Protocol to Pass or Replate Mammalian Cells (HEK 293 Cells)

Lan Zhou 11/01/00; Updated by JK Park 6/15/04; Commented by TCH

1. Remove the complete medium of HEK 293 cell flask as completely as possible (**Note**: There is absolutely **no need** to wash your cells with PBS or plain medium prior to adding trypsin; otherwise you actually hurt the cell viability).

2. Add trypsin to the flask (**3 ml** for T-75 Flask, or **1 ml** for T-25 Flask).

3. Place it to 37℃ incubator for 1-5 minutes (**Note**: Different cell lines may need different time; you need to check the flask every 1-2 min).

4. Rock the flask and rinse down cells by adding complete medium (i.e., **10ml** for T-75 or **5ml** for T-25 Flask).

5. Make sure completely dissociate cell clusters into single cells by pipetting up and down.

6. According to the desired cell confluence, add the desired volume of resuspended cell mix into new flasks or plates

7. Add appropriate complete medium (final volume: 6-10 ml for T-25 or 15-20 ml for T-75).

8. Shake flasks/plates gently to mix well and place them back to 37℃ CO2 incubator.