

## Passing Cells

1. Warm cell media, PBS, and trypsin in hot water bath
2. Prepare the tissue culture hood by spraying ethanol and turning on the light and fan (fan should be running for at least 20 mins. prior to any work done in the hood)
3. Retrieve cells from incubator and put in the hood. Spray media, PBS, and trypsin bottles with ethanol before putting in hood.
4. Using a sterile glass pipette on the aspirator suck out old media, or pour it out into a waste beaker.
5. Add 8 ml of PBS and gently slosh the flask. Suck out PBS using aspirator or pour into water beaker.
6. Add 2 ml of trypsin to flask and put back into the incubator for about several minutes. Examine the flask every few minutes to see how many cells are in the solution.
7. Meanwhile, prepare new flask by adding 17 ml of media and labeling with date, passage number, passage ratio, and your initials.
8. Tighten the cap of the flask with trypsin/cells, and hit the belly of the flask to make sure that all of the cells detach.
9. Add 8 ml media to flask. Using a 10 ml pipette, transfer cell solution to a 15 ml centrifuge tube. Centrifuge solution for approximately 3 minutes, or until a definite clump of cells forms at bottom of tube. (Setting on centrifuge should be 1100, and don't forget to balance out the solution with 10 ml de-ionized water in a tube facing opposite it)
10. Remove the old media and trypsin using the aspirator. Be sure not to suck up any cells.
11. Add 10-15 ml media (depending on passage ratio) to the tube using a 5 ml pipette. resuspend the cells.
12. Count the cells using the Olympus Cell Counter or a hemocytometer and pipette a volume of the supernatant based on the flask size:  
T75 Flask:  $1 \times 10^6$  to  $2 \times 10^6$  cells per T75  
T25 Flask:  $3 \times 10^5$  to  $5 \times 10^5$  cells per T25
13. Put flask(s) in incubator, and open the cap by a quarter of a turn (unless using vented caps).
14. Tape up any packaging so as to keep it sterile, and take everything out of the hood. Spray the hood down with ethanol and turn on the UV light.