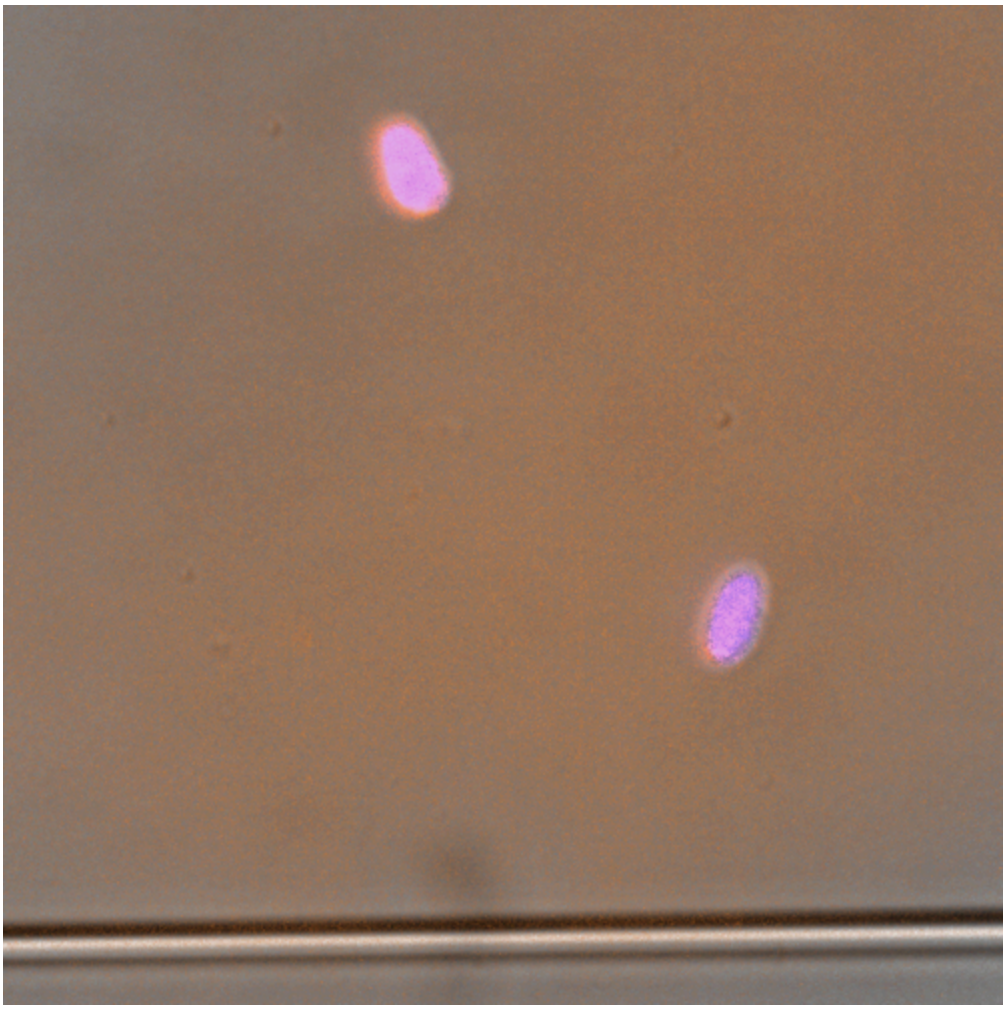
Note

If working with a single-cell platform, e.g. 10x Genomics, the nuclei should be suspended in PBS + 1% BSA + 0.2 U/ul RNase Inhibitor

7.1 If red bloold cells are present in fresh tissue nuclei suspension, dilute the suspension with NSB to 1 ml. Add 2 ml RBC lysis buffer and incubate on ice for 5 minutes. Pellet nuclei by centrifugation 600 g x 5 mins, 4 Celsius. Suspend nuclei in 100 ul NSB with gentle pipetting.

600 x g, 4°C, 00:05:00

8 Take 10 ul nuclei suspension and mix with 10 ul DAPI or Hoechst dye at 10 ug/ml and 10 ul WGA dye at 1 ug/ml. Count the nuclei.



DAPI/Hoechst staining-blue; WGA staining-orange Intact nuclei are co-stained by DAPI/Hoechst and WGA.

Nuclei preparation

9 Dilute nuclei to the desired density using NSB.