VERSION 2

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

In 1 collection

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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

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⊗ 5M Sodium Chloride, 1000ml **Promega Catalog #V4221**

⊗ BSA **Sigma Aldrich Catalog ##A8806**

⊗ RiboLock RNase Inhibitor (40 U/μL) **Thermo Fisher Catalog #E00381**

⊗ 0.5M EDTA **Fisher Scientific Catalog #2482-500**

⊗ 10 x PBS no calcium no magnesium **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 **Sigma Aldrich Catalog #P7949**

⊗ 1M Tris-HCl pH 7.5 **Thermo Fisher Scientific Catalog #15567027**

OPEN ACCESS

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

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Protocol status: In development

We are still developing and optimizing this protocol

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☒ 1M CaCl₂ Sigma-aldrich Catalog #21115

☒ 1M MgCl₂ 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

Sample preparation

- 1 Rinse fresh samples in ice-cold PBS twice.

Note

Biopsy tissue can be stored 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 µl 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 µl 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 blood 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.