Enteroids/colonoids from cryopreserved biopsies

Last updated: 12/12/2023 (TK)

Reagents and critical equipment:

- Recovery wash: DPBS + 10% FBS
- Liberase TH (Sigma #5401135001): for a stock solution of 13 Wunsch units (WU)/mL (2.5 mg collagenase/mL), reconstitute 1 vial in 2 mL sterile HBSS, store 100 aliquots at -20ºC. After thawing one 100 µL tube, make 15 µL aliquots and freeze. After thawing a 15 µL aliquot, discard the leftovers and don't refreeze.
- DNAse I (Roche # 10104159001): reconstitute 100 mg in 4 mL sterile PBS, yielding a 50U/mL solution (100x). Aliquot 100 µL/tube and store at -20ºC
- Matrigel (Corning #354234): keep on ice! After thawing the 10 mL bottle (on ice, will take a while - best to do overnight), aliquot 500 µL/Eppendorf tube (pre-chill the tubes on ice) and store at -20ºC
- Organoid growth medium formulated with high WNT activity for line expansion. A reliable commercially available reagent is Human IntestiCult Organoid Growth Medium (StemCell # 06010): we recommend thawing the OGM Basal Medium and Supplement (one bottle each) aliquoting 5-10 mL/conical and storing at -20ºC. Do not freeze-thaw after this. To prepare complete IntestiCult:
    - Combine equal volumes (e.g. 5 mL) each of OGM Basal Medium and Supplement
    - ROCK inhibitor (Y-26732, to 10 µM final concentration) should be used through entire P0, then for the first feeding of every passage
    - Prophylaxis: use Primocin in P0-P1, then switch to Anti-anti after seeding P2
- Y-27632 (Selleck # S1049): to make 50 mM solution, reconstitute 50 mg in 3.1mL sterile DMSO, dispense into 500 µL aliquots. Once thawed, dispense each into 20x25 µL aliquots to minimize freeze-thaw cycles. Store at -20ºC
- Primocin (Invivogen # ant-pm-05): Comes as 500x concentrate, aliquot 50 µL/tube to minimize freeze-thaw cycles and store at -20ºC
- Anti-anti (Thermo Fisher 15240062)
- Sterile HBSS+4%BSA or DPBS+4%BSA
- ThermoMixer (Eppendorf 05-412-501)

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Protocol:

If working with a fresh biopsy, begin with Step 3 (in this case, DPBS or HBSS can be used instead of the recovery wash solution).

1. Quickly thaw the cryovial in a 37°C water bath
    - *If using a bead bath, place a small beaker with pre-warmed water into the beads and put the cryovial in the beaker*
2. Transfer the thawed suspension to an Eppendorf tube
3. Spin 700g – 90 s and discard the supernatant
4. Rinse the tissue fragments 3 times:
    1) Resuspend in 1 mL recovery wash solution, mix by inverting the tube
    2) Spin 700g – 30 s and discard the supernatant
5. *Only if the biopsy was cryopreserved as a single piece: mince the tissue fragments with sterile scissors, spin briefly and remove supernatant*
6. Resuspend the pellet in 0.5 mL working solution of Liberase TH (1:100 of stock-> 0.13 WU/mL) + DNAseI (1:100 of 50 U/mL stock) in HBSS
7. 37°C – 10 m (thermomixer, 800RPM)
    - During the incubation, prepare a 100 μm strainer by placing it in a 50 mL conical tube and applying 8 mL of HBSS+4%BSA
8. Spin the tube with tissue briefly and flow the supernatant through the prepared strainer (keep this tube on ice until all tissue is digested)
9. Resuspend the pellet in another 0.5mL of Liberase+DNAse
10. 37°C - 10 m (thermomixer, 800RPM)
11. Pass 10 times through a P1000 tip, spin briefly
12. Flow the supernatant through the strainer from step 5
13. Resuspend the pellet in another 0.5mL of Liberase+DNAse
14. 37°C - 10 m (thermomixer, 800RPM)
15. Pass 10 times through a P1000 tip (at this point, the remaining tissue fragments, if any, should be very small)
16. Transfer the digested mix onto the strainer from step 5
17. Use a plunger from 1 mL syringe to force the larger fragments through the strainer
18. Pellet the cells: 500g – 5 m, discard the supernatant
19. Reconstitute the pellet in 500 µL of HBSS-4%BSA and count the cells using Trypan Blue exclusion
20. Seed up to 10^5 live cells per dome of 50 µL 80% Matrigel (e.g. 10 µL of cell suspension [10^7 cells/mL] mixed with 40 µL Matrigel) into one well of a pre-warmed 24-well plate
21. Let the domes solidify at 37°C for 30 m (incubator), then add 500 µL/well of pre-warmed complete organoid growth medium
22. Change media every other day (ex. Monday, Wednesday, Friday), monitor organoid formation and growth
23. The organoids are ready for harvest/passage on day 11-14 of culture.