Day 1

1. Make media
   1. Mix and autoclave 700 mL YPDaW medium. 10 g/L Y, 20 g/L P, 20 g/L D, 0.1 g/L adenine.
   2. Mix and autoclave 700 mL YPDaW dietary restriction medium—use 5 g/L dextrose instead of 20 g/L.
   3. Remember to save room (7 mL) for adding tryptophan after autoclaving.
   4. Add tryptophan after autoclaving.
2. Dispense media
   1. Use glass pipettes to make aliquots of both media types in 50 mL centrifuge tubes.
   2. Use the 5 mL pipette to add 2.5 mL YPDaW into 44 sterile culture tubes.
   3. Repeat (b) with DR media.
3. Label tubes
   1. There are 4 replicates for each of the following 11 strains: 5W1,2,3,4,5; LY1; 20W1,2,3,4,5
   2. Label all 44 YPD tubes
   3. Label all 44 DR tubes
   4. Make LARGE labels so that the Sharpie doesn’t rub off on the shaker
4. ROY: Set up experiment files on the flow cytometer.
5. KELSEY: When making media, FIRST dispense 9.8 mL of each type of media 50 times into 50 flasks. After autoclaving the flasks, we need to add 100 μL Trp to each flask.

Day 2

1. Inoculate tubes
   1. Fetch plates that were streaked 09/04 from JH 353 or from our bench space. (Ask Roy where it is.)
   2. For each of the 44 YPD tubes, use a loop to pick up 1 colony and inoculate it into a corresponding labeled tube.
   3. Put all 44 tubes on the shaker in JH 353. Please arrange them in a sensible manner so that we would be able to infer which tube is which if some labels get rubbed off.
2. DANNI: Restreak plates
   1. Label new plates in the same way that the plates provided are labeled.
   2. Streak each line onto its corresponding sector on the new plate. Do not use just 1 colony for this part.
   3. Parafilm and incubate in JH 353.
   4. If you ask nicely and with enough lead time, Danni will probably be willing to do this for you.
3. DANNI: Label 88 culture tubes and 88 flasks. E.g., 5 1 1, 5 1 2, 5 1 3, 5 1 4 for the 4 technical replicates for 5W1.

Day 3

1. Set up the well plate
   1. Pipette 990 μL YPD medium into all 48 wells of a 48 well plate.
   2. On the growth curve computer, create your experiment file from the correct protocol file. Roy can help you find it if you don’t remember.
   3. Turn on the plate reader. Turn on the computer.
   4. Run the Gen5 program. Open the experiment file 20180907\_YPD\_W\_KLM.xpt. It is in Documents --> Experiments --> Roy folder
2. Inoculate the well plate
   1. Inoculate the wells in the below pattern. Each X represents one well. Wells with no X are negative controls—they should have media but not get inoculated with yeast. The A1 corner is in the upper left.
   2. 
   3. Put the well plate into the plate reader with well A1 in the correct corner.
   4. Remove the lid.
   5. Do NOT push the plate in.
   6. Start the run by clicking the green arrow on the screen. You have to wait until the chamber warms to 30 °C, then press ok, and the run will actually begin.
   7. The run will last for 48 hours
3. INOCULATE 44 FLASKS—From each tube, transfer 100 μL of culture to the corresponding flask. Incubate on 30 °C shaker.

Day 4

1. Inoculate next set of culture tubes
   1. repeat all steps for “Inoculate tubes” from Day 2, except using DR media
   2. Do this at a slightly later time of day than you inoculated the well plate—because the run lasts 48 hours and we want the tube cultures to be 24 hours old when we inoculate the next 48 well plate.
2. DANNI: Set up 88 sets of 10^-5 dilutions using sterile Epure water. So, 88 tubes with 990 μL water for 10^-2 and then 88 tubes with 999 μL water for 10^-5. Label them. Also label 88 YPDaW plates from the fridge downstairs.
3. KELSEY AND ROY: PLATING. Dilute all 44 flask cultures 10^-5. Spread plate 100 μL onto labeled plates. VORTEX before each transfer and before plating.

Day 5

1. Fetch the previous 48 well plate from the plate reader
   1. This means that you will have to do things at a later time of day for the DR well plate than for the YPD well plate—see previous comments
   2. WHEN YOU FETCH THE WELL PLATE, you have to save the experiment file!!!!!
2. Set up the well plate
   1. See instructions for Day 3, but use DR media. The experiment file is called 20180909\_DR\_W\_KLM.xpt
3. Inoculate the well plate
   1. See instructions for Day 3, but use DR media
4. KELSEY OR ROY: INOCULATE 44 flasks as previously (Day 3), but with DR media.

Day 6

1. (KELSEY or DANNI) AND ROY: Do dilutions and plating as previously (Day 4), for with DR flasks.

Day 7

1. Fetch the plate from the plate reader.
2. Save the experiment file with a helpful filename.
3. COUNT COLONIES from Day 4.

Day 8

Day 9

1. Count colonies from Day 6