Human Gastrointestinal Tissue Processing for Organoids
HDDC Organoid Core, Breault Lab
Updated 10/24/2019

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.

Punch Biopsy Processing

1. Biopsies should be collected on ice in Advanced DMEM/F-12 medium, and processed or frozen within 24 hours (refer to freezing protocol)
2. Transfer biopsy from collection medium into 500uL Collagenase Type 1
   Note: With multiple biopsies or larger pieces, consider using 1mL Collagenase Type 1
3. Break up and pipette biopsy piece until fragments easily pass through P1000 tip
4. Incubate in 37C water bath for 40 minutes
5. 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
6. Dilute Collagenase with 1mL Advanced DMEM/F-12, spin at 500 x g for 5 minutes at 4C
7. Aspirate supernatant, add appropriate amount of Matrigel
   Note: For small biopsies ~150-200uL Matrigel, for larger pieces add ~300uL
8. Resuspend pellet in Matrigel, plate 50uL droplets into each well of 24-well plate. Incubate in 37C/5% CO_2 incubator for at least 10 minutes to polymerize.
9. Add 500uL culture medium to each well
   Note: Frozen biopsies will take longer to form organoids at first

Intestinal Resection Processing

1. Prepare a few 10cm dishes to use while processing. Autoclave forceps, scissors, and razor blade.
2. Remove resected tissue and wash vigorously in dish of cold Advanced DMEM/F-12
3. Move tissue to new dish and identify orientation. The quality and amount of tissue will vary piece to piece so it may be difficult to identify specific layers. All white fatty tissue should be removed as well as any residual muscle tissue, which is typically striated and denser. You will only want to have lamina propria, submucosa, and mucosa remaining for processing, color varying from pink to red depending on inflammation.
4. Once isolated, chop into biopsy size pieces ~0.5-1mm in size.
5. Break up and pipette biopsy piece until fragments easily pass through P1000 tip
6. Incubate in 37C water bath for 40 minutes
7. 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
8. Dilute Collagenase with 1mL Advanced DMEM/F-12, spin at 500 x g for 5 minutes at 4C
9. Aspirate supernatant, add appropriate amount of Matrigel
   Note: For small biopsies ~150-200uL Matrigel, for larger pieces add ~300uL

10. 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.
11. Add 500uL culture medium to each well
    Note: Frozen biopsies will take longer to form organoids at first
    Note: Due to the nature of resected tissue, there can likely be contamination of fibroblasts upon primary culture. Fibroblasts will migrate through the Matrigel and adhere to the bottom of the well plate. They should not transfer in subsequent passages.

Reagents
Cell Recovery Solution (Corning)
Matrigel GFR PR-free Basement Membrane Matrix (Corning)
Primocin (Invivogen)
Pen/Strep (Gibco/Life Tecnologies)
Collagenase Type I, 2mg/mL in HBSS (Life Technologies)
Advanced DMEM/F-12 (Life Technologies)