FLOWERS Protocol

Day 1

- □ Make Stigma-Based Medium (adapted)
- □ Make starters of jdc_isolates and *E. amylovora* in LB; incubate at 30°C

Day 2

- □ To make standarized starters, transfer to Stigma-Based Medium:
  - □ jdc_isolates starters (6µL to 600µL of SBM per well in 96 deep well plate)
  - □ *E. amylovora* starter (20µL to 2mL of SBM in snap cap Falcon tube)

  ...and incubate at 30°C

Day 3

- □ Wait for standarized starters to grow

Day 4

- □ Measure OD of jdc_isolates and *E. amylovora* standarized starters
- □ Assemble communities using standarized starters of jdc_isolates:
  - □ plate_singles_1 = jdc_isolates
  - □ plate_singles_2 = jdc_isolates.rotate_left()
  - □ plate_pairs_1 = plate_singles_1 + plate_singles_2
  - □ plate_pairs_2 = plate_pairs_1.shuffle_rows()
  - □ plate_quad_1 = plate_pairs_1 + plate_pairs_2
  - □ plate_quad_2 = plate_quad_1.shuffle_rows()
  - □ plate_quad_3 = plate_quad_2.rotate_left()
  - □ plate_communities = plate_quad_1 + plate_quad_3
- □ Inoculate communities and standarized starter of *E. amylovora* into fresh SBM (2µL of each into 200µL of SBM per well in 96 deep well plate)
- □ In a different control plate, inocululate communities into fresh SBM (2µL into 200µL of SBM per well in 96 deep well plate)
- □ Incubate at 30°C with transpirable filter

Day 5

- □ Wait for communities to grow

Day 6

- □ Wait for communities to grow

Day 7

- □ Transfer 100μL per well to a standard 96 well plate
- □ Measure OD
- □ Measure GFP fluorescence
- □ Use FACS to quantify abundance of *E. amylovora* in each community
    - □ If needed: dilute 30μL of the communities into 270μL of PBS per well in 96 deep well plate (1:10 dilution for a final 10-100 million cells per mL as required by FACS specifications)