**Preparation of Human Biopsies for Shipment and Crypt Culture Establishment**

1. Collect biopsies and store on ice in washing medium (see recipe below).
2. Within 1-2 hours, pour the medium with the biopsy sample into a 100 mm Petri dish. Use fine forceps to transfer the tissue to a new, clean 40 mm Petri dish. Place on ice or immediately begin Step 3.
   1. Note 1: We perform Steps 2-6 on the lab bench top. The use of a tissue culture hood is not required at these stages. The washing medium contains antibiotics, which typically contain the low level bacterial loads associated with the biopsy material.
   2. Note 2: Tools must be rinsed in bleach, rinsed in sterile water and then wiped down with an alcohol swab and allowed to briefly air dry before and after touching human samples. These steps prevent bacterial/yeast contamination of cultures. We typically pour bleach and sterile water into separate 100 mm Pertri dishes and use these solutions to rinse our tools.
   3. Note 3: All tools must be autoclaved after the experiment to ensure elimination of any potential pathogenic organisms. All disposable materials that came into contact with the human samples should be disposed of in the biohazard bin.
3. With fine dissection scissors, mince the biopsy tissue until pieces are small enough to be able to easily pipette with a P1000 tip. Scissors will be held near horizontal while cutting.
4. Add 1 mL washing medium to the tissue and use a P1000 pipette to transfer the minced tissue and medium to a 5 mL Eppendorf tube.
5. Add an additional 4 mL washing medium plus 10 µM ROCK inhibitor (Y-27632) (5 µL of a 10 mM stock) to fill the 5 mL Eppendorf tube.
6. Place on ice in a Styrofoam container. Ship O/N.

***Recipe for Washing Medium:***

DMEM/F12 with HEPES (SIGMA D6421-500ML) 500 ml

100X L-glutamine (SIGMA: G7513-100ML) 5 ml

100X Penicillin/Streptomycin (SIGMA: P4333-100ML) 5 ml

FBS (SIGMA: F6178) 50 ml

*Instructions for washing medium:* Can be stored at 4ºC for up to one month. It is possible to make ~40 mL aliquots in 50 mL conical tubes that can be stored at -20ºC for at least 6 months. These aliquots can be individually thawed and stored at 4ºC if desired.

***Other Materials:***

Eppendorf Tube® 5.0 mL, clear, Sterile, CS/200 (10x PK/20)

Company: Eppendorf

Cat No: 0030119487

Cost: $56

100 mm sterile Petri dish with clear lid (can be from any company)

Company: Fisher

Cat No: FB0875713 (case of 500)

Cost: $214.20

TPP Tissue Culture Dish (40 mm Petri dish)

Company: MidSci

Cat No: TP93040 (case of 900, 20/pk)

Cost: $399.69

Y-27632 dihydrochloride (10 mg)

Company: R&D Systems

Cat No: 1254/10

Cost: $225

*Preparation of Y-27632 inhibitor compound (MW = 329.27):* To make a 10 mM stock, dissolve 10 mg Y-27632 in 3.04 mL sterile H20. Aliquot 50-100 µL per 0.6 mL eppendorf tube. Store aliquots long-term at -80ºC. We keep one aliquot at a time at -20ºC (can withstand several freeze/thaw cycles) as our working stock. Final concentration to use in medium is 10 µM (a 1:1000 dilution).

**Contact and Shipping Information**

Send all biopsies after preparation to:

Kelli Van Dussen

Washington University

4940 Parkview place

Pathology/CSRB room 1020

St. Louis, MO 63110