FACS Analysis of Propidium Iodide Stained Cells to Assess DNA content

1) Spin down 1 x10^{7} cells (about 100µl of an overnight culture) and remove supernatant.
2) Add 1 ml of ice cold 70% Ethanol and vortex (cells will appear clumped, this is normal), leave for 10 mins (this stage fixes the cells by dehydration).
3) At this stage these cells can be stored at 4^{0}C indefinitely if required (good for time courses)
4) Spin down cells, remove ethanol solution and re-hydrate by adding 1 ml of 50mM Na Citrate.
5) Spin down again, discard supernatant and resuspend pellet in 0.5ml of 50mM Na Citrate containing 1mg/ml RNAase A (as PI binds to all nucleic acids, this step removes the background by digesting RNA in the cell) and incubate for 2h at 37^{o}C.
6) Add PI to a final concentration of 6µg/ml.
7) Load entire contents of the tube into a FACS tube and record fluorescence using the FL2 (red) detector.