Freezing and Resurrecting Gastrointestinal Organoids & Biopsies
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Freezing Intestinal Biopsies

1. Prepare intestinal biopsy freezing medium (recipe below)
2. Prepare cryovial with any critical information about patient
3. Add 1mL freezing medium to cryovial
4. Remove biopsy from storage medium with P1000, eject into cryovial
5. Place cryovial in Mr. Frosty/Corning CoolCell or any slow freezing device, put in -80C overnight
6. After 24hrs move vial to liquid nitrogen storage

Freezing Organoid Lines

1. Prepare appropriate organoid freezing medium (recipe below)
2. Prepare cryovial with any critical information about patient
3. Aspirate medium from wells that will be frozen
   Note: For typical plating density, use 3 wells of a 24-well plate per 1mL cryovial. For less dense cultures consider 4-6 wells per tube.
4. Distribute 1mL of freezing medium across the number of wells per tube to be frozen
5. Gently scrape Matrigel from plate to suspend it in the freezing medium and move to cryovial
6. Place cryovial in Mr. Frosty, Corning CoolCell, or any slow freezing device, put in -80C overnight.
7. After 24hrs move vial to liquid nitrogen storage

Intestinal Biopsy Freezing Medium
90% FBS
10% DMSO

Intestinal Organoid Freezing Medium
70% Organoid Culture Medium (SI medium for duo, Colon medium for colon, rectum)
20% FBS
10% DMSO

Processing Frozen Intestinal Biopsies

1. Thaw 1mL of Collagenase Type I, move to 15mL conical tube
2. Prepare a second 15mL conical tube with 3-4mL Advanced DMEM/F-12
3. Thaw frozen biopsy in water bath for ~2 minutes until small piece of ice remaining
4. With P1000 remove biopsy from tube and place into tube of Advanced DMEM/F-12
   Note: Important to help dilute residual DMSO from freezing
5. Remove biopsy from medium and place into tube of Collagenase Type 1
6. Break up and pipette biopsy piece until fragments easily pass through P1000 tip
7. Incubate in 37C water bath for 40 minutes
8. Remove from water bath and pipette up and down ~25x to break up tissue and dissociate crypts
   Note: There may be residual fat and tissue left after this step
9. Dilute Collagenase with 1mL Advanced DMEM/F-12, spin at 500 x g for 5 minutes at 4C
10. Aspirate supernatant, add appropriate amount of Matrigel
    Note: For small biopsies ~150-200uL Matrigel, for larger pieces add ~300uL
11. Resuspend pellet in Matrigel, plate 50uL droplets into each well of 24-well plate. Incubate in 37C/5% CO2 incubator for at least 10 minutes to polymerize.
12. Add 500uL culture medium with 1000X ROCK inhibitor (Y-27632) to each well
    Note: 3mL culture medium + 3uL Y-27632 for 6 wells
    Note: Frozen biopsies will take longer to form organoids at first

Resurrecting Frozen Organoid Lines

1. Per vial to be thawed, prepare 2-4mL aliquots of appropriate culturing medium. Pre-warm in 37C water bath.
2. Thaw frozen organoids in water bath for ~2 minutes until small piece of ice remaining
3. With a P1000 move thawed organoids to 1 tube of pre-warmed culturing medium. Pipette up and down gently to dislodge organoids from residual Matrigel
4. Spin at 500 x g for 5 minutes at 4C
5. Aspirate supernatant
   Note: Due to the residual Matrigel from the freezing procedure, organoids may not pellet as easily as normal. In this case, aspirate as much as possible while minimizing loss. The new Matrigel addition will dilute the residual medium enough to not affect polymerization or culturing.
6. Add 150-300uL Matrigel to tube, depending on freezing density, and resuspend organoids gently without mechanically breaking them up
7. Place 50uL droplets into each well of 24-well plate. Incubate in 37C/5% CO2 incubator for at least 10 minutes to polymerize.
8. Add 1000X ROCK inhibitor (Y-27632) to second aliquot of pre-warmed culturing medium. Add 500uL medium to each well.

Reagents:

Collagenase Type I, 2mg/mL in HBSS (Life Technologies)
Advanced DMEM/F-12 (Life Technologies)
FBS (Life Technologies)
DMSO (Millipore-Sigma)
Matrigel GFR PR-free Basement Membrane Matrix (Corning)
Y-27632, 10mM (Tocris)