Sep 06, 2020

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

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

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ABSTRACT

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

DOI

dx.doi.org/10.17504/protocols.io.bkxqkxmw

PROTOCOL CITATION

Ran RZ Zhou 2020. Nuclei isolation from human intestinal biopsic tissue for single-cell genomic applications. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bkxqkxmw

KEYWORDS

gut, intestine, human, nuclei, single cell

CREATED

Sep 06, 2020

LAST MODIFIED

Sep 06, 2020

PROTOCOL INTEGER ID

41680

GUIDELINES

The human intestinal tissue are obtained after patients' consents and approval from 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

NAME	CATALOG #	VENDOR
UltraPure™ DNase/RNase-Free Distilled Water	10977023	Thermofisher
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
Top up to 10 ml by 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
Top up to 10 ml by water

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

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

Nucleus suspension buffer 10 ml (make fresh)
10 ul RNAse Inhibitor stock
50 ul 2% BSA
Top up to 10 ml by 1x PBS

1x PBS 500 ml (filter through 0.2 uM filter top)
50 ml 10x PBS
450 ml 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 Wash fresh ro frozen samples in ice-cold PBS twice.

Biopsy tissuse can be store for 3 days in liquid nitrogen following the steps below:
Tissues are rinced in ice-cold PBS twice then RNAlater once;
Flash freeze the tissue in liquid nitrogen.

Citation: Ran RZ Zhou (09/06/2020). Nuclei isolation from human intestinal biopsic tissue for single-cell genomic applications. https://dx.doi.org/10.17504/protocols.io.bkxqkxmw

Tissue lysis

2 Mince the tissues in 200 ul lysis buffer in 1.5 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.

4 Wet a 40 micron cell strainer by 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).

Nuclei collection

6 Spin the down the nucleus in filter-through at 600 g x 5 mins, at 4 celsius in a 15 ml conical tube or 5 ml conical tubes if samples are processed by more downstream assay.

600 x g, 4°C, 00:05:00

7 Suspend nucleus 100 ul NSB.

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

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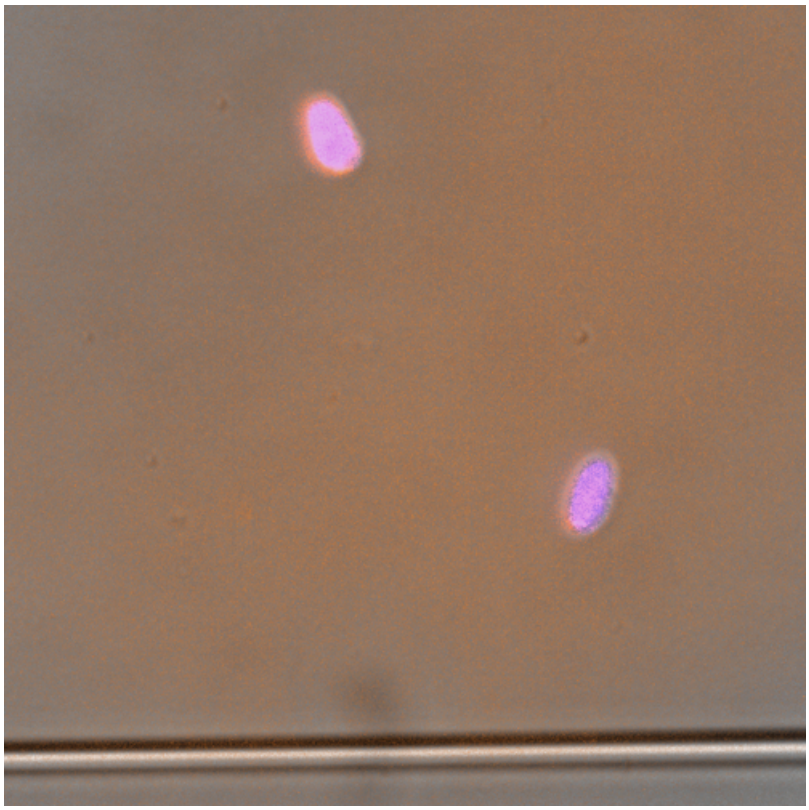

DAPI/Hoechst staining-blue; WGA staining-orange Intact nuclei are stained by DAPI/Hoechst and WGA.

8.1 If red blood cells are present, dilute nucleus suspension by NSB to 1 ml. Add 2 ml RBC lysis buffer and incubate on ice for 5 minutes. Pellet nucleus by centrifugation 600 g x 5 mins, 4 Celsius. Suspend nucleus in 100 ul NSB

600 x g, 4°C, 00:05:00

Nuclei preparation

9 Dilute nucleus to desired density for NSB or proceed with downstream application.

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