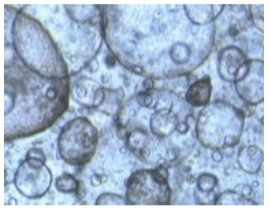
Aug 03, 2020

Cryopreservation of organoid cultures



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Cellular Generation and P… | Organoid and Assembloid

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We use this protocol and it's working

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Abstract

This protocol defines the procedure for cryopreservation 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 cancer organoids derived from colon, pancreas and oesophageal tumours.

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.
- Appropriate freezing containers will ensure that the liquid freezes at a controlled rate of around -1 °C per minute at -80 °C .

Materials

MATERIALS

DPBS, no calcium, no magnesium **Thermo Fisher Catalog #**14190136

Eppendorf tube- 5ml **Eppendorf Catalog #**0030122321

Recovery Cell Freezing Media **Gibco - Thermo Fisher Catalog #**12648-010

Nunc™ Biobanking and Cell Culture Cryogenic Tubes **Thermo Fisher Scientific Catalog #**375418

Nunc™ Cell Scrapers **Thermo Fisher Scientific Catalog #**179693

Equipment

- Sterile cell culture hood
- Centrifuge
- P1000, P200, tips
- Cell counting equipment
- CoolCell or appropriate freezing container
- -80^{O}C freezer
- Liquid Nitrogren storage

Before start

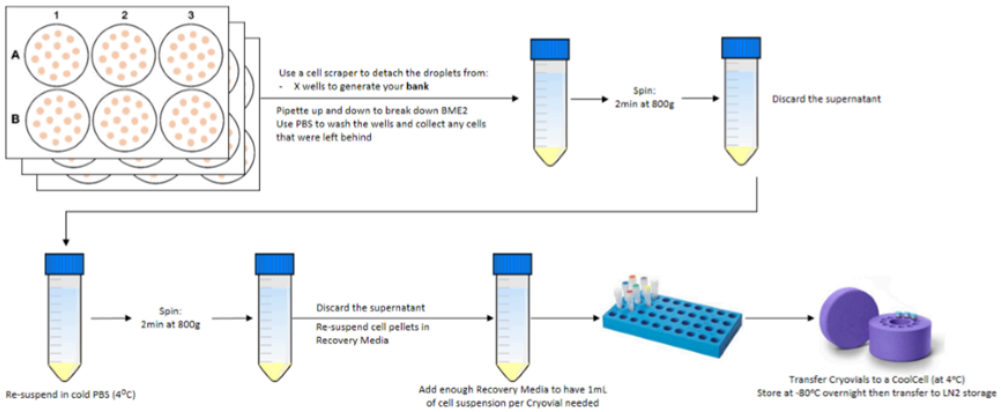
- Before cryopreservation of the line, ensure the organoids are of good quality and avoid banking organoids that have become very large or dark.
- Place a labelled CoolCell or appropriate freezing container in the fridge and make sure it is at 4 °C before commencing work.
- Thaw an appropriate amount of freezing media (1ml per cryovial) and keep at 4 °C before use.

Note

The freezing media can be kept at 4 °C for a maximum of 7 days.

Process diagram

1 **Banking of organoid cell cultures:**



Protocol

2 Using a P1000 pipette, or a cell-scraper, detach BME2 drops from as many wells as required (generally 1 well into 1 cryovial- we recommend aiming for a density of $1x10^6$ cells per cryovial).

Note

If you plan to bank a specific number of cells, be sure to take a cell count at Step 7.

The count can be taken from the cell suspension, or alterntively you could detach a representative well at this stage, from which to take the count.

3 Transfer the organoid suspension to a 5ml Eppendorf or 15ml Falcon tube.

4 Wash the wells with cold PBS (~ 4 °C) to collect any remaining organoids and add to the collection tube.

Make sure organoids are dissociated from BME2 drops by aspirating up and down multiple times with a stripette.

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

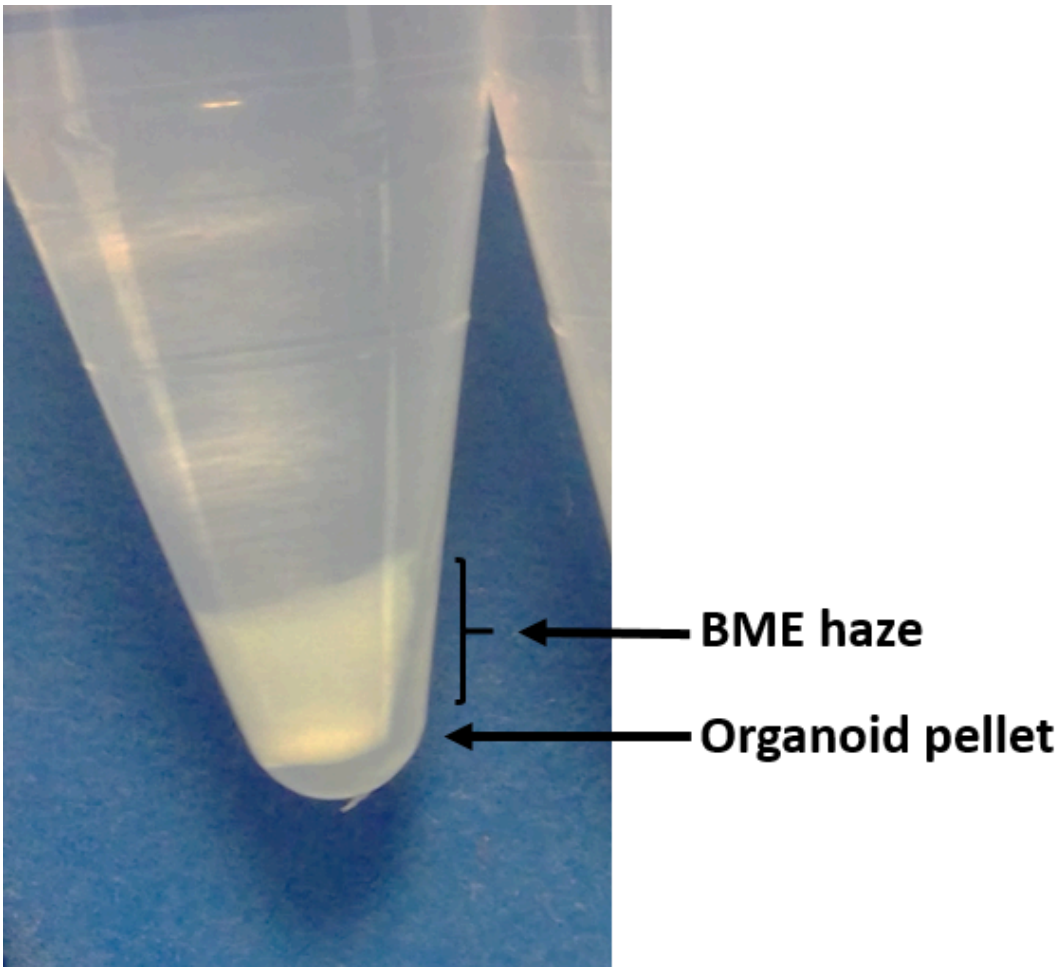
Safety information

Centrifuge buckets must be seales using safety caps, which must only be opened inside a microbiological safety cabinet.

6 Aspirate off the supernatant, leaving the organoid cell pellet at the bottom of the tube. Organoid cell pellets are loose pellets so take care when aspirating.

Note

If the pellet is not clear, aspirate as close to the BME2 'haze' as possible and re-suspend in cold PBS, spin down again (800 x g for 2 minutes, brake set on 4) and then aspirate the supernatant.



BME2 layer above organoid pellet after centrifugation.

7 If you **do not** want to bank a specific number of cells, proceed to Step 8.

If banking a specific number of cells, either take a cell count from your whole organoid suspension, or from a 'representative well' by resuspending the pellet in an appropriate amount of organoid media. Take a cell count from this suspension.

From this cell count you can calculate how much organoid suspension to use to freeze the desired cell density.

Note

Organoids are typically counted as aggregates because they are difficult to break down to single cells.

We take two aggregate cell counts which much be within 10% of each other, otherwise a third count is taken and an average is calculated between the three counts.

We recommend freezing a cell density of $1x10^6$ cells per cryovial.

8 Resuspend the organoid pellet in as much Recovery Cell Freezing Media as required (1ml per cryovial) and transfer into the prelabelled cryovials.

9 Place the cryovials into a CoolCell or appropriate freezing container and place into -80 °C freezer.

Note

If there is an odd number of cryovials, use a 'balance' cryovial for better cryopreservation.

10 After 24 hours (up to 72 hours) transfer cryovials to liquid nitrogen storage.