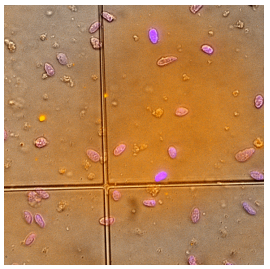
Sep 14, 2020

# Nuclei prep for single cell RNA/ATAC seq from intestinal surgical samples



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Human Cell Atlas Metho... | Helmsley project_Basu lab

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

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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

Isolating high-quality nuclei from intestinal surgical tissues is critical for single cell RNA/ATAC-seq. This protocol provides details on nuclei preparation for such application, with an overall working time of less than an hour, if FACS sorting is not incorporated.

## Guidelines

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

## Materials

MATERIALS

- 5M Sodium Chloride, 1000ml **Promega** **Catalog #**V4221
- BSA **Merck MilliporeSigma (Sigma-Aldrich)**
- RiboLock RNase Inhibitor (40 U/µL) **Thermo Fisher** **Catalog #**EO0381
- Cell strainer 100 micron **Corning** **Catalog #**431752
- 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
- 40um Cell Strainer **Fisher Scientific** **Catalog #**22363547
- 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 ultra pure 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 ultra pure water

**RBC lysis buffer** 10 ml
1 ml Red blood cell lysis buffer 10x (warmed to room temperature)
9 ml ultra pure water

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

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

## Troubleshooting

## Before start

Record the wait time between tissue removal from the patient and arrival at the lab. We don't recommend processing tissue older than 3 hours since the RNA will degrade.

## Pre-processing of surgical samples

1 Rinse surgical samples in ice-cold PBS for three times.
00:02:00

2 Place the surgical samples in a 100 mm petri dish with cold HBSS, and isolate mucosa layer by Iris scissors.
00:03:00

3 Weigh mucosa layer and cut the tissue into small pieces (<200 mg, 8mm x 8mm) on ice.
00:05:00

4 Place each small piece in a 1.7 ml Eppendorf tube on ice.

Note

Flash freeze the unused tissus in liquid nitrogen and store those tissues in liquid nitrogen/at - 80 Celsius.

## Lyse surgical tissues for nuclei

5 Mince the tissue (fresh or frozen) in 0.5 ml lysis buffer by Iris Scissors on ice for 5 minutes.
00:05:00 On ice

6 Transfer the minced tissue in lysis buffer to a 5 ml conical tube. Add an additional 3 ml lysis buffer to the tube.

7 Mix the tissue with lysis buffer by inverting the tube and incubate on ice for 10 minutes.
00:10:00 On ice

Note

Agitate tissue in lysis buffer by inverting the tube every 2 minutes.

## Enrich nuclei from the lysate

8 Wet a 100 micron cell strainer with 1 ml lysis buffer.

9 Filter the lysate through the strainer on ice and wash the strainer with 3 ml NSB. Keep the flow through as this is where your nuclei are.

00:05:00 On ice

Note

If FACS sorting is incorporated after nuclei enrichment, NSB in this experiment is supplemented with Hoechst 33342 10 ug/ml and WGA-Texas Red 1 ug/ml.

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

11 Filter the flow-through on this strainer on ice and wash the strainer by 3 ml NSB. Keep the flow through as this is where your nuclei are.

00:03:00 On ice

12 Spin the final flow-through in a 15 ml conical tube, 500 g x 5 minutes at 4 Celsius.

500 x g, 4°C, 00:05:00

13 For fresh tissues, suspend the pelleted nuclei in 1 ml NSB with gentle pipetting and continue with step 14.
For frozen tissues, suspend the pelleted nuclei in 200 ul NSB with gentle pipetting and continue with step 18.

14 Add RBC lysis buffer 10 ml to the fresh tissue nuclei suspension.

15 Invert the tube three times to mix and incubate at room temperature for 2 minutes.
00:02:00

Note

The incubation may be extended (up to 10 minutes) to fully lyse RBCs.

16 Spin the nuclei suspension in a 15 ml conical tube, at 600 g x 5 minutes at 4 Celsius.
500 x g, 4°C, 00:05:00

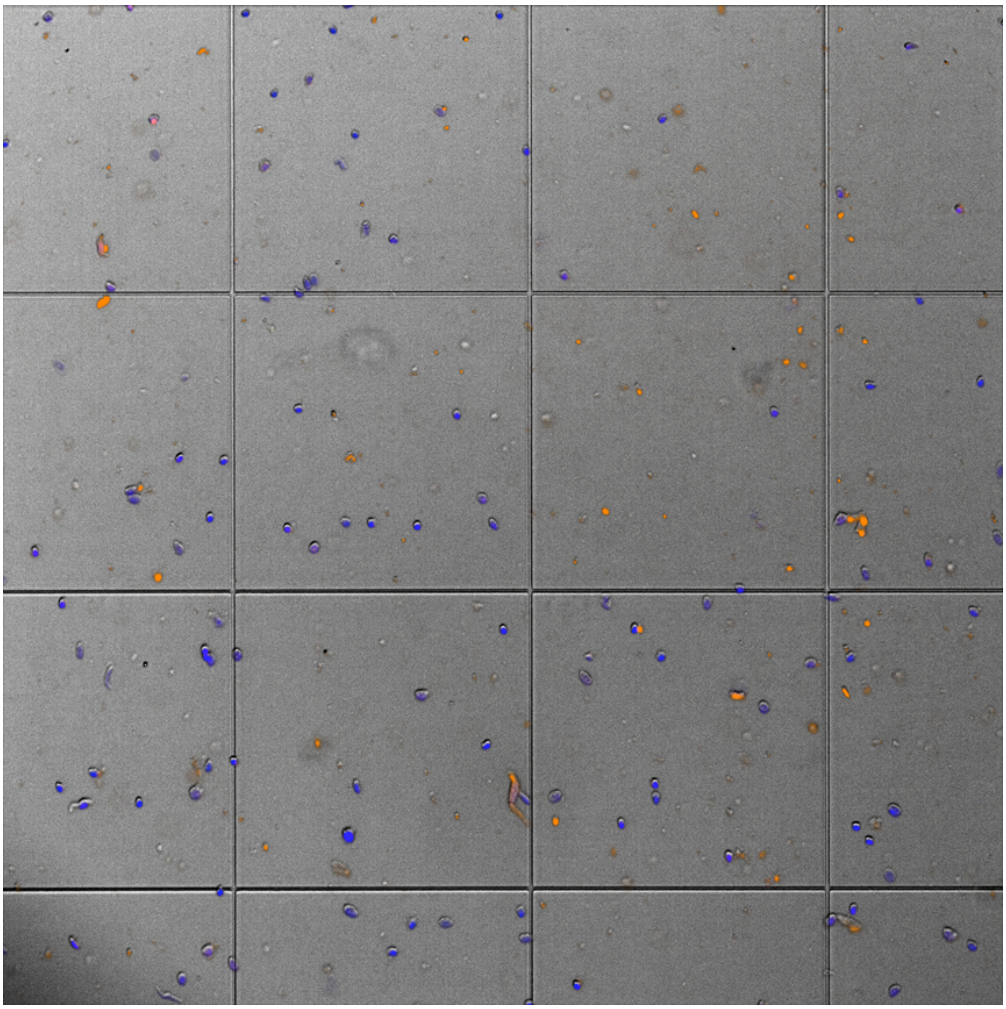
17 Suspend pelleted nuclei in 200 ul NSB or other nuclei suspension buffer as recommended by the single cell platform. Use gentle pipetting to resuspend as the nuclei are very fragile.

## Prepare nuclei for down stream applications

18 Stain the nuclei with Hoechst 33342 10 ug/ml and WGA-Texas Red 1 ug/ml. Count the nuclei.
00:05:00

Note

Skip this staining step for nuclei prepared for sorting.



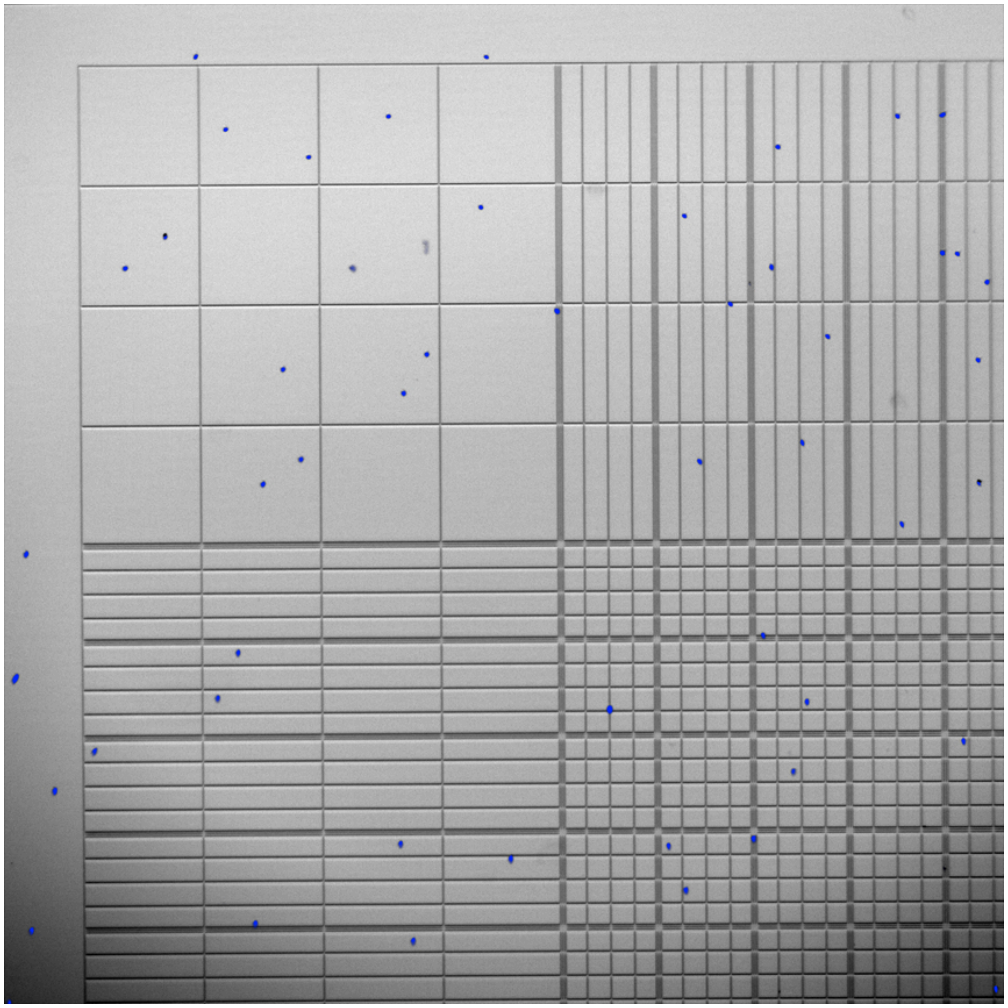
Objective lens 10x_ Nuclei are stained by Hoechst 33342-blue. Nuclei with better integrity are co-stained by WGA-orange.

19 Adjust the nuclei concentration to the desired concentration using nuclei suspension buffer.

20 Proceed with downstream applications.

Note

After FACS sorting, nuclei are spun down at 700 g x 5 minutes at 4 celsius and stained by Hoechst 33324 10 ug/ml. Count the nuclei again and adjust the nuclei concentration accordingly.



Objective lens 4x _Count sorted nuclei with Hoechst 33324 staining.