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 log₂ 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 OD₆₀₀ 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°C with shaking.
- 7) Read the OD₆₀₀ for each flask. Continue to grow SUB328 (WT ub) at 30°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 τ to doubling time

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