Digesting Skin

- For digestion, we utilize **Miltenyi Whole Skin Dissociation Kit, human #130-101-540**: https://www.miltenyibiotec.com/en/~/media/images/products/import/0007500/im0007590.ashx
- Work is performed in a sterile tissue culture hood
- Utilize fresh 3mm punch biopsies straight from patient into PBS on ice
- Wash 3 mm biopsy again in PBS in one well of 6-well plate
- Blot additional PBS from biopsy on bottom of clean wells in the 6-well plate
- Transfer biopsy to another clean, dry well
- In a 1.5 mL Eppendorf tube, combine 100 microliters of Enzyme D + 5 microliters of Enzyme A and mix
- Apply 25 microliters of Enzyme D+A mix to biopsy
- Using tweezers and scalpel, slice biopsy as many times as possible to dissociate it into small slivers
- Transfer these slivers to a **Miltenyi gentleMacs C tube**
- Wash well in which biopsy was cut (containing residual Enzyme D + A) with 870 microliters of Buffer L and transfer all fluid to the C tube
- Add remaining 80 microliters of Enzyme D + A mix to the C tube containing Buffer L and slivers of biopsy
- Add 25 microliters of Enzyme P to C tube
- Flick C tube gently to mix
- Incubate C tube in 37 degree water bath for 2 hours, shaking the tube gently every 15 minutes
- After 2 hours, add 1 mL of Buffer L to C tube; from this point on, keep cells cool (at 4 degrees or on ice) as much as possible
- Run gentleMACS Program h_skin_01 on **Miltenyi OctoDissociator**
- Place a **70 micron Miltenyi Macs SmartStrainer** over a 15mL conical and wet the membrane with 2mL of cold PBS containing 0.04%BSA kept on ice
- Make sure to keep PBS/BSA cold
- Wash C tube with 4 mL of PBS containing 0.04%BSA and pass 6mL of contents (2mL of cells in Buffer L + 4 mL of PBS containing 0.04%BSA) through 70 micron strainer into 15 mL conical
- Wash C tube with another 3.5 mL of PBS containing 0.04%BSA and pass this through the 70 micron strainer
- Using the plunger of a 10 mL syringe gently apply pressure to the top of the strainer to pass any residual cells through
- Wash C tube with another 3.5 mL of PBS containing 0.04%BSA and pass this through the 70 micron strainer
- Spin the 15 mL conical down for 8 minutes at 1200 rpm in a pre-cooled centrifuge (4 degrees)

- You should see a pellet. Using a serological pipette, remove the supernatant without disturbing the pellet.
- Obtain a new 15 mL conical and place a **30 micron Miltenyi Macs SmartStrainer** over the new 15mL conical and wet the membrane with 2mL of PBS containing 0.04%BSA
- Resuspend the cell pellet with 5 mL of PBS containing 0.04%BSA and pass this through the 30 micron Miltenyi strainer
- Wash the old 15 mL conical with 3.5 mL of PBS containing 0.04%BSA and pass this through the 30 micron strainer.
- Repeat the 3.5 mL wash step
- The new 15 mL conical should contain your filtered cells and ~14 mL of PBS containing 0.04%BSA
- Spin the 15 mL conical down for 8 minutes at 1200 rpm in a pre-cooled centrifuge (4 degrees)
- You should see a pellet. Using a serological pipette, remove the supernatant without disturbing the pellet. Leave approximately 50-80 microliters of PBS containing 0.04%BSA in which to re-suspend your pellet
- Flick the 15 mL conical to resuspend your pellet
- You can also scrape the bottom of the 15 mL conical back and forth against the grating of the hood quickly to resuspend the cells
- Place the 15mL conical of cells in 50-80 microliters of PBS containing 0.04%BSA on ice.
- Count cells