Anita’s Protocol For Spliting/Plating NIH-3T3 Cells

1) Flask should be close to confluence at the time of splitting. If you will be plating cells on coverslips, begin by coating them with poly-D-lysine:
   a) Place coverslip in each well of 6-well plate or dish to be used.
   b) Add 700ul of poly-D-lysine to the top of the coverslip.
   c) Incubate at RT for ~10min.
   d) Remove lysine by aspiration.
   e) Wash plates 5 times with 2-3ml sterile water.
2) Remove media from confluent flask by aspiration.
3) Add 3ml of Trypsin/EDTA.
4) Incubate at 37C for ~3min.
5) During incubation, add 15ml of media to a fresh TC-75 flask and 45ml to a sterile 50ml tube.
6) Remove cells from incubator and knock several times to dislodge cells.
7) Add 7ml media to flask and agitate to ensure all cells are in solution.
8) Add 2ml of media/cell suspension to new flask/media and place at 37C.
9) Add 5ml of cells to 45ml media in 50ml tube; add 2-3ml from this tube to each well/plate (it is not necessary to have the suspension form a “bubble” over the coverslips).
10) Incubate plated cells overnight prior to transfection.