SYTO9 & PI FACS Viability Assay

SYTO9/PI Viability Protocol

- stock solutions of SYTO9 (3.34 mM) and Propidium Iodide (PI, 20 mM) stored at -20°C in the Gresham Lab
- collect desired cell samples by spinning @10,000g for 5mins and resuspended in water
- sonicate samples and measure cell density by coulter counter
- resuspend cells in 1XPBS with desired volume to achieve the cell concentration 10^6 ~ 10^7 cells/ml
- prepare two 1 ml sample with 10^6 ~ 10^7 cells. one for staining and one for blank control
- allow the dye to thaw at room temperature in the dark
- add 1ul of STYO9 and PI in 1ml staining sample (final conc. SYTO9: 3.34uM; PI: 20uM) and vortex gently
- incubate samples at room temperature for 20mins in the dark
- run sample through Flow cytometry or FACS

*note about use of blanks: one blank can be collected for each condition that will be measured (i.e. different strains, different media) OR can collect a blank for each SAMPLE (i.e. each time point) being measured.

*can store samples in media and dye at 4°C protected from light for approximately 7 days until FACS analysis (may affect signals)

testing the assay:
if desired, the assay can be tested for the conditions under which viability is being measured by doing a heat killed calibration experiment:

- grow up cell samples in the desired condition and create 1 mL samples of mixtures of 0%, 25%, 50%, 75%, and 100% viable cells
- 0% sample consists of cells that are completely non-viable (heat killed in 70-80°C water bath for 20-30 min.)
- 25% viable sample consists of 25% live cells from your culture and 75% heat killed cells
- 100% sample consists of all viable cells from your culture, etc.