Human Gastrointestinal Organoid Culture
HDDC Organoid Core, Breault Lab
Updated 10/23/2019

Important Notes/Considerations

- Matrigel should be kept at 4C or on ice at all times. Once polymerized, the Matrigel will not re-liquify.
- It is very important to avoid bubble formation when working with Matrigel. Do not fully eject your pipette tip when working with Matrigel.
- Use antibiotics when culturing primary human tissue, as the biopsy or resection is likely to not be sterile during processing. Primocin and Pen/Strep are good options.
- Passaging ratio and frequency is heavily dependent on the cell line and needs of the researcher, so there are no set limits. Typically based on number of wells needed to be generated, and common ratios are 1:2, 1:3, and 1:4.
- Human enteroids have demonstrated to be karyotypically normal up to passage 25. Consider freezing low passage enteroids to maintain and karyotypically healthy line in the future.
- Consider testing your organoid lines regularly for mycoplasma contamination.
- Culture typically done in 24-well plates, but volumes can be adjusted for 48-well and 96-well plate layouts.

Organoid Passaging (24-well plate format)

1. Aspirate medium from wells
2. Add 500uL of Cell Recovery Solution to each well. Resuspend Matrigel dome in solution and transfer to 15mL conical tube.
3. Incubate on ice for 1 hour.
4. Invert tube several times as organoids have likely sunk to the bottom. Spin at 500 x g for 5 minutes at 4C.
5. Aspirate supernatant, careful to not disturb pellet.
   Note: In some cases, after incubation, Matrigel may not be completely dissolved resulting in a pellet gradient dispersed in Matrigel at the bottom of the tube. Resuspend organoids and matrix in tube with P1000, and re-spin.
6. Add fresh Matrigel to tube based on how many wells that will be generated.
7. Bend/kink a P1000 tip and mechanically break up organoids in Matrigel at least 50x. This process fragments the organoids, with each fragment resulting in formation of a new organoid.
   Note: Set the pipet 150-200uL lower than Matrigel volume before breaking up to prevent bubble generation.
8. Seed 24-well plate with 50uL droplets into each well. Place in 37C/5% CO2 incubator for at least 10 minutes to polymerize Matrigel.
9. Add 500uL culturing medium to each well. Feed every 2-3 days and passage as needed.

Reagents
Cell Recovery Solution (Corning)
Matrigel GFR PR-free Basement Membrane Matrix (Corning)
Primocin (Invivogen)
Pen/Strep (Gibco/Life Tecnologies)