FACS Analysis of Bacteria from Infected Macrophages
(*protocol from A.K. Lee, Falkow Lab, modified by I. Brodsky*)

Infection:

Seed 24-well dishes at desired density

(I like to seed at $5x10^4$ and let them grow for 2 days, assuming a 24hr doubling time)

Day before infecting, inoculate standing O/N bacterial cultures with desired strains

Take OD_{600} of bacteria

Dilute bacteria to desired cfu/mL based on OD_{600} 1.0 = $1.2x10^9$ cfu/mL

Infect cells with bacteria in 50 μL volume for 30’

For FACS infect at moi 25-50 to ensure enough bacteria inside cells

At 30’, aspirate bacteria, add 1 mL DMEM/well

Analysis:

After adding 1 mL DMEM to each well, take 0 time point:

Each timepoint and each strain has 2 data points

a) outside bacteria

b) inside bacteria

Outside bacteria:

Remove 1 mL DMEM from well, put in FACS tube

Inside bacteria:

Aftrer removing DMEM for outside bacteria, add 200 μL 0.1% Triton X-100 in PBS; incubate 5 min

Lyse cells by pipetting up-and-down 20 times.

Add 800 μL DMEM

Remove entire 1 mL, put in FACS tube

I usually take time points can every 30’ although this can be a little hectic

FACS collection:

Histogram should be formatted slightly differently than for bacteria in medium:

Scale should be 30-50 (not 90)

It may help to have the histogram plot only the gated data rather than all of it