Cell Cycle Analysis: Ethanol Fixation and Propidium Iodide staining

Notes on different protocols

1. Final ethanol concentration should be ~60-70%.
2. RNAse A can be used at 0.2 – 0.5 mg/ml final concentration
3. PI used at 10-50 ug/ml final.
4. DAPI used at 2 ug/ml final.

Notes on cell counting

1. Starting with the same concentration of cells in each sample makes for prettier data – and can be important for comparing subtle differences between samples. The ratio of cells to PI will shift the location of the peak (FL2-A on the FASCaliber, or PE-Cy5.5-A on the LSR2) and so you will not need to do as much adjusting of the laser voltage if the cell concentration is similar in the beginning.

Staining Buffer (e.g. for 5ml)

- RNAse. https://www.thermofisher.com/order/catalog/product/12091021
- Propidium Iodide. https://www.thermofisher.com/order/catalog/product/P3566?SID=srch-srp-P3566
- Triton X-100. https://www.sigmaaldrich.com/catalog/product/sigma/t8787

Reagent	*Stock*	*Fold*	*Final*	*5 ml*
RNAse A	20 mg/ml	50x	0.4 mg/ml	100 ul
Propidium Iodide (PI)	1 mg/ml	100x	10 ug/ml	50 ul
Triton X-100	20% w/v	200x	0.1%	25 ul
				5000 ul PBS

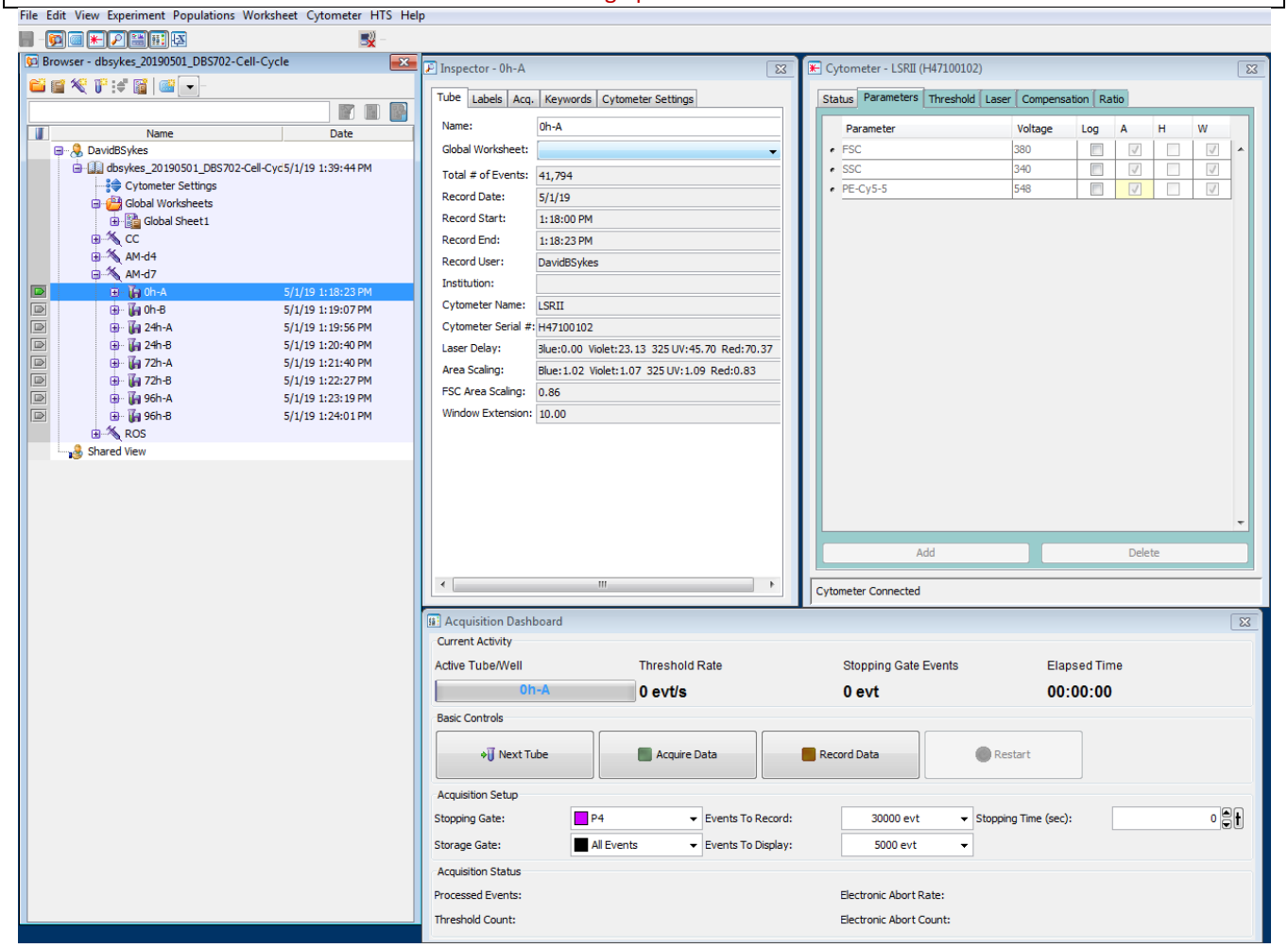
Protocol – Fixation

1. Count cells and resuspend at ~10 million/ml in PBS with no FBS.
   a. This is a good starting concentration if you have enough cells.
2. Transfer 300 ul of cells (3 million – 6 million) to a FACS tube.
3. Chill on ice for 5'.
4. While vortexing continuously on low, add 700 ul of ice-cold 100% ethanol
   a. Final EtOH 70%, final volume 1 ml
5. Transfer the cells to -20-degrees for a minimum of 4-hours and generally at least overnight.

Protocol – Staining

1. Add 3 ml of PBS or FACS buffer and pellet the cells.
2. Aspirate.
3. Resuspend in 300 ul of **Staining Buffer**.
4. Mix well and transfer through a filter-top FACS tube using a P1000.
5. Incubate 15' at 37-degrees.
   a. There is no need to wash the cells
6. Flow cytometry
   a. FACSCalibur – FL2
   b. LSR2 – PECy5.5
   c. Remember to record in LINEAR.
   d. Remember to adjust the voltage so that the G1 peak is exactly 100 for each sample.

Setting up the LSR2



Gating strategy