Goal:
To determine the concentration of your perturbation that will inhibit the growth of SUB328 transformed with WT ubiquitin in glucose containing media

Materials Provided:

- SUB328(WT ub) liquid culture
- Synthetic Complete Liquid Media (SC-D) -His/-Leu/-Ura
- Your Perurbation:

  TBD

- 250 mL flasks
- Plastic Cuvettes
- Spectrophotometer

Protocol:

1) Design an experiment to determine concentration of your perturbation that will inhibit SUB328 (WT Ub) by 25%. Use 4 different concentrations of your perturbation. A $\log_2$ dilution for the other 3 concentrations will be sufficient, but I encourage you to be creative with your experimental design. **Remember to grow 1 flask without any perturbation.**
2) Create a stock of your perturbation in SC-D at 2X the concentration of your highest condition. Use that stock as a starting point for a serial dilution series making up at least 15 mL of perturbed media for each condition.
3) Determine the OD_{600} of the starting SUB328 (WT ub) culture provided to you.
4) Calculate how much SUB328 (WT ub) culture to add per flask to achieve OD_{600} = 0.2 in a final volume of 20 mL.
5) Add the required amount of yeast culture and the complementary amount of SC-D in each flask to a final volume of 10 mL. Add 10 mL of SC-D + perturbation to each culture.
6) Grow your SUB328 (WT ub) for 30 minutes at 30^{o}C with shaking.
7) Read the OD_{600} for each flask. Continue to grow SUB328 (WT ub) at 30^{o}C with shaking. These are your zero time point measurements.
8) Every hour for eight hours measure the OD_{600} of your culture. I would suggest measuring a 1:1 to 1:10 dilution of your sample at the later time points as readings over OD_{600} = 2 are not trustworthy and to preserve your sample.
9) Plot your measurements and calculate the doubling time of each SUB328 (WT ub) culture:

   To extract a growth rate it is easiest to plot log(OD_{600}) vs time. The slope of best fit line is the parameter “k”. (On google drive I find it is easiest to use the slope function) To get at doubling time from k, simply take the inverse to determine "τ". Because we are working in log space you will need to convert $\tau$to doubling time

(“t”) by multiplying τby the log(2). (for example I always work with natural logs so: $t = \tau * ln(2)$)