

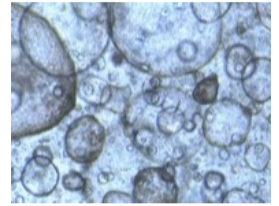
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## Derivation of organoids from primary tumour tissue

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Cellular Generation and ...

Organoid and Assembloid



Hazel Rogers

Dr

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**Protocol status:** Working

**We use this protocol and it's working**

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**Keywords:** Organoid, Cancer, Derivation, extensive experience in organoid derivation, organoid derivation team within the cellular generation, organoid derivation team, derivation of organoid model, derivation of organoid, organoid derivation, organoid model, organoids from colon, organoid, primary tumour tissue this protocol, primary tumour tissue, oesophageal tumour, cellular generation, pancrea, tissue,


## Abstract

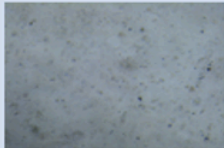


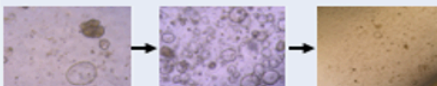
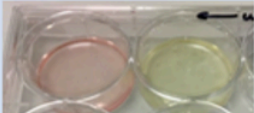
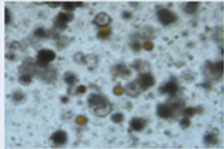
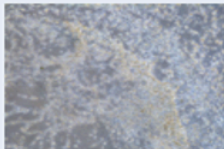
This protocol describes the derivation of organoid models from primary tumour tissue. It has been developed by the organoid derivation team within the Cellular Generation and Phenotyping Group at the Wellcome Sanger Institute. We have used the process to derive organoids from colon, pancreas and oesophageal tumours. The team has extensive experience in organoid derivation and have successfully banked over 100 models.



## Guidelines

### General Information and tips

- The tissue samples we process are transported from clinical sites in Advanced DMEM-F12 containing primocin antibiotic (final concentration 100 ug/ml). Samples are shipped chilled at  4 °C .
- We use 5 ml Eppendorf tubes to help with sterility. However, if you do not have access to these tubes any alternative sterile tubes of appropriate volume can be used.
- We have experience of deriving organoid models from colon, oesophagus and pancreatic tumour tissue.
- We recommend using glass rather than plastic petri dishes for tissue dissection as tissue can get stuck in grooves cut into the plastic dish.
- Plate digested cells as close together as possible.
- Be very cautious at initial passages after derivation. Organoids can grow well for a few passages and then significantly drop off. We generally keep organoids in the same number of wells or reduce the area plated in if growth is slow or some cellular material has died.
- Not all derivations will be successful. Listed below are some common reasons we see for failure.

Reason	How do you know?	What does it look like?
Lack of cells	Insufficient starting material. No/minimal organoid formation seen.	
No organoid formation	Viable cells present but do not form organoids.	
Unable to propagate	Organoids form but unable to expand.	
Growth has dropped off	Organoids were present and expanding but have since stopped reforming.	
Bacterial contamination	Media turns very yellow and probably also cloudy. Small bacteria can be seen under high magnification.	
Fungal contamination	May manifest as very round, bright yellow dots within BME2 droplets or white colonies on top.  Media may turn yellow but can remain clear.	
Mycoplasma contamination	Cannot be detected visually although organoid growth may be affected.  Repeatedly test positive for mycoplasma.	-
Fibroblast contamination	Excessive fibroblast outgrowth takes over the culture.	

## Trouble Shooting



Problem	Possible Solution
BME2 is setting too quickly whilst plating.	Try keeping your solution in a cooling rack whilst plating.
Cells plated too densely resulting in BME breaking up.	Harvest cells, BME2 and media in a tube. Pipette to break up BME2. Spin, then aspirate supernatant. If a lot of BME2 is left (grey haze above pellet) re-suspend in ice cold PBS, then repeat spin. If this does not work, re-suspend in TrypLE and incubate at 37°C for a few minutes before spinning. Re-suspend cell pellet in appropriate amount of BME2 and re-plate.
One well is contaminated but rest of plate looks normal.	Aspirate media from contaminated well. Add 2 ml chlorohexidine gluconate and leave for 30 min. Aspirate entire contents of well and wash out with PBS. Keep an eye on remaining wells for the next few days.
Culture is taken over by fibroblasts.	Use a P1000 pipette to harvest organoids and media in a tube and wash wells with PBS. Fibroblasts tend to attach to the surface of the culture plate so should be left behind when the organoids are harvested. Spin then aspirate supernatant. Re-suspend in TrypLE and continue with passaging protocol.



## Materials

### MATERIALS

✕ Falcon 15 mL Polystyrene Conical Tube **Fisher Scientific Catalog #352095**

✕ Penicillin Streptomycin **Invitrogen - Thermo Fisher Catalog #15140 122**

✕ DPBS no calcium no magnesium **Thermo Fisher Scientific Catalog #14190144**

✕ Collagenase, Type II, powder **Thermo Fisher Catalog #17101015**

✕ Cultrex® Reduced Growth Factor Basement Membrane Matrix Type 2 (BME  
2) **Trevigen Catalog #3533-010-02**

✕ Falcon 50mL Conical Centrifuge Tubes **Fisher Scientific Catalog #14-432-22**

✕ Costar 6-well Clear TC-treated Multiple Well Plates Bulk Packed Sterile **Corning Catalog #3506**

✕ Eppendorf Tubes 5.0 ml **Eppendorf Catalog #0030122321**

✕ Anumbra Glass Petri Dish 100×15mm **Scientific Laboratory Supplies Ltd Catalog #PET1008**

✕ Surgical Scalpel Blade No. 21 **Swann Morton Catalog #0507**

✕ Cell Strainers 100 µm pore size **VWR International (Avantor) Catalog #732-2759**

✕ Pestle for Cell Strainer **Merck MilliporeSigma (Sigma-Aldrich) Catalog #Z742105**

✕ Primocin **InvivoGen Catalog #ant-pm-1**

✕ Y-27632 dihydrochloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #Y0503**

### Equipment

- Sterile cell culture hood
- Centrifuge
- 1000 µl and 200 µl pipettes and tips
- Pipetteboy
- Stripettes
- 37°C waterbath
- 37°C humidified incubator (5% CO<sub>2</sub>)
- Light microscope
- Tube rotator

## Troubleshooting



## Safety warnings



### Note

**For full safety information refer to individual COSHH and MSDS forms**

- **Primocin** can cause possible respiratory and skin sensitisation.
- **Penicillin Streptomycin** can cause possible respiratory and skin sensitisation. May also damage fertility or the unborn child.
- **Rock inhibitor (Y-27632)** is harmful if swallowed, inhaled or splashed on skin.
- Organoids derived from **primary samples** may contain uncharacterised adventitious agents, including blood-borne viruses.



## Before start

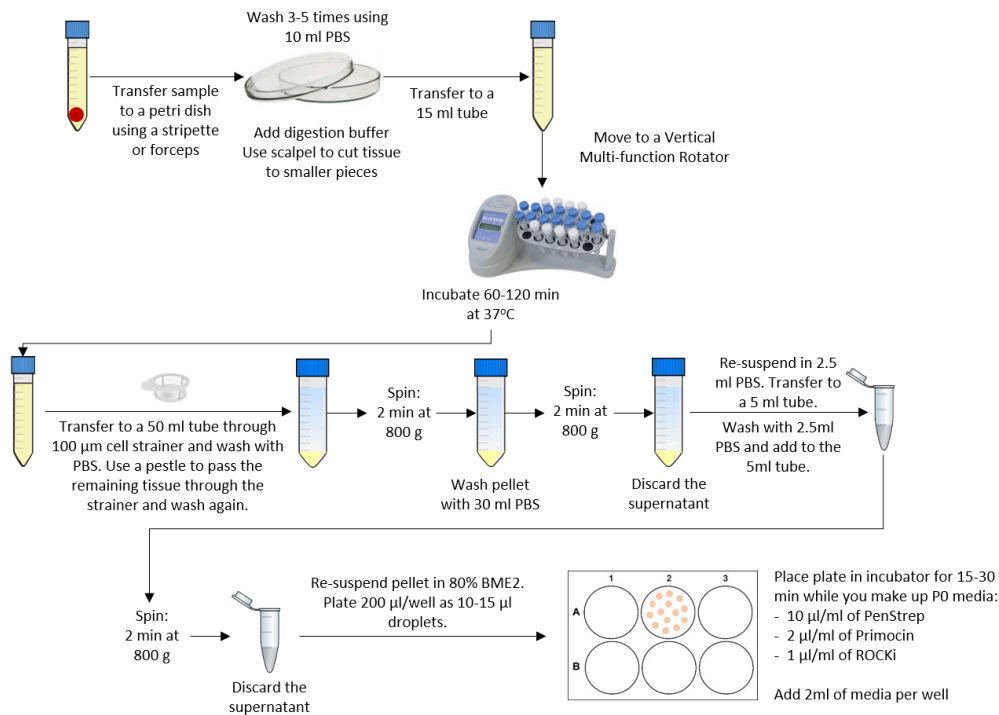
- Thaw BME2 aliquot overnight at  $4^{\circ}\text{C}$  and dilute 4:1 with appropriate organoid media (tissue specific) to make an 80% stock
- Ensure cell culture plates have been stored overnight in  $37^{\circ}\text{C}$  incubator
- Pre-warm organoid culture media to room temperature
- Prepare 100 mg/ml collagenase stock. Re-suspend  $1\text{ g}$  collagenase II in  $10\text{ mL}$  PBS. Aliquots can be stored at  $-20^{\circ}\text{C}$  for up to one year.
- Prepare digestion buffer:

Reagent	Stock Concentration	Volume
Organoid Media	-	9.5 ml
Collagenase	100 mg/ml	0.5 ml
Primocin	50 mg/ml	0.02 ml
Penicillin Streptomycin	100X	0.1 ml
Rock inhibitor (Y-27632) (10 mM)	10 mM	0.01 ml



## Process Diagram

1



## Protocol

1h


- 2 Pour or pipette tissue, and media sample has been transported in, into a glass petri dish.

### Safety information

**If tissue has unknown infection status, only open the container the sample has been transported in within a microbiological safety cabinet.**

### Note

**We recommend using glass rather than plastic petri dishes as tissue can get stuck in grooves cut into the plastic dish whilst cutting up the sample.**



- 3 Aspirate as much media as possible. Add  10 mL PBS to wash the tissue sample. Aspirate PBS and repeat wash at least two more times (we perform 3 washes for pancreas and oesophagus and 5 washes for colon).




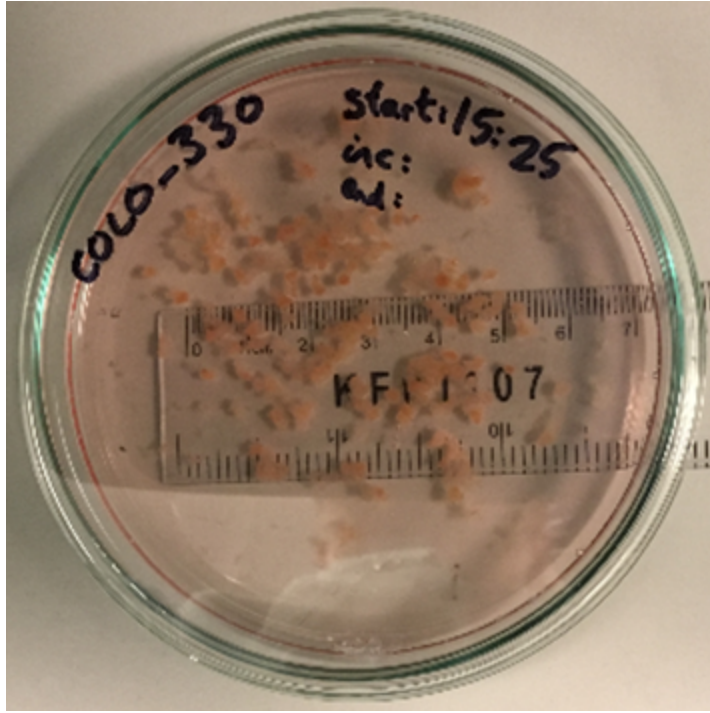
#### Note

**After last wash make sure to aspirate as much PBS as possible to avoid diluting the digestion buffer.**

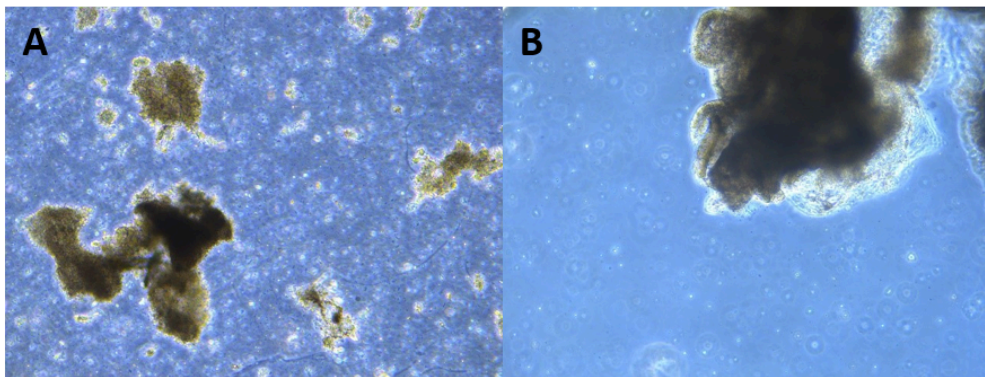
#### Note

**If tissue is breaking up, making aspiration difficult without losing the sample, transfer tissue and media back to the  15 mL tube and centrifuge (  800 x g 2 min). Aspirate supernatant and re-suspend in PBS to wash. Repeat these steps for appropriate number of washes.**





- 4 Add  10 mL digestion buffer. Using a scalpel, cut sample into small pieces of approximately 1-2 mm in diameter.



Tissue cut into small pieces

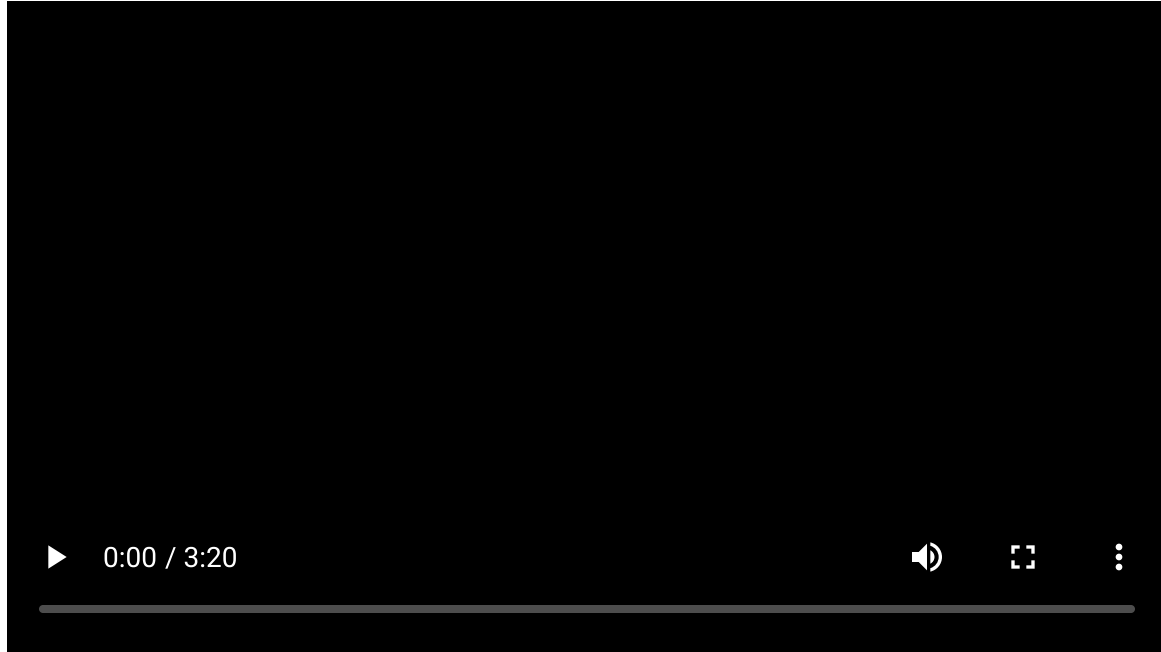


Examples of tissue samples with high (A) and low (B) cellularity (post cutting with a scalpel)

- 5 Transfer tissue and digestion buffer to a  15 mL tube. Place sample in a tube rotator and incubate at  37 °C for 60-120 minutes.
- 6 Following incubation, assess tissue fragments under a microscope to confirm sufficient digestion. The sample should look cloudy to the eye and appear as single cells or small clumps under a microscope.
- 7 Transfer digested sample to a  50 mL tube through a 100 µm cell strainer. Use a pestle to pass any remaining tissue through the strainer. Wash the  15 mL tube with



🧪 10 mL PBS and add to the 🧪 50 mL tube through the cell strainer. Repeat the wash step.



8 Centrifuge at 🌀 800 x g for 2 minutes.

9 Aspirate supernatant and re-suspend pellet in 🧪 30 mL PBS. Repeat spin at 🌀 800 x g for 2 minutes.

#### Note


**When aspirating, you do not need to worry about getting too close to the pellet at this stage.**

10 Aspirate supernatant and re-suspend pellet in 🧪 2.5 mL PBS. Transfer to a 🧪 5 mL tube (or 🧪 15 mL tube). Wash 🧪 50 mL tube with another 🧪 2.5 mL PBS and transfer to 🧪 5 mL tube. Repeat spin at 🌀 800 x g for 2 minutes.



## Note



**Transferring to a smaller volume tube helps with re-suspension in a small volume of BME2 in the next step.**




- 11 Aspirate as much supernatant as possible. Re-suspend cell pellet in appropriate amount 80% BME2 (  200  $\mu\text{L}$  per well of a 6 well plate).

## Note






**BME2 must be dispensed as quickly as possible as it will begin to set at room temperature. A cool block could be used to help keep the temperature down while plating.**

## Note

**Volume of BME2 to re-suspend in must be determined from size of cell pellet. Aim to plate cells as close together as possible. If unsure re-suspend in a small volume. Pipette one or two  15  $\mu\text{L}$  -  20  $\mu\text{L}$  droplets and check under the microscope. If too dense increase BME2 volume.**

- 12 Using a P200 pipette, dispense organoid/BME2 suspension as small  15  $\mu\text{L}$  -  20  $\mu\text{L}$  droplets into a 6 well plate (seed  200  $\mu\text{L}$  per well).



- 13 Place in a  37 °C incubator (5% CO<sub>2</sub>) for 15-30 minutes to allow BME2 to set.
- 14 Prepare media containing antibiotics and Y-27632 (rock inhibitor). Add volumes below per ml of appropriate culture media:
-  2 µL primocin
  -  10 µL penicillin streptomycin
  -  1 µL Y-27632
- 15 Add  2 mL of appropriate prepared media per well of a 6 well plate.
- 16 Return to incubator. Media change twice a week until ready to passage. Keep in media containing antibiotics and Y-27632 until first passage.

## Full Process Video

1h

17

