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EXPERIMENTAL PROTOCOL: Maintenance, passaging and cryopreservation of colon cancer organoids

Author/s: Mantrana A., Guil-Luna S., Aranda-Aguilar E., Rodríguez Ariza A.

Contact information: ana.mantrana@imibic.org; *v22gulus@uco.es*

Introduction

This protocol is for the maintenance, passaging and cryopreservation of colorectal cancer organoids.

Reagents

- Colorectal Cancer Organoid Media (see below)
- Dimethyl sulfoxide (DMSO) (Sigma-Aldrich, cat. no. 34943)
- Falcon tubes, 15 mL (Sarstedt, cat. no. 62.554.502)
- FBS (Biowest, S1810)
- Matrigel (Corning #354230)
- Organoid Culture Media (see below)
- TrypLE Express (Gibco, cat. no. 12604-013)

Instruments and tools

- Mr Frosty freezing container (ThermoFisher, 5100-0036)
- Nunc Cryovials (ThermoFisher, cat. no. 375418)
- Plates, 24 well (Greiner, cat. no. 662160)

Before start

Preparation of Reagents:

Component	Company/Cat#	Stock Conc.	Volume	Final Conc.
Advanced DMEM/F12	Sigma #D6421	500 mL	486.9 mL	1x
HEPES	Media Kitchen (Life Technologies #15630080)	1 M	5 mL	10 mM
Glutamax	Media Kitchen (Life Technologies #35050-061)	100x (200 mM)	5 mL	1x (2 mM)
Zell Shield	Minerva Biolabs GmbH, Berlin, Germany	100x	5 mL	1x

Colorectal Cancer Organoid Media (CRC Medium)

Component	Company/Cat#	Final Conc.
OC Medium	See above	1x
A 83-01	TOCRIS #2939-10mg	0.5 µM
B27	ThermoFisher Sc. #12587010	2x
EGF	Sigma Aldrich #SRP3027-500UG	0.25µg/ml
Gastrin	Sigma Aldrich #G9145-0.5MG	1 ug/mL (480.7461nM)
N acetyl Cyst	Sigma Aldrich #A9165-5G	1.23mM
SB202190	Sigma Aldrich #S7067-5MG	5. 0296 µM
Y27632	Sigma Aldrich #Y0503-5mg	10 µM

PROCEDURE

Priorities:

1) Maintenance organoids
2) Expansion organoids
3) Production of cryopreserved organoids biobank

Maintencance of Organoids (Media change every Monday and Thursday)

1. Carefully aspirate 500 µL medium from each well containing organoids.
2. Add 500 µL of pre-warmed (37°C) CRC medium to each well.

Passaging of organoids

Passaging organoids without dissociation:

1. Carefully remove culture medium from each well and add 400 µL of cold PBS per well.
2. Scrap matrigel with p1000 and transfer organoids to a 15 mL falcon tube.
3. Wash each well again with 400 µL of cold PBS and transfer remaining organoids to the 15 mL falcon tube.
4. With a 21g syringe, separate matrigel from organoids mechanically.
5. Centrifuge at 300g for 5 minutes at 4°C and remove supernatant.
6. Resuspend pelleted organoids in the desired matrigel volume (dilution from 1 well ⇨ 2 wells or from 1 well ⇨ 4 wells depending on the initial density obtained)
7. Allow matrigel to settle for 30 minutes at 37°C.
8. Add 500 µL of colorectal cancer organoid medium per well.

Passaging organoids with Dissociation:

1. Carefully remove culture medium from each well and add 500 µL of Tryple per well.
2. Scrap matrigel with p1000 and incubate for 15 minutes at 37°C.
3. Check dissociation every 5 minutes and pipette with p1000 to enhance cell detachment.
4. Collect dissociated organoids into a 15 mL falcon tube and top up to 15 mL with PBS.

5. Centrifuge at 300g for 5 minutes RT, and remove supernatant.
6. Resuspend pelleted organoids in the desired matrigel volume (dilution from 1 well ⇨ 2 wells or from 1 well ⇨ 4 wells depending on the initial density obtained)
7. Allow matrigel to settle for 30 minutes at 37°C.
8. Add 500 µL of colorectal cancer organoid medium per well.

Cryopreservation of organoids

1. Starting from passage 2, set aside 2 wells from each passage for cryopreservation.
2. Scrap the matrigel at the bottom of each well and transfer to 15 mL Falcon tubes.
3. Rewash each well with 500 µL of organoid culture medium.
4. Top remaining Falcon tube with organoid culture medium up to 10 mL.
5. Centrifuge at 1500 rpm for 5 minutes at 4°C
6. Aspirate supernatant to the top of matrigel
7. Add FBS with 10% DMSO (900 µL FBS + 100 µL DMSO), using 1 mL of this mix per 2 wells.
8. Gently resuspend organoids and transfer 1mL of freezer mix to each cryopreservation vial.
9. Place the vials into a Mr. Frosty and store at -80°C for at least 24h. Within several days, transfer vials to liquid nitrogen storage.