Subcloning Protocol for 5, 1, and 0.2 cells per well of a 96 well plate

Besides reagents to trypsinize cells, you will need:
In hood:

75 ml	pre-warmed cell maintenance media (100ug/ml G418)
3	TC treated 96-well plates
2	Small tubes (1.5 ml is fine) and rack
3	25 ml serological pipets
	200 ul pipetor
	12 channel pipetor, 200 ul each channel
37	200 ul pipet tips (3 rows of 12)
	Waste box for pipet tips (old tip box works well)
	Pen

At microscope:
Haemocytometer
Pipet and pipet tip for dispensing 10 ul

Note: cells sink in media, so make sure to mix solution by pipetting up and down immediately before taking cells

Rinse (DPBS), trypsinize, & resuspend cells in same volume media

To count cells
Pipet >10 ul cells into small tube, remove from hood
Pipet 10 ul cells into haemocytometer on microscope
Count cells in haemocytometer, the total # of cells in one of the 9 big squares = ***count***
As one big square = 0.1 ul volume, ***count*** cells/0.1 ul = cells/ul

To dilute cells and plate into 96 well plates
Pipet 1 ml media into 1.5 ml conical tube
Add 625/***count*** ul of cell suspension into the 1 ml of media, this makes a 62.5 cells/ul suspension
For example, with a cell count of 101, add 625/101cells ul = 6.188ul to the 1ml of media

For 5 cells per 200 ul well, you will use a 25 cells/ml dilution:
Add 100 ul of 62.5 cells/ul suspension to a sterile trough
Add 25 ml media, mix by pipetting twice
Plate 200 ul/well into a 96 well plate, use autoclaved 12 channel pipet with 300 ul unstuffed pipet tips

For 1 cells per 200 ul well, you will use a 5 cells/ml dilution:
Add 20 ml media to remaining ~5 ml in trough, mix by pipetting twice
Put 200 ul/well into a 96 well plate

For 0.2 cells per 200 ul well, you will use a 1 cell/ml dilution:
Add 20 ml media to remaining ~5 ml in trough, mix by pipetting twice
Put 200 ul/well into a 96 well plate
Place cells in incubator, check for colonies in a week