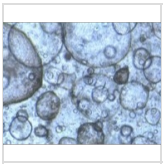

Aug 03, 2020

Thawing an organoid cryovial

Emily Souster[1], Hazel Rogers[1], Laura Letchford[1], Sara Vieira[1], Maria Garcia-Casado[1], Mya Fekry-Troll[1], Charlotte Beaver[1], Rachel Nelson[1], Hayley Francies[1], Mathew Garnett[1]

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1 Works for me dx.doi.org/10.17504/protocols.io.bh3jj8kn

Cellular Generation and Phenotyping

Emily Souster

ABSTRACT

This SOP defines the procedure for thawing a frozen cryovial of organoids into 1 well of a 6 well plate for further culture. 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 cancer organoids with successful propagation of organoids derived from colon, pancreas and oesophageal tumours.

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GUIDELINES

- 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.
- It is useful to keep a bottle of cold PBS in the fridge as this can be used to resolve pelleting issues. Resuspending a 'hazy' pellet in cold PBS can help to re-melt the BME2, resulting in a more distinct cell pellet after centrifugation.

MATERIALS

NAME	CATALOG #	VENDOR
Costar® 6-Well Flat-Bottom Plate, Tissue Culture-Treated 50 Plates	38015	Stemcell Technologies

NAME	CATALOG #	VENDOR
DPBS no calcium no magnesium	14190144	Thermo Fisher Scientific
Cultrex® Reduced Growth Factor Basement Membrane Matrix Type 2 (BME 2)	3533-010-02	Trevigen
Eppendorf tube- 5ml	0030122321	Eppendorf
Y-27632 dihydrochloride (made to 10mM stock concentration)	Y0503	Sigma Aldrich

MATERIALS TEXT

Equipment

- Sterile cell culture hood
- Centrifuge
- P1000, P200, P20 pipettes and tips
- 37^{O}C waterbath
- 37^{O}C humidified incubator (5% CO2)

SAFETY WARNINGS

Y-27632 is harmful if swallowed, inhaled or splashed on skin. Ensure correct use of PPE e.g. gloves, lab coat, safety glasses.

BEFORE STARTING

- Thaw an aliquot of Basement Membrane Extract type 2 (BME2) on ice, overnight at **4 °C** .
- Ensure 6 well plates have been stored overnight in **37 °C** incubator.
- Pre-warm 4ml of tissue specific organoid media in a 5ml eppendorf tube to **37 °C** (media can be placed in a **37 °C** waterbath for 10 minutes).

Process Diagram

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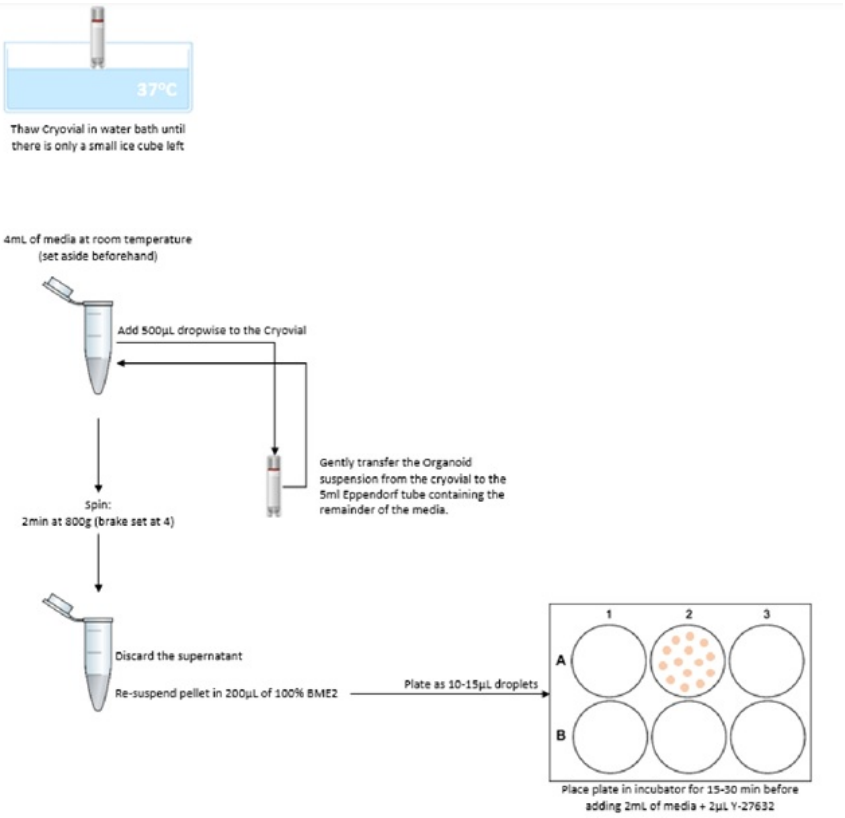

Protocol

2 Remove cryovial from liquid nitrogen storage and place on dry-ice for transfer to cell culture lab.

3 Thaw the cryovial in a **37 °C** waterbath until only a small ice crystal remains.

4 Add 500µl of the warmed organoid media, dropwise, to the vial and then transfer everything gently into the 5ml eppendorf tube containing the remainder of the warm organoid media.

5 Centrifuge at **800 x g** for 2 minutes (with the brake set on 4).

Centrifuge buckets must be sealed using safety caps, which must only be opened in a microbiological safety cabinet.

6 Aspirate the supernatant to leave the organoid cell pellet.

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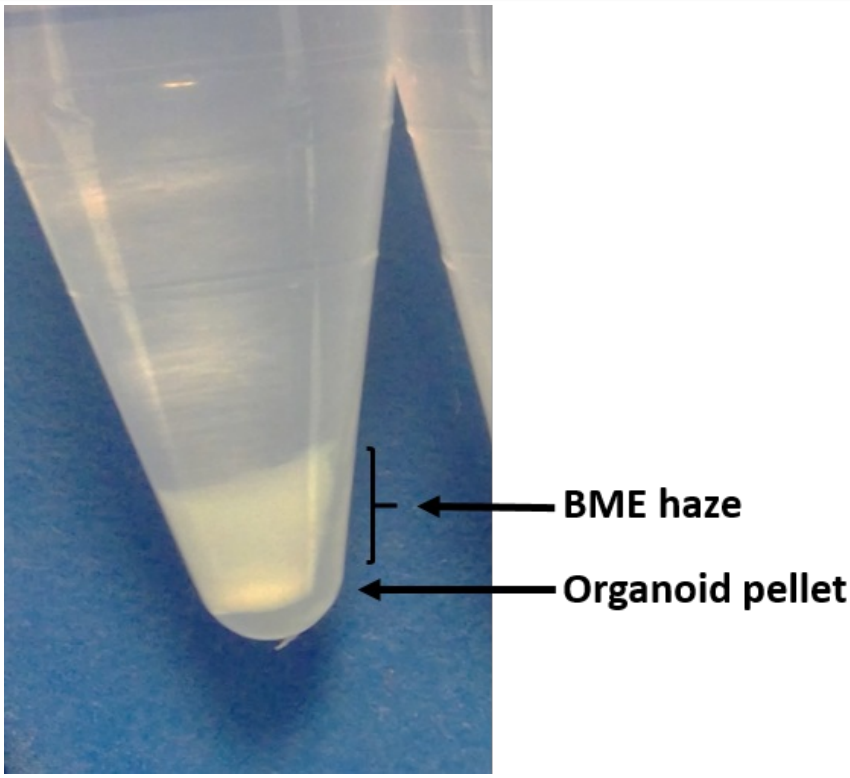
Sometimes after centrifugation, a BME2 'haze' will remain above the pellet. If this has happened, or if the culture has not pelleted fully or correctly, aspirate as much of the supernatant as possible and top up with ice cold PBS. Centriuge the culture again at 800xg for 2 minutes (brake set to 4).



BME2 layer above organoid pellet after centrifugation.

7 Resuspend the organoid pellet in 200µl of 100% BME2, being careful not to introduce bubbles (200µl is the recommended volume of 100% BME2 for thawing a single cryovial).

It is important that 100% BME2 is used here. When plating out whole organoids, 100% BME2 helps to prevent the organoids sinking and attaching to the surface of the plate (whereas 80% BME2 is used when plating dissociated cells in further culture).

BME2 must be dispensed as quickly as possible as it will begin to set as it reaches room temperature. The stock must be returned to ice as soon as possible.

A cool block can be used to help keep the temperature down while plating.

8 Using a P200 pipette, dispense 200µl organoid/BME2 suspension as small 10-15µl droplets onto 1 well of a warmed 6 well plate.

Place the plate upside down in a **37 °C** incubator for 15-30 minutes to allow BME2 to set.

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Inverting the plate ensures that the large organoids are well dispersed through the BME2 blob as it sets, rather than sinking to the bottom.

9 While waiting for BME2 to set,

Thaw an aliquot of 10mM Y-27632 (ROCK inhibitor).
Prepare an aliquot of organoid media for the appropriate number of wells (2ml per well).
Add Y-27632 to the media (1µl Y-27632 per 1ml media to achieve a final concentration of 10µM).

Y-27632 is harmful if swallowed, inhaled or splashed on skin.

10 After the incubation period, remove the plate from the incubator and add 2ml organoid media containing Y-27632 to each well.

Return plate to a **37 °C** , 5% CO_2 incubator, right side up.

Citation: Emily Souster, Hazel Rogers, Laura Letchford, Sara Vieira, Maria Garcia-Casado, Mya Fekry-Troll, Charlotte Beaver, Rachel Nelson, Hayley Francies, Mathew Garnett (08/03/2020). ThawingÃÂ an organoid cryovial. https://dx.doi.org/10.17504/protocols.io.bh3jj8kn