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Isolating human intestinal crypts from biopsies for organoid generation

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1. Jung P, Sato T, Merlos-Suárez A, Barriga FM, Iglesias M, Rossell D, Auer H, Gallardo M, Blasco MA, Sancho E, Clevers H, Batlle E. Isolation and in vitro expansion of human colonic stem cells. Nat Med. 2011 Sep 4;17(10):1225-7. doi: 10.1038/nm.2470. 2. Middendorp S, Schneeberger K, Wiegerinck CL, Mokry M, Akkerman RD, van Wijngaarden S, Clevers H, Nieuwenhuis EE. Stem Cells. Adult stem cells in the small intestine are intrinsically programmed with their location-specific function. 2014 May;32(5):1083-91. doi: 10.1002/stem.1655. 3. Sasaki N, Sachs N, Wiebrands K, Ellenbroek SI, Fumagalli A, Lyubimova A, Begthel H, van den Born M, van Es JH, Karthaus WR, Li VS, López-Iglesias C, Peters PJ, van Rheenen J, van Oudenaarden A, Clevers H. Reg4+ deep crypt secretory cells function as epithelial niche for Lgr5+ stem cells in colon. Proc Natl Acad Sci U S A. 2016 Sep 13;113(37):E5399-407. doi: 10.1073/pnas.1607327113. Epub 2016 Aug 29. 4. Sato T, Stange DE, Ferrante M, Vries RG, Van Es JH, Van den Brink S, Van Houdt WJ, Pronk A, Van Gorp J, Siersema PD, Clevers H. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology. 2011 Nov;141(5):1762-72. doi: 10.1053/j.gastro.2011.07.050. Epub 2011 Sep 2. PMID: 21889923

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We are still developing and optimizing this protocol

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Abstract

This protocol provides details on crypt isolation from human terminal ileum and colon to robustly generate organoids.

Guidelines

Human intestinal biopsies are obtained from endoscopic procedures. All the biopsies are performed after patients' consents and approval from Institutional Review Board at the University of Chicago (IRB Number: 15573A).Intestinal organoids are maintained in human organoid media (details in Materials) and are not prone to differentiation until cultured in differentiation media (unlisted in this protocol).

Materials

MATERIALS

- 100 mg Ciprofloxacin (Cipro) **biorbyt** **Catalog #**orb134677
- SB202190 25 mg **STEMCELL Technologies Inc.** **Catalog #**72634
- Nicotinamide **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**N0636
- 6-well plate **Corning**
- 0.5M EDTA solution **Thermo Fisher Scientific** **Catalog #**15575020
- Y-27632 **STEMCELL Technologies Inc.** **Catalog #**72303
- DPBS no calcium no magnesium **Thermo Fisher Scientific** **Catalog #**14190144
- Advanced DMEM **Thermo Fisher** **Catalog #**12491015
- Penicillin-Streptomycin (10,000 U/mL) **Thermo Fisher** **Catalog #**15140148
- Glutamax 100x **Thermo Fisher Scientific** **Catalog #**35050061
- HEPES 1M **Thermo Fisher Scientific** **Catalog #**15630080
- N2 supplement 100x **Thermofisher** **Catalog #**17502048
- N-Acetyl-L-( )-cysteine **Fisher Scientific** **Catalog #**O1049-25
- EGF recombinant mouse protein **Thermofisher** **Catalog #**PMG8043
- Jagge-1 **Anaspec** **Catalog #**AS-61298
- A 83-01 **STEMCELL Technologies Inc.** **Catalog #**72024
- CHIR99021 **Stemgent** **Catalog #**04-0004-10
- LY2157299 **Cayman Chemical Company** **Catalog #**15312
- [Leu15]-Gastrin I human **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**G9145
- Recombinant Human R-Spondin-1 **peprotech** **Catalog #**120-38
- L-WRN **ATCC** **Catalog #**CRL-3276
- Recombinant murine WNT-3A **peprotech** **Catalog #**3115-20
- Recombinant human Noggin **peprotech** **Catalog #**120-10C
- B27 supplement minus Vitamin A **Thermofisher** **Catalog #**12587010
- Matrigel Growth factor reduced **Becton Dickinson (BD)** **Catalog #**356230
- ART™ Wide Bore Filtered Pipette Tips **Thermofisher** **Catalog #**2079G
- Cell strainer 100 micron **Corning** **Catalog #**431752
- 12-well TC treated plate **Corning**
- 24-well plate **Corning**

15 ml corniacal tube **Corning Catalog #**352095

Dissociation medium 100 ml
8 mM EDTA in DPBS without Calsium and Magnesium

Complete ADF media with conditioned media is composed of:
a. Advanced DMEM/F12
b. 1X Glutamax
c. 10mM HEPES
d. 1X Pen/Strep
e. 1X N2 supplement
f. 1X B-27 Supplement Minus Vitamin A
g. N-acetyl-L-(+)-cysteine (1.25 mM)
h. Nicotinamide (10 mM final)
i. 50% L-WRN conditioned media
j. Murine EGF (50 ng/ml)
k. Jagged-1 (1 uM)
l. Y-27632 (10 uM)
m. SB202190 (30 uM)
n. A-8301 (500 nM)
o. Chir99021 (2.5 uM)
p. LY2157299 (500 nM)
q. Leu15-Gastrin I (10 nM)
r. Recombinant human R-spondin1 (500 ng/ml)

Alternatively, only with recombinant proteins, complete ADF media is composed of:
s. Advanced DMEM/F12
t. 1X Glutamax
u. 10 mM HEPES
v. 1X Pen/Strep
w. 1X N2 supplement
x. 1X B-27 Supplement Minus Vitamin A
y. N-acetyl-L-(+)-cysteine (1.25 mM)
z. Nicotinamide (10 mM)
aa. Murine Wnt3A (100 ng/ml)
bb. Murine epidermal growth factor (50 ng/ml)
cc. Noggin (100 ng/ml)
dd. R-spondin-1 (500 ng/ml)
ee. Jagged-1 (1 uM)
ff. Y-27632 (10 uM)
gg. SB202190 (30 uM)
hh. A-8301 (500 nM)
ii. Chir99021 (2.5 uM)

jj. LY2157299 (500 nM)
kk. Leu15-Gastrin I (10 nM)

Ciprofloxacin 10 ug/ml is added freshly when feeding cells.

Stocks of small molecules and recombinant proteins are prepared and stored according to the manufacturer's instruction.

P1000 tips
Customize the pipet tip. If needed, tips can be altered by cutting it to slightly smaller than the size of the largest biopsy, using a sterilized razor blade. Cut the pipet tip at a straight angle, not biased, to keep the hole as small as possible.

Troubleshooting

Before start

Human organoid media also listed as complete Advanced DMEM/F12 (complete ADF) is supplemented with L-WRN (murine cell line) conditional media, alternatively complete ADF can be supplemented with recombinant proteins.

1 Collect biopsies in PBS or culture media in 1.5 ml Eppendorf tubes.

2 Prechill PBS and 8mM EDTA in PBS on ice. Pre-warm human organoid medium and 12-well/24-well plate in the tissue-culture incubator.

3 Once biopsies received, wash 3 times with 25 ml ice cold PBS each in 50 ml conical tubes, or until PBS looks clear.

Note

Biopsied tissues range from 6 to 20 mm3 in size. All the tissues biopsied from the same site are pooled for downstream procedures.

4 Incubate all the tissues from each biopsy in 25mL of 8 mM EDTA/PBS in a 50 ml conical tube for 30 min, rocking (not shaking) at 4C.

4 °C

00:30:00

5 Place one 100 um cell strainer on each well of a 6-well plate. Pre-wet the cell strainer with 1mL culture media.

6 Transfer biopsies from EDTA/PBS into 1-2 ml ADF.

7 Using a customized P1000 *aerosol resistant* tip, pipet up and down vigorously, making sure that the biopsy tissue goes in and out of the tip, but with some difficulty. This helps release the crypts.

Note

Customize the pipet tip: tips can be altered by cutting it to slightly smaller than the size of the largest biopsy, using a sterilized razor blade. Cut the pipet tip at a straight angle, not biased, to keep the hole as small as possible.

8 Use the 100 um cell strainer to filter the tissue and large pieces from the crypts. Collect the crypts into a 1.5 ml microfuge tube. Check to see whether enough crypts were

released from the tissue on a microscope.

9 Centrifuge the crypts at 300-400 g at 4C for 5 min in a tabletop swinging bucket centrifuge

300 x g, 00:05:00 4 °C

Note

A microcentrifuge is not recommended here because it is a fixed angle and we want the pellet to be at the bottom of the tube, not on the side

10 Remove the supernatant and resuspend the crypts in pre-warmed and CO2-equilibrated human organoid media (complete ADF) containing growth factors

11 Mix crypts with thawed matrigel in a ratio of 1:2 (cells:matrigel). Pipet up and down; avoid creating bubbles

12 Plate crypt/matrigel mixture onto a pre-warmed 12- or 24-well tissue culture plate, 100 or 50 ul/well respectively.

13 Incubate 45-60 min in 37C, 5% CO2 incubator

37 °C

00:45:00 Incubation

14 Add complete ADF into each well (24-well: 0.5 ml/well; 12-well: 1 ml/well)

15 Feed organoids with complete ADF every other day. Also add ciprofloxacin (10 ug/ml) to make sure that there are no remaining bacteria in your culture.

Note

Media is changed every other day. Organoids are split and expanded as needed, about once per week.