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Preparation of single cell suspensions from human intestinal biopsies for single cell genomics applications V.1

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Manuscript citation:
1. Fujii M, Matano M, Toshimitsu K, Takano A, Mikami Y, Nishikori S, Sugimoto S, Sato T. Human Intestinal Organoids Maintain Self-Renewal Capacity and Cellular Diversity in Niche-Inspired Culture Condition. Cell Stem Cell. 2018 Dec 6;23(6):787-793.e6. doi: 10.1016/j.stem.2018.11.016. 2.Smillie CS, Biton M, Ordovas-Montanes J, Sullivan KM, Burgin G, Graham DB, Herbst RH, Rogel N, Slyper M, Waldman J, Sud M, Andrews E, Velonias G, Haber AL, Jagadeesh K, Vickovic S, Yao J, Stevens C, Dionne D, Nguyen LT, Villani AC, Hofree M, Creasey EA, Huang H, Rozenblatt-Rosen O, Garber JJ, Khalili H, Desch AN, Daly MJ, Ananthakrishnan AN, Shalek AK, Xavier RJ, Regev A. Intra- and Inter-cellular Rewiring of the Human Colon during Ulcerative Colitis.Cell. 2019 Jul 25;178(3):714-730.e22. doi: 10.1016/j.cell.2019.06.029.

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We are still developing and optimizing this protocol

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Abstract

The protocol is adapted from Fujii's and Smillies's reports for single cell transcriptome analysis from human intestines. It provides details on acquirement of single cell suspension from epithelium and lamina propria. This methods is modified to generate appropriate meterials from patient's intestinal biopsies for sinlge-cell transcriptome and genomic applications.

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

MATERIALS

- BSA **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**A7906
- Liberase TM **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**000000005401119001
- FBS **Invitrogen - Thermo Fisher**
- Corning® 40µm Cell Strainer **Corning** **Catalog #**431750
- TrypLE™ Express Enzyme **Thermo Fisher Scientific** **Catalog #**12604013
- Trypan Blue Solution 0.4% (w/v) in PBS pH 7.5 ± 0.5 **Corning** **Catalog #**25-900-CI
- Eppendorf tubes 1.5 mL uncolored **Eppendorf** **Catalog #**022363204
- NxGen® RNAse Inhibitor **Lucigen** **Catalog #**30281-2
- Penicillin-Streptomycin (10,000 U/mL) **Thermo Fisher** **Catalog #**15140163
- DNase I, RNase-free, HC (50 U/µL) **Thermo Fisher** **Catalog #**EN0523
- HEPES 1M **Thermo Fisher Scientific** **Catalog #**15630080
- 15 ml corniacal tube **Corning** **Catalog #**352095
- 0.5M EDTA **Fisher Scientific** **Catalog #**2482-500
- HBSS no calsium no magnesium **Thermo Scientific** **Catalog #**14175-095
- 10 x PBS no calsium no magnesiusm **Fisher Scientific** **Catalog #**BP399500
- UltraPure™ DNase/RNase-Free Distilled Water **Thermofisher** **Catalog #**10977023
- 50 ml Conical tube **Genesee Scientific** **Catalog #**28106
- 0.2 micron syring filter **Catalog #**7232520
- Filter top vaccum unit 1000 ml **Thermo Scientific** **Catalog #**5670020
- Red blood cell lysis buffer 10x **Miltenyi Biotec** **Catalog #**130-094-183

1x PBS 1000 ml
10x PBS 100 ml
Water 900 ml
Filtered through 1000 ml Filter-top vaccum unit and stored at room temperature.

Wash media 100 ml
HBSS/PBS 97 ml
FBS 2 ml
Pen/Strep 1 ml (final concentration 100 units/mL of penicillin and 100 µg/mL of streptomycin)
Filtered through 0.2 micron syring media and prepare freshly

Dissociation media 50 ml

HBSS	47.2 ml
EDTA 0.5M	0.8 ml (final concentration 8 mM)
FBS	1 ml
Pen/Strep	0.5 ml (final concentration 100 units/mL of penicillin and 100 µg/mL of streptomycin)
HEPE 1M	0.5 ml (final concentration 10 mM)

Digestion media 20 ml

Wash media (recipe above)	48.6 ml
Liberase TM (1 mg/ml)	2 ml (final concentration 100 ug/mL)
DNase (5 mg/ml)	0.4 ml (final concentration 100 ug/mL)

Quench media 20 ml

Wash media	19.6 ml
FBS	0.4 ml

1x RBC lysis buffer 50 ml

10x RBC lysis buffer	5 ml
Water	45 ml

Cell suspension buffer 100 ml

PBS	100 ml
BSA	10 mg

Troubleshooting

Safety warnings

Sharp-end forceps and Iris scissors are used in the protocol. Primary tissues from patients are treated with cautions for unknown infectious status.

Before start

Generally two samples are obtained from each patient. The sample information from tissue containers (1.5 ml Eppendorf tube in our facility) are recorded. Each sample contains 2-3 pinches. Samples from the same patients but from different biopsy location (ileum and ascending colon) are processed separately. Wet wight of the tissues from each sample are measured by a micro-scale and recorded.

Pre-Dissociation

1 Chill wash media and dissociation media on ice. Samples are transfered with Advanced DMEM/F12 based medium in 1.5 ml eppendorf tubes on ice. Once received in lab, sample are transfered to 15 ml conical tube using sharp-end forceps after the media are chilled. Alternatively, tissues can be transferred using a P1000 pipette.

On ice

Note

Pinched tissues range from 6 to 20 mm3 in size.

Sample Wash before dissociation

2 Place 10 ml ice cold wash media in each 15 ml conical tube with each sample (containing 2-3 pinches). Invert tubes 5 times to rinse off mucus.

3 Repeat step 2 four more times or until the wash media becomes clear.

4 Transfer tissues from 15 ml conical tube to a clean 1.5 ml Eppendorf tube and mince the tissues with Iris scissors for 2-3 mins on ice.

Note

The minced tissues range from 1 to 3 mm3 in size.

00:03:00 On ice

Dissociate Epithelial layers from Lamina propria

5 Add 1 ml ice-cold Dissociation media to each eppendorf tube and transfer the mixture to 15 ml conical tubes. Place 9 ml ice-cold Dissocaition media in each conical tube to agitate the tissues settled in the bottom.

6 Rotate the tubes (end over end) on a rotator in a cold room at 15 rpm for 30 mins. Alternatively place the tubes on ice on a linear rocker and rock the tubes (end to end) at

100/min for 30 mins.
On ice | 4 °C | 00:30:00

7 Pipet the tissue in dissociation media in a 5 ml serological pipet 5 times and let the tissue settle in the tube on ice for 1-2 mins. Collect the supernatant and continue with Epithelium Digestion. Add 10 ml dissociation media to the tissue and repeat step 6.

8 Collected supernantant will be processed in the following section. Sedimented tissue represents the lamina propria and will be processed in the "Lamina propria Digestion" section.

Note

The epithlial sheets isolated from lamina propria remain in the dissociation media supernatant. The lamina propria will be processed separately from epithelium.

Epithelium Digestion

9 Centrifuge the epithelium in the supernatant in 15 ml conical tubes at 300 g x 3 mins at 4 celsius.
300 x g, 4°C, 00:03:00

10 Remove supernatant and add 5 ml TrypLE express to the pellet. Incubate the mixture at 37 celsius for 10 mins with rotation at 20 rpm.

11 Neutralize TrypLE express by adding 5 ml wash media. Centrifuge at 400 g x 3 mins at 4 celsius.
400 x g, 4°C, 00:03:00

12 Remove supernatant. Resuspend cells in 1 ml wash media and place on ice.

13 Take 20 ul cell suspension with 20 ul Tryplan blue and mix well. Check the cell viability on a light microscope.
If viability is higher than 85%, proceed with red blood cell removal.

Note

Either hemocytometer or Countess automated cell counter can be used for viability assessment. Live cells are excluded from staining blue and the numbers of live and dead cells are both counted for viablity rate = 100% x N of live cells/(N of live cells + N dead cells). At least 50 cells are counted in total.

Lamina propria Digestion

14 Wash the tissue three times with 5 ml Wash media. Transfer the tissue with P1000 pipette to 1.5 ml Eppendorf tubes.

15 Add 1 ml Digest media. Place tissue at 37°C with 300 rpm on the thermomixer for up to 20 mins.

Note

Check the digestion after 15 mins incubation with Digest media. If cell clumps remains and cell viability > 85%, incubate for additional 5 minutes.

16 Neutralize TrypLE by 0.5 ml Quench media. Leave tissue on ice for 5 mins. Agitate the tissue by pipetting up and down.

17 Take 20 ul cell suspension with 20 ul Trypan Blue. Mix well and check the viability on a light microscope. Proceed with red blood cell removal.

Red blood cell removal

18 Dilute one volume of cell suspension (epithelium or lamina propria; keep tissue types separate) by ten volumes of Miltenyi Biotec 1× Red Blood Cell Lysis Solution.

19 Gently mix by 5 ml serological pipet for 5 seconds and incubate for 2 minutes at room temperature.

20 Centrifuge at 400×g for 10 minutes at room temperature.

400 x g, Room temperature, 00:10:00

21 Aspirate supernatant completely. Resuspend the cell pellet in 5 ml cell suspension buffer and filter through a 40 micron cell strainer. Proceed with Cell suspension preparation.

Cell Suspension Preparation

22 Pellet the cells by centrifugation 400 g x 3 mins at 4 celsius.

400 x g, 4°C, 00:03:00

23 Remove supernatant. Resuspend cells in 1 ml cell suspension with RNase Inhibitor 0.1 U/ul. Check the viability with Tryplan blue again. Place the cells on ice for downsteam applications.