Expt date:_____

Mice used:_____

Mouse primary intestinal myofibroblast prep

Reagents & Equipment:

- Mouse of choice (best to do no more than 2 mice per prep)
- DMEM + 10% FBS + P/S (Dulbecco's Minimal Essential Medium –H (high glucose) + 10% fetal bovine serum + 1% pen/strep); plan ~150 ml per mouse
- DMEM + P/S (Dulbecco's Minimal Essential Medium-H + 1% pen/strep, NO SERUM); plan ~200 ml/mouse
- 50 ml tubes
- 2 ml pipet or syringe with yellow tip (for flushing intestines)
- dissecting tools (especially – sharp small scissors)
- sterile petri dishes – whatever size is available (35 mm, 60 mm, whatever)
- sterile razor blades
- 75 cm^2 tissue culture flasks (2 per tissue)
- collagenase type I (Worthington Biochemicals, Freehold, NJ, Cat #CLS-1)
- shaker
- pipets (10 ml)

Procedure:

1. Dissect colon and/or small intestine.
2. Flush the tissue out with DMEM+P/S. If it gets stuck, you can smush it out with your finger, but be as gentle as possible in order to avoid damaging the tissue.
3. Open lengthwise with scissors and cut into 2-3 mm pieces. Transfer fragments to a 50 ml tube with about 10 ml media + P/S.
4. Shake or rock gently for 2-3 minutes. Allow to settle for a few seconds, then pipet off supernatant, replace with fresh media + P/S (no serum), and repeat. Shake 8 times total (roughly 20-25 minutes). After the last time, take off as much media as possible.
5. During this time – make up collagenase mix in DMEM + P/S:
   0.5 mg/ml collagenase type I
   Make about 5 ml per tissue sample in DMEM + P/S (no serum). Serum will inhibit collagenase activity so be sure to leave it out at this point.
6. Pour tissue into a small sterile dish and mince into a fine slurry with a sterile razor blade. Add 5 ml collagenase mix and transfer the tissue + media/collagenase into a 50 ml tube.
7. Shake gently for 20-25 minutes at room temp.
8. Add 10 ml media + P/S + 10% FBS to inactivate collagenase. Pipet up and down with a 10 ml pipet 20-30 times to disperse the cells and break up the tissue. Allow tissue to settle for a minute or two and then pipet the supernatant to a new 50 ml tube. Save the settled tissue for step 10.
9. Spin the supe tube down at low speed (about 250xg) for 5 minutes. Aspirate the supernatant. The pellet contains cells that were dispersed in step 8 – mostly epithelial and immune cells, but possibly some myofibroblasts as well.
10. Add 10 ml DMEM + P/S + FBS to each pellet (supe pellet and tube with settled tissue chunks from step 8) and plate each separately in T-75 flasks. You should have two flasks per tissue (= 4 flasks per mouse if you do both small intestine and colon). The "supe" flasks (less tissue fragments) often don't contain many myofibroblasts, but it's best to save both fractions. I've had cells grow out of either fraction, or both.
11. Allow cells to adhere for 48 hours. Tap flask gently to dislodge tissue fragments, aspirate off old media + tissue fragments + non-adherent cells, and add fresh media (DMEM + P/S + 10% serum). Most contamination will appear by this point if it's going to, although some slow-growing yeasts which might not appear for a week or so.
12. Media should be changed every 5 days or so thereafter.

13. Cells may take 2-6 weeks to become confluent for the first time but will usually grow more quickly after that. After a few weeks, if colonies have appeared but are not spreading out very well, cells can be trypsinized to promote growth. They can be left in the same flask or moved to a new flask if there are not enough cells to split.
14. Begin freezing stocks of cells at passages 2-6. Cells should be used for assays ideally between passages 3 and 9. Sometimes cells just stop growing around passages 2-5.

References:

Theiss AL, Simmons JG, Jobin C, and Lund PK. Tumor necrosis factor (TNF) alpha increases collagen accumulation and proliferation in intestinal myofibroblasts via TNF receptor 2. *J Biol Chem* 2005;280(43):36099-109.

Fruchtman S, Simmons JG, Michaylira CZ, Miller ME, Greenhalgh CJ, Ney DM, and Lund PK. Suppressor of cytokine signaling-2 modulates the fibrogenic actions of GH and IGF-I on intestinal mesenchymal cells. *Am J Physiol: GI & Liver Physiol* 2005;289:G342-350.