Splitting Cells

Everything must be done with sterile technique in the hood.
Before beginning spray hood down with 70% ethanol and wipe clean.

Pre-Warm Trypsin and Media in 37C waterbath.

-Aspirate old media (tilted plate)
-Rinse gently with 5 ml sterile room temperature D-PBS.
-Aspirate off PBS (tilted plate)
-Add 2.5 ml trypsin
-Put back in incubator (2-5 mins; depending on cell line).
-Tap dish to help release cells.
-Add 7.5 ml of media to cells to make volume an even 10 ml.
-To maintain the cells - dilute cells the appropriate amount:

    Ex, for 1:10, do 1ml of cells, 9 ml of media in new plate
    For 1:4, do 1 ml of cells, 3 ml of media in new plate.

-To plate the cells for a new experiment (i.e. transfection):
-Spin down remaining cell solution for 5 minutes at 1000 rpm at room temperature.
-Aspirate the supernatant
-Resuspend cells in 10 ml fresh media.
-Put 10 ul of trypan blue stain into centrifuge tube. Add to that 10 ul of your cells.
-Count the number of non-blue cells in the 4, 4x4 squares

    Example, say you count 304 cells:

304/4=92.25 x 10= 922.5 x 2 (The Dilution factor) = 1845 cells/ul

-Plate appropriate number of cells. Make sure to mix the cells by pipetting to prevent cells from settling at the bottom while counting.

-Wash the counter by rinsing in water.