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Isolating human intestinal crypts from biopsies for organoid generation

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ABSTRACT

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

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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

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

NAME	CATALOG #	VENDOR
100 mg Ciprofloxacin (Cipro)	orb134677	biorbyt
SB202190 25 mg	72634	Stemcell Technologies
Nicotinamide	N0636	Sigma Aldrich
6-well plate		Corning
0.5M EDTA solution	15575020	Thermo Fisher Scientific
Y-27632	72303	Stemcell Technologies
DPBS no calcium no magnesium	14190144	Thermo Fisher Scientific
Advanced DMEM	12491015	Thermo Fisher
Penicillin-Streptomycin (10,000 U/mL)	15140148	Thermo Fisher

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NAME	CATALOG #	VENDOR
Glutamax 100x	35050061	Thermo Fisher Scientific
HEPES 1M	15630080	Thermo Fisher Scientific
N2 supplement 100x	17502048	Thermofisher
N-Acetyl-L-( )-cysteine	O1049-25	Fisher Scientific
EGF recombinant mouse protein	PMG8043	Thermofisher
Jagge-1	AS-61298	Anaspec
A 83-01	72024	Stemcell Technologies
CHIR99021	04-0004-10	Stemgent
LY2157299	15312	Cayman Chemical Company
[Leu15]-Gastrin I human	G9145	Sigma Aldrich
Recombinant Human R-Spondin-1	120-38	peprotech
L-WRN	CRL-3276	ATCC
Recombinant murine WNT-3A	3115-20	peprotech
Recombinant human Noggin	120-10C	peprotech
B27 supplement minus Vitamin A	12587010	Thermofisher
Matrigel Growth factor reduced	356230	BD Biosciences
ART™ Wide Bore Filtered Pipette Tips	2079G	Thermofisher
Cell strainer 100 micron	431752	Corning
12-well TC treated plate	View	Corning
24-well plate	View	Corning
15 ml corniacal tube	352095	Corning

MATERIALS TEXT

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)

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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.

BEFORE STARTING
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.

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

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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.

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**

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)

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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.

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

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