Human Gastrointestinal Fibroblast Culture
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Fibroblast Isolation and Culture

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. Incubate biopsy pieces in Liberase TM in 37C water bath for 40 minutes
6. Dilute with 10mL Advanced DMEM/F-12. Pipette up and down ~25x.
7. Spin at 1000 x g for 5 minutes at 4C, aspirate supernatant. Wash the pellet with 10mL Advanced DMEM/F-12 and pipette up and down ~25x.
8. Spin at 1000 x g for 5 minutes at 4C, aspirate supernatant.
9. Resuspend pellet in 3mL Fibroblast Growth Medium and add to new T25 flask
10. Culture at in 37C/5% CO_2 incubator until confluent, feed every 3-5 days.
11. Check flask daily for fibroblasts and medium color changes. If isolation successful, fibroblasts will begin to crawl from tissue fragments and attach to the plate. This process may take 2-4 weeks.
12. Passage and freeze as necessary.

Reagents

Liberase TM (Sigma #5401119001) – 2mL H_2O per 5mg vial (13W/mL stock)
161.5uL aliquot diluted in 1338.5uL Ham's F12 (working concentration 1.4W/mL)

Fibroblast Growth Medium (100mL):
5mL FBS
4mL Pen-Strep
1mL Glutamax (100X)
Up to 100mL Advanced DMEM/F-12