**Materials**

* YPDaW plates for streaking
* SDC+ade+W medium for culturing
  + You will need 36 5 g/L (28 mM) and 36 20 g/L (111 mM) flasks. Make extra. Remember to leave room for W.
* 48 green tubes w/ 970 μL Epure for diluting for FC
* 1 96 well plate for FC
* FC experiment file, prepared ahead of time
* 48 labeled 15 mL centrifuge tubes for centrifugation
* 48 labeled molecular microtubes for supernatant samples

**Day T -3: Thawing**

1. Streak out 11 lines from freezers. Use 3 plates.

**Day T -1: Preculturing**

1. You need to grow lines to stationary phase for 24 hours.
   1. You need 24 preculture flasks, 12 of each medium. 1 for each strain plus 1 NC.
   2. Inoculate each strain into each medium, from plate, in duplicate.

**Day 0: Culture to log phase and SAMPLING**

1. You need 48 culture flasks. You are also going to need 48 15 mL centrifuge tubes; and 48 FC tubes or green tubes.
2. Culturing:
   1. Fetch your stationary phase flasks.
   2. Dilute 1:100 (so, 100 μL) into fresh medium.
   3. **Incubate with shaking for 4 or 5 hours at 30 °C. Check after 2 or 2.5 hours.**
3. Sampling for cell counts:
   1. Diluting 3:100 (30 and 970) into FC tubes; or green tubes + use a 96 well plate.
   2. >>>*If you are not able to use FC immediately, fix with glutaraldehyde and wash out; preserve samples in the dark at 4 °C* <<<
   3. Sample perhaps 45 μL on flow cytometer. You should get perhaps 5000 events, perhaps a little less.
4. Sampling for HPLC:
   1. Pour each flask into the corresponding 15 mL centrifuge tube.
   2. Centrifuge at 5000 g for 3.5 minutes. If the pellet looks pelleted and the supernatant looks clear, then this is enough; if there is any cloudiness, then centrifuge more!
   3. Sample 1000 μL of supernatant into a molecular grade tube. Freeze it at -20 °C until the next step.

**Day X: Performing HPLC**

1. Thaw your supernatants at 4 °C.
2. Vortex all supernatants vigorously—according to Jake, the molecules do precipitate when you freeze things down.
3. Bring your samples up to McKinlay laboratory.