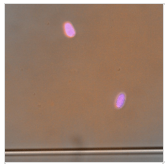

Version 2

Sep 15, 2020

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

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In Development dx.doi.org/10.17504/protocols.io.bma2k2ge

Human Cell Atlas Method Development Community | Helmsley project_Basu lab

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ABSTRACT

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

DOI

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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 Zhou

KEYWORDS

gut, intestine, human, nuclei, single cell

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LAST MODIFIED

Sep 15, 2020

PROTOCOL INTEGER ID

42042

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

NAME	CATALOG #	VENDOR
5M Sodium Chloride, 1000ml	V4221	Promega
BSA	#A8806	Sigma Aldrich
RiboLock RNase Inhibitor (40 U/µL)	EO0381	Thermo Fisher
0.5M EDTA	2482-500	Fisher Scientific
10 x PBS no calsium no magnesiusm	BP399500	Fisher Scientific

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

NAME	CATALOG #	VENDOR
UltraPure™ DNase/RNase-Free Distilled Water	10977023	Thermofisher
Red blood cell lysis buffer 10x	130-094-183	Miltenyi Biotec
Tween 20	P7949	Sigma Aldrich
1M Tris-HCl pH 7.5	15567027	Thermo Fisher Scientific
1M CaCl2	21115	Sigma-aldrich
1M MgCl2	63069	Sigma-aldrich

MATERIALS TEXT

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

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

Sample preparation

1 Rinse fresh samples in ice-cold PBS twice.

Biopsy tissuse can be store up to 3 days in liquid nitrogen/at - 80 Celsius following the steps below:

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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.

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.

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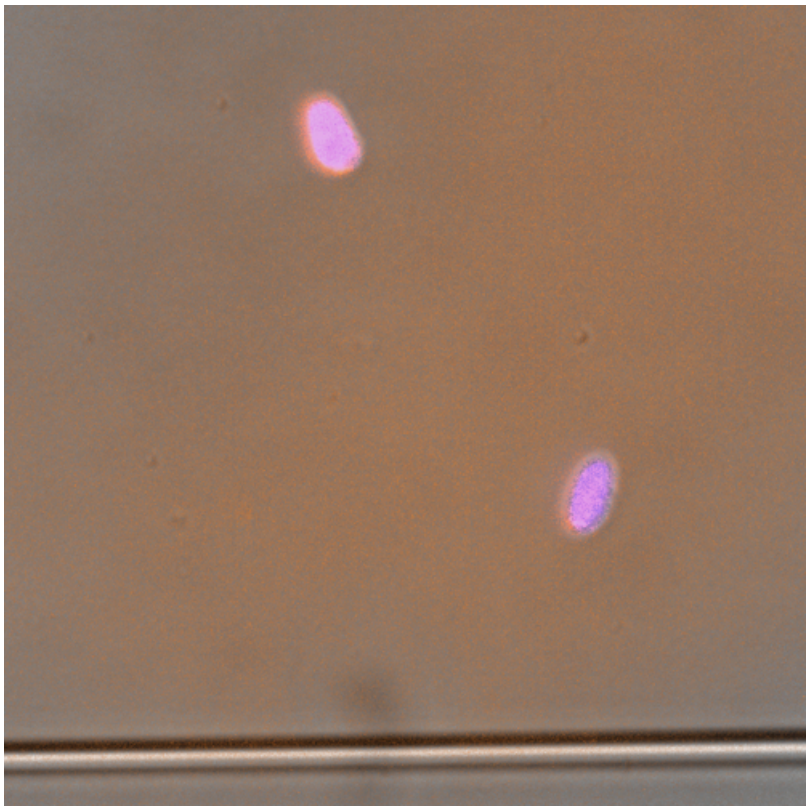

DAPI/Hoechst staining-blue; WGA staining-orange Intact nuclei are co-stained by DAPI/Hoechst and WGA.

Nuclei preparation

9 Dilute nuclei to the desired density using NSB.

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