Cell Splitting Protocol
Sack Lab

Notes: Steps should be done inside of a Biological Safety Cabinet in Sterile conditions. Make sure that the area has been cleaned with 70% ethanol, the UV light is off, and that the blower has been on for at least 10 min before starting. Once finished close clean the area off with 70% ethanol, close the cabinet and turn on the UV light.

Procedures (done in T25 flask)**:**

1. Check under microscope to make sure that cells are less than 80% confluent
2. Remove the solution from the flask
3. Rinse with DPBS (Divalent free Dulbecco's Phosphate Buffered Saline) ~ 5 mL
4. Remove the solution
5. Add 1 mL 0.05% Trypsin EDTA
6. Place in 37° C incubator until cells detach from the dish ~ 5 min
   -**Check** under the microscope to make sure cells have detached
7. Add **5** mL of Cell Media into a new dishes
8. **Check** under the microscope to make sure the cells are dispersed into single cells.
9. Add the appropriate amount of cells into the new dishes
10. Swirl the dish or pipet up and down
11. Label the Dish: Name, Date, Cell Line, Passage #, Ratio

*Cell Media: Ham's – F12 from Gibco, 10% FBS, Penicillin/Streptomycin
To make add 50 mL Media + 50 mL FBS