CELL SPLITTING PROTOCOL

For a 100mm TC plate:

1. Look at cells under the microscope to determine what the split ratio should be.
2. Aspirate off the old media.
3. Add 2 ml 1xPBS to rinse, and gently rotate plate.
4. Aspirate off 1xPBS.
5. Add 2 ml 1xTrypsin and gently mix around the plate.
6. Place plate in 37°C CO_2 incubator (for about 5 min).
7. Prepare the new plate by adding fresh media.

split ratio	1:10	1:5	1:2
cells	1 ml	2 ml	5 ml
media	9 ml	8 ml	5 ml

8. Remove plate from 37°C incubator; make sure that the cells are detached.
9. Add 8 ml of media to the old plate and gently mix around. Pipet up all of the cells.
10. Dispense the proper split volume of the cells to the new plate. Gently mix the cells around.
11. Place plate back into the 37°C CO_2 incubator.