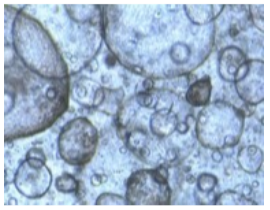
Jul 07, 2020

Passaging cancer organoid cultures



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Cellular Generation and P… Organoid and Assembloid

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Protocol status: Working
We use this protocol and it's working

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Abstract

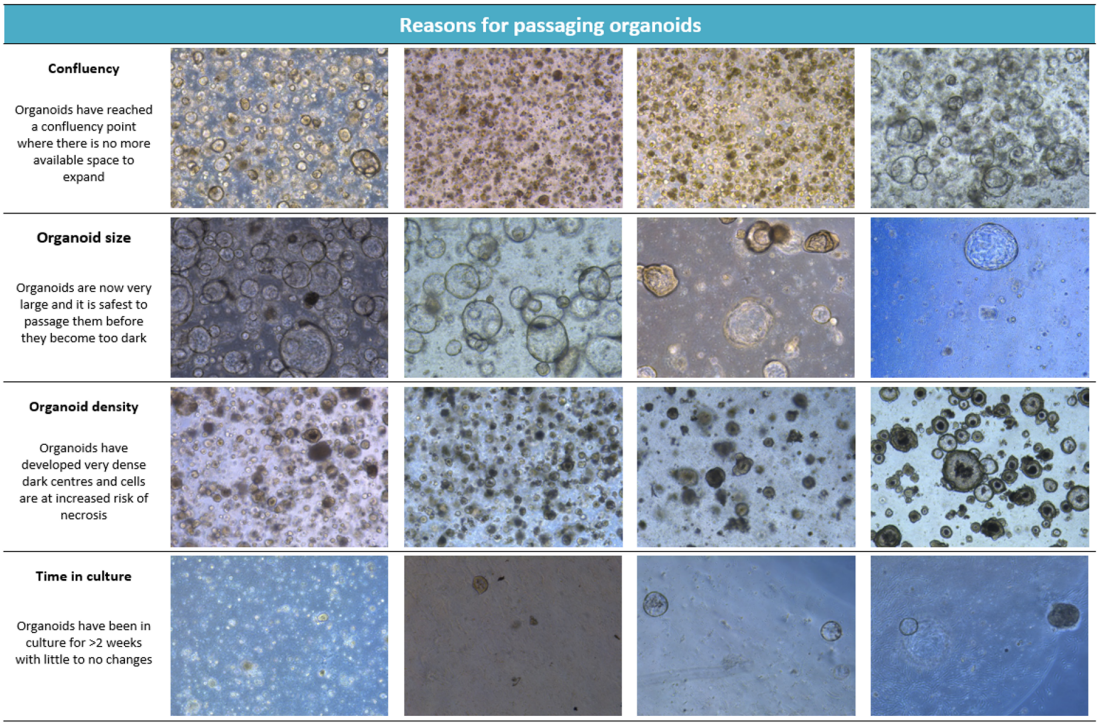
This protocol describes the passaging of organoid cultures. It has been developed by the organoid derivation team within the Cellular Generation and Phenotyping Group at the Wellcome Sanger Institute. The team has extensive experience passaging and expanding organoid models. The method described has mainly been used for the passaging cancer organoids with successful propagation of organoids derived from colon, pancreas and oesophageal tumours.

Guidelines

General Tips

- We generally use low split ratios of 1:3 or 1:2 with our organoid cultures. We will also passage to the same or a lower number of wells if organoids are not growing (see troubleshooting below).
- 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.
- Cold PBS is helpful for resolving some pelleting issues (see toubleshooting below). Therefore it is useful to keep a bottle of PBS in fridge.

Reasons for passaging organoids



Troubleshooting

Problem	Possible Solution
Organoids not breaking down in TrypLE (after approx. 20 min).	Spin down the suspension, remove old TrypLE, add fresh TrypLE and incubate at 37°C.
Organoid suspension not pelleting during centrifugation.	Aspirate as much of the supernatant as possible, re-suspend in ice cold PBS and repeat centrifugation step.
Organoid suspension still not pelleting.	Aspirate as much of the supernatant as possible, top-up with TrypLE, pipette up and down and repeat centrifugation step.
Organoids sticking to bottom of the plate rather than being suspended in BME2.	When setting BME2 in incubator post plating, invert the plate. Additionally 100% BME2 rather than 80% can be used.
BME2 is setting too quickly whilst plating.	Try keeping your solution in a cooling rack whilst plating.
Organoids are not growing very well.	Try plating organoids in a smaller number of wells so they are closer together. This often helps their growth.
Droplets of BME2 have lifted from the well or have broken down.	If line does not need passaging, re-plate without breaking down the organoids. If passaging, harvest the media and droplets in a tube, spin then aspirate the media and re-suspend in TrypLE. Proceed with passaging protocol.
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.
Line was growing well but is now dropping off after expansion.	Your cells may have been plated too far apart. Passage line to a smaller volume to see if this will help it recover.

Materials

MATERIALS

Costar® 6-Well Flat-Bottom Plate, Tissue Culture-Treated 50 Plates **Stemcell Technologies Catalog #**38015

TrypLE™ Express Enzyme **Thermo Fisher Scientific Catalog #**12604013

Fisherbrand™ Cell Scrapers **Fisher Scientific Catalog #**11587692

Falcon™ 15mL Conical Centrifuge Tubes **Fisher Scientific Catalog #**14-959-53A

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

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

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

Equipment

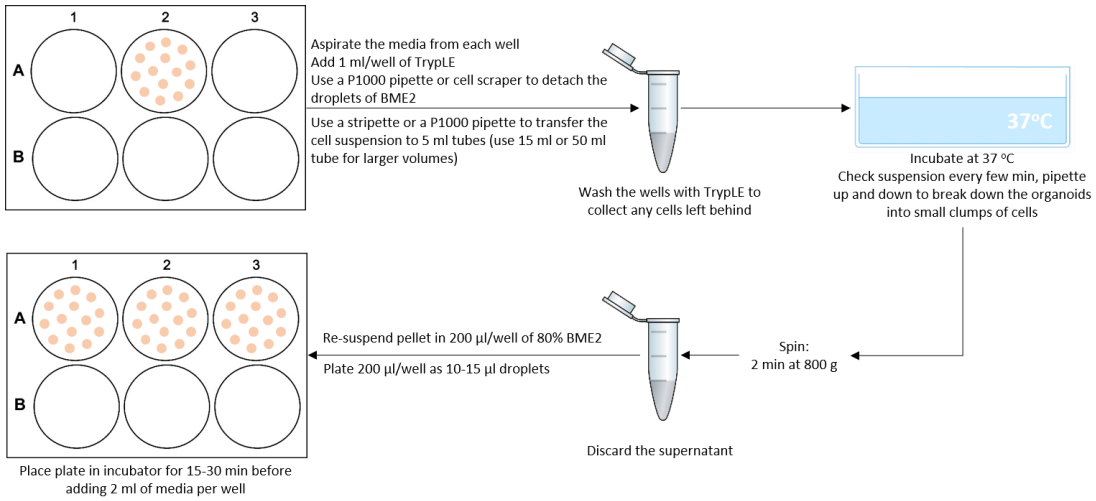
- Sterile cell culture hood
- Centrifuge
- 1000 µl and 200 µl pipettes and tips
- Pipetteboy
- Stripettes
- 37^{o}C waterbath
- 37^{o}C humidified incubator (5% CO_2)
- Light microscope

Before start

- Thaw BME2 aliquot overnight at 4 °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 °C incubator
- Pre-warm organoid culture media to room temperature

Process Diagram

1



Protocol

2 Aspirate media from wells and add 1 mL or 2 mL TrypLE to each well.

> **Note**
>
> **If any of the following are true collect organoids and media in a tube and centrifuge to pellet before adding TrypLE**
> 1. **BME2 droplets detached from plate and floating in media**
> 2. **Lots of cells or organoids floating in the media**
> 3. **Lots of fibroblasts attached to the plate**

3 Using a P1000 pipette, detach BME2 drops from the plate. (Optional - detach BME2 drops using a cell-scraper; recommended for passaging a large number of plates).

4 Pipette suspension up and down multiple times to dissociate organoids from BME2 and transfer to an approproate size tube using a P1000 pipette or 10 mL stripette.

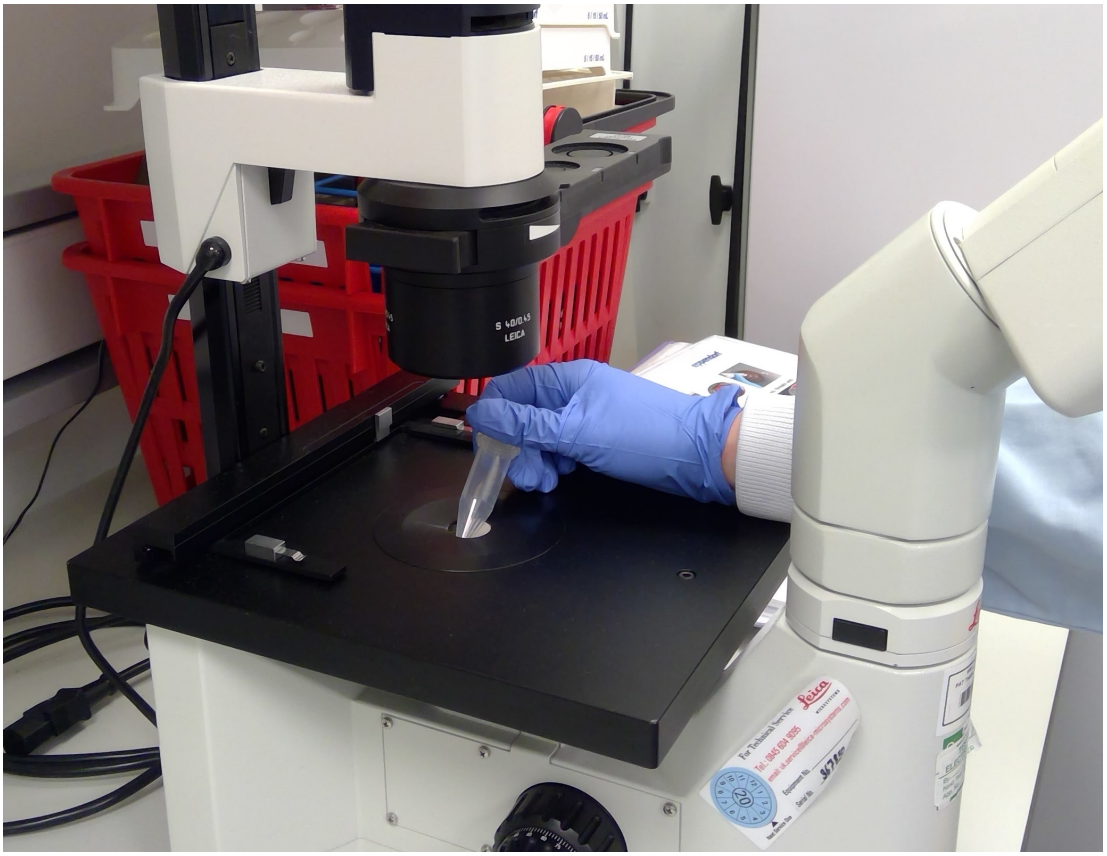
5 Wash wells with TrypLE (approx 1 mL per well of a 6 well plate) to collect any remaining organoids and add to the collection tube.

6 Incubate at 37 °C

7 Check organoid suspension under microscope after 5 minutes and then as required to assess the dissociation of organoids. Use a P1000 to pipette the cell suspension up and down to help dissociate organoids. Stop incubation when organoids broken down to small clumps of cells.

Note

Avoid breaking organoids down to single cells



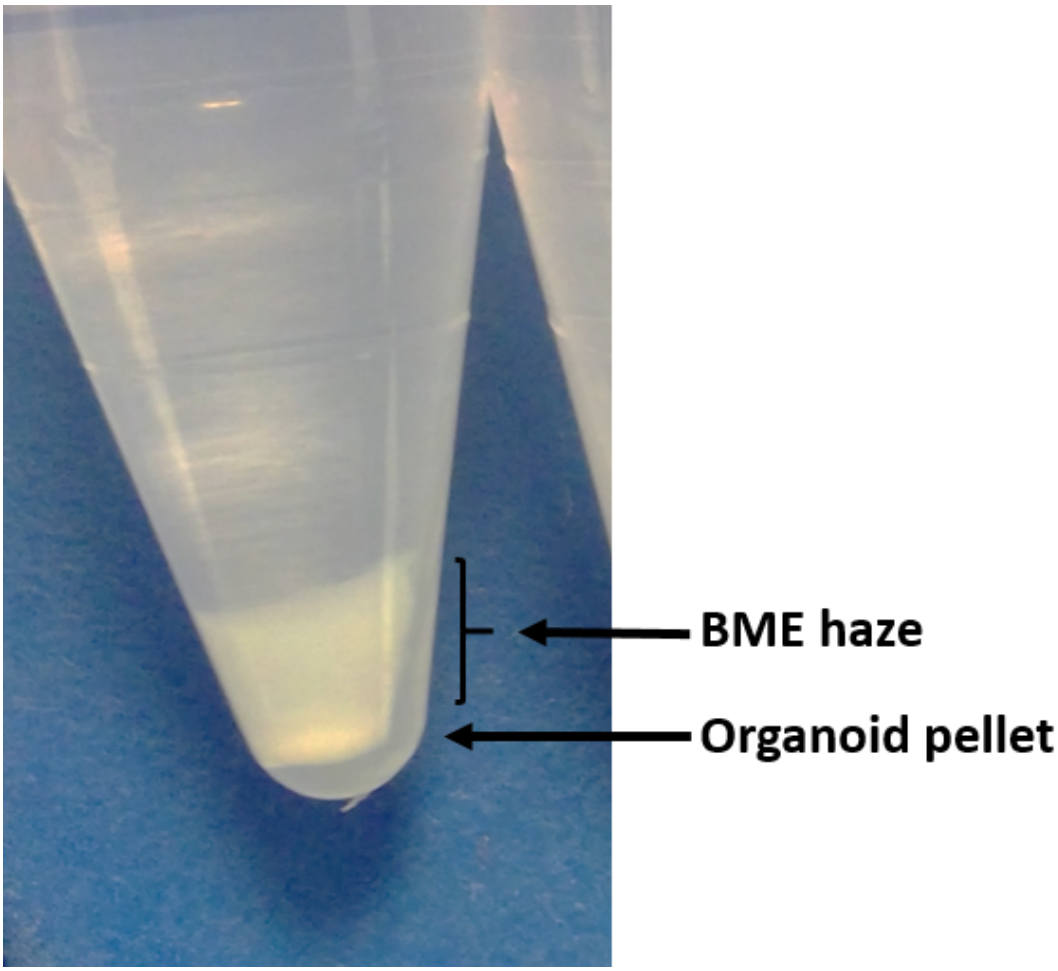
Check if organoid suspension has broken down to small clumps of cells on a microscope.

8 Centrifuge at 800 x g for 2 minutes.

9 Aspirate supernatant to leave organoid cell pellet.

Note

If the organoid suspension does not pellet during centrifugation or there is a grey haze above the pellet (residual BME2), aspirate as much supernatant as possible. Re-suspend in ice cold PBS and repeat centrifugation.



BME2 layer above prganoid pellet after centrifugation

10 Re-suspend pellet in appropriate amount of 80% BME2 (200 µL per well of a 6 well plate).

Note

Start by re-suspending pellet in a small volume to break down, then add additional BME2 to required volume.

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

Avoid creating bubbles while re-suspending pellet.

11 Using a P200 pipette dispense a total of 200 µL organoid/BME2 suspension per well of a 6 well plate as 10 µL - 15 µL droplets.

12 Place in a 37 °C incubator (5% CO_2) for 15-30 minutes to allow BME2 to set.

13 Add 2 mL of appropriate organoid media per well of a 6 well plate.

14 Return to incubator. Media change twice a week until ready to passage.