Tissue Culture

- Wear gloves
- Spray hood and bottles with 70% EtOH
- Put cells in hood
- Use tips on glass Pasteur pipette
- Tilt plates towards you and aspirate off media, careful not to touch bottom where cells are attached
- Pipette 2mL 1xPBS onto each plate, swirl around and aspirate off PBS
    - PBS – is a salt solution and primes the cells to be lifted without them exploding
- Add 1mL Trypsin to each plate and return to incubator for up to 5 minutes
    - Trypsin – breaks up the cell attachments to the plates
- Tap the sides of the plates to loosen the cells
- Put in hood
- Add 4mL of Media to plate to bring total media+trypsin to 5mL
    - Pipette up and expel at top of plate to bring the cells down to bottom, then pipette up and down to mix
- To split…. It is like you are splitting one plate into either ten or five or split evenly amongst any number of plates
    - 1:10 – put 9.5 mL of media into new plate, then add .5mL of your 5mL cell mixture into the new plate and swirl
    - 1:5 – put 9 mL of media into new plate, then add 1mL of your 5mL cell mixture into the new plate and swirl
    - 2 – put 8 mL of media into 2 new plates, then add 2.5mL of your 5mL cell mixture into each plate and swirl
    - Return to incubator, and aspirate the rest of the cell mixture **UNLESS** you are using the remaining cells for an experiment (ie: Soft Agars, Clonogenics, Proliferation, shRNA) then put in a 15mL conical to count!!!!!!!!!!!!