Passaging or subculturing tissue culture cells

Cells that grow on plastic divide until they cover the entire surface of the dish, at this point they must be removed from the dish, diluted and replated into fresh dishes, usually every 3 to 4 days. In most cases removal of the cells from the plastic is facilitated by the use of the enzyme trypsin which digests away the extracellular matrix deposited by the cells as they grow. Always keep 2 dishes of each cell type going at any one time to guard against accidental contamination of one dish that would wipe out your stocks of growing cells.

To remove cells from a confluent 10cm dish:

1. Remove medium, wash with 5mls PBS, remove.
2. Add 1ml Trysin.EDTA (1X)**, incubate 2 to 5mins at either room temperature or 37oC untill the cells can be seen to be detaching, check under the microscope.

   How long and at what temperature has to be determined for each cell type, try 1 to 2 mins at room temp. if nothing much is happening try incubating at 37oC.
3. Add 4mls fresh, complete medium (ie medium + serum + antibiotics) and pipette around the dish to remove the cells.
4. For routine subculturing place 1ml cell suspension into 10mls fresh, complete medium in a new 10cm dish.

   This level of dilution (equivalent to 1 dish into 5) is suitable for most cell types With fast growing, transformed lines a 1 : 10 dilution may be better, ie 0.5ml into a fresh dish. However, most cell types cannot tolerate being seeded too sparsely so don't try to dilute them more to avoid passaging them twice a week.
5. If precise cell numbers are required, pipette the cell suspension into a sterile 15ml conical tube and count the cells using the hemacytometer (see next page)

** Use 10X Trypsin.EDTA for the MDCK cells, they are fairly trypsin resistant.

** 293 cells do not require trypsin to remove them from the dish, just pipette around with fresh medium.

Cells that are growing in suspension obviously do not require this treatment. HeLa cells grown in suspension are maintained at a density of 5 x 105 to 106 cells/ml. Count the cells daily and dilute with fresh, complete medium (ie medium + serum + antibiotics) as required.