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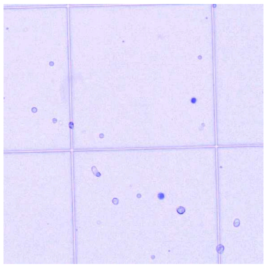
Preparation of single cell suspensions from human intestinal biopsies for single cell genomics applications V.4



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We use this protocol and it's working

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Disclaimer

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
- RBC lysis buffer **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #11814389001

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	98 ml
2% BSA	2 ml

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
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

Cell suspension buffer 100 ml

PBS	100 ml
2%BSA	0.5 ml

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 (1.7 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 weight 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 media in 1.7 ml eppendorf tubes on ice. Once received in lab, samples are transfered to 35 mm dish using sharp-end forceps after the media are chilled. Alternatively, tissues can be transferred using a P200 pipette.

🌡 On ice

Note

Pinched tissues range from 6 to 20 mm^3 in size.

Sample Wash before dissociation

2 Place 3 ml ice cold wash media in each dish with each sample (containing 2-3 pinches). Shake the dish on ice to rinse off mucus.

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

4 Transfer tissues from the 35 mm dish to a clean 5 ml conical tube. Add 0.2 ml Dissociation media and mince the tissues with Iris scissors for 1 minute on ice.

Note

The minced tissues range from 1 to 3 mm^3 in size, can easily pass a P1000 tip.

⏱ 00:01:00 🌡 On ice

Dissociate Epithelial layers from Lamina propria

5 Add 3 ml ice-cold Dissociation media to each 5 ml tube.

6 place the tubes on ice on a linear rocker and rock the tubes (end to end) at 100/min for 60 mins. Alternatively rotate the tubes in a 37 Celsius oven for 15 mins. 15m

37 °C 00:15:00

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 5 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 epithelial 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 5 ml conical tubes at 300 g x 5 mins at 4 celsius.

300 x g, 4°C, 00:05:00

10 Remove supernatant and add 2 ml warmed TrypLE express to the pellet. Incubate the mixture at 37 celsius for 5 mins with rotation at 20 rpm.

Note

A hybridation oven with a rotisserie is recommended.

11 Neutralize TrypLE express by adding 2 ml wash media, agitate the tissue by pipetting up and down and filter through a 40 micron cell strainer.

12 Centrifuge at 300 g x 5 mins at 4 celsius.Remove supernatant. Resuspend cells in 1 ml wash media and place on ice.

300 x g, 4°C, 00:05:00

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 one times with 3 ml Wash media. Aspire the Wash media as much as possible.

15 Add 2 ml Digest media. Incubate the tissue at 37°C with end-over-end rotation 20 rpm for up to 30 mins.

Note

Check the digestion after 20 mins incubation with Digest media. If cell clumps remains and cell viability > 95%, incubate for additional 5 minutes.

16 Neutralize Liberase TM by 1 ml Quench media. Leave tissue on ice for 5 mins. Agitate the tissue by pipetting up and down and filter through a 40 micron cell strainer.

17 Centrifuge at 300 g x 5 minus at 4 Celsius. Remove supernatant and suspend cells in 1 ml wash media.

300 x g, 4°C, 00:05:00

18 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

19 Dilute one volume of cell suspension (epithelium or lamina propria; keep tissue types separate) by two volumes of Red Blood Cell Lysis Solution.

20 Gently mix by inverting the tubes and incubate for 2 minutes at room temperature.

21 Centrifuge at 300 g for 5 minutes at 4 Celsius.

300 x g, 4°C, 00:05:00

22 Aspirate supernatant completely. Resuspend the cell pellet in 0.1 ml cell suspension buffer with RNase Inhibitor 0.1 U/ul (RNase Inhibitor is not needed if loading on 10x Genomics platform). Proceed with Cell suspension preparation.

Cell Suspension Preparation

23 Count the cells with Trypan Blue staining.

24 Adjust cell density by cell suspension buffer with RNase Inhibitor 0.1 U/ul (RNase Inhibitor is not needed if loading on 10x Genomics platform).