**Growth Curve with Countess (6 well plate)**

Collect the media from each well into a 15 ml conical (collection tube)

Wash cells in PBS and collect wash into the collection tube

Add 500ul trypsin to each well. Place in incubator unless all cells are floating.

Quench trypsin by adding 1 ml media to each well

Collect trypsin/media into the corresponding collection tube

Wash each well with 1 ml media and add to collection tubes

Spin tubes down. 400rcf for 3.5 mins

Aspirate supernatant but leave 500ul in each tube

Resuspend the cell pellet in the remaining 500 ul media and move it to an Eppendorf

Spin the Eppendorf’s down at 400 rcf for 5 min

Remove the supernatant. Try not to double dip

Resuspend in 50-200ul depending on your cell concentration (pick a volume and stick with it for all samples)

Spin down the trypan blue (only pipette off the top aqueous part)

Get a 0.6ml tube for each sample. Add 20ul trypan blue to each one

Vortex your cell sample and mix very well. Add 20ul of cells to corresponding trypan blue tube. Mix well

Add 10ul trypan blue/cell mix to an EV slide. (take 2 reads per sample so each sample will use a whole slide)

Read on countess.