Protocols for human breast cancer patient-derived organoids (PDO) (09/23)

(also see Dekkers et al. 10.1038/s41596-020-00474-1 for additional details)

Thawing breast cancer-derived organoids –

Materials required

1. Thawed Matrigel (Corning) or other BCM matrix
2. Advanced DMEM
3. Complete breast cancer organoid media
4. 1%BSA in PBS
5. Prewarmed 24 well plate
6. Swinging bucket rotor with attachments for 15 mL and 1.5 mL conical tubes
7. 37-degree water bath
8. 37-degree, prewarmed water bottle, warming block etc.

Process

1. Thaw the freezer vial in a clean 37 degree water bath
2. Using a pipette precoated with sterile 1%BSA in PBS (PBS-B) transfer media into a PBS-B coated 15 mL conical vial containing 5 ml sterile AD-DMEM
3. Precipitate organoids by centrifugation at 1200 RPM for 5 minutes
4. Aspirate 4.5 ml of DMEM, and resuspend organoids in 1.5 ml of Ad-DMEM
5. Transfer organoids into a PBS-B coated 1.5 ml conical tube and pellet organoids again in a swinging bucket rotor
6. Gently aspirate all but about 10 ul of DMEM taking care not to disturb the cell pellet
7. Gently resuspend cells in remaining media by gentle pipetting with a precoated 10uL pipette tip (keep pipette tip)
8. Add 30uL of thawed Matrigel to the resuspended cells
9. Using a 10ul tip, gently mix the cells into the matrigel without creating bubbles
10. Using a 10 ul tip, add cells to prewarmed a 24 well plate in small Matrigel domes (5-10uL estimate) (keep the plate on top of warming block/water bottle to help Matrigel solidify)
11. Invert plate to keep cells suspended in Matrigel domes
12. Return the plate to the incubator and put warming block on top
13. After 20 minutes of incubation, add appropriate pre-warmed organoid media to Matrigel domes and return to the incubator

Feeding breast cancer-derived organoids

1. Organoids should be fed 2x/week
2. Prewarm media to limit Matrigel dissociation
3. Gently aspirate current media
4. Replace with fresh media
5. If Matrigel is dissociating (floating) should proceed to “passaging organoids steps” and use Cell Recovery solution method to gently remove degraded Matrigel for replating

Passaging organoids

Materials required

1. TrypLE express (for dissociating organoids into single cells)
2. Cell recovery solution (for keeping organoids in tact during passaging) (Corning)
3. Thawed Matrigel (Corning) or other BCM matrix
4. Advanced DMEM
5. Complete breast cancer organoid media (prewarmed to 37 degrees)
6. 1%BSA in PBS
7. Prewarmed 24 well plate
8. Swinging bucket rotor with attachments for 15 mL and 1.5 mL conical tubes
9. 37-degree water bath
10. 37-degree, prewarmed water bottle, warming block etc.

Procedure

1. Remove medium by aspiration being careful not to disturb Matrigel droplets
2. For organoid dissociation using TrypLE express
    a. Add TrypLE express directly to wells (0.25mL/24 well, 0.5mL/12 well)
    b. Return to the incubator for 5-10 minutes
    c. Using a 1 ml tip precoated with PBS-BSA disperse Matrigel and organoids by pipetting up and down
    d. Visualize under a microscope to ensure organoids are dissociated,
    e. If not return the plate to the incubator for 2-5 more minutes and repeat the dissociation
    f. After all organoids are dissociated add them to a precoated 1.5mL tube on ice
    g. Wash wells with Media, PBS, or TrypLE express to ensure all organoids are recovered from the well
    h. Add wash to 1.5mL tube with dissociated organoids
3. For organoid preservation and release using a cell recovery solution
    a. Add 125-250uL cold cell recovery solution to each well and disrupt Matrigel by pipetting up and down
    b. After all organoids are released from the plate add them to a precoated 1.5mL tube on ice
    c. Wash wells with cell recovery solution to ensure all organoids are recovered from well
    d. Incubate Matrigel-organoid mixtures on ice for 30 minutes with gentle shaking
    e. Pellet (1200RPM, 5 minutes, 4 degrees) and check for residual Matrigel (fluffy cloudy precipitate typically above the cell pellet)
    f. If residual Matrigel is present, continue dissociating on ice, (can add fresh Cell recovery solution to speed this process up)
    g. Check again in 10-15 minutes- repeat until no Matrigel is visible
4. Wash cell/organoid pellet with Ad-DMEM/F12
5. Spin down pellet at 1200RPM 5 minutes 4 degrees in a swinging bucket rotor
6. Repeat wash (steps 4 and 5)
7. If needing to count cells for the experiment – count and aliquot at this point, pellet cells as above
8. Aspirate media as possible from the cell pellet being careful not to disrupt the pellet (A 20uL pipette is helpful for this) (can leave 10-15uL or more for even larger pellets)
9. Resuspend cells in media with a PBS-BSA precoated tip
10. Add an appropriate amount of Matrigel and gently pipette up and down to mix being careful to not introduce bubbles
11. Plate Matrigel-organoids onto a prewarmed dish in 10uL droplets (rest plate on a stable warming pad or block to help keep the plate warm- especially when working with larger volumes)
    a. Check droplets to ensure that further dilution with more Matrigel is not needed
12. Invert the dish and set in the incubator with a warming pad on top for 30 minutes
13. Add fresh media as needed and return to the incubator

Culture Media Composition:

Component	Media 1	Media 2
Wnt3a	—	20% conditioned medium*, **
R-spondin1	10% conditioned medium*	10% conditioned medium*, **
Noggin	10% conditioned medium*	10% conditioned medium*, **
B27 + VitA	1×	1×
Nicotinamide	10 mM	10 mM
N-acetylcysteine	1.25 mM	1.25 mM
Primocin	100 µg/ml	100 µg/ml
Hydrocortisone	—	0.5 µg/ml
β-estradiol	—	100 nM
Forskolin	—	10 µM
Y-27632* **	5 µM	5 µM
Heregulin B1	5 nM	5 nM
FGF-7	5 ng/ml	—
FGF-10	20 ng/ml	20 ng/ml
A83-01	0.5 µM	0.5 µM
EGF	5 ng/ml	5 ng/ml
SB202190	1 µM	—

* can be substituted with purified growth factors (RSPO3 – 250ng/mL, Noggin – 100ng/mL, Wnt3 – 0.2nM)
** we have had success replacing Wnt, Noggin, RSPO conditioned media with 10% conditioned media derived from the L-WRN cell line for Media 2
*** - Y-27632 is only required for the first day after thawing - but has a very short half-life at 4 degrees, so should be added fresh day of use when thawing organoids