Yeast Showdown Rematch: Growth Curves

NOTE: The exercises below are intentionally open-ended and possibly difficult. If you are confused about what the question is asking, ask a teaching assistant or take a peak in the hints document; however, you'll learn the most if you try to solve the problem before looking at the hints or solutions.

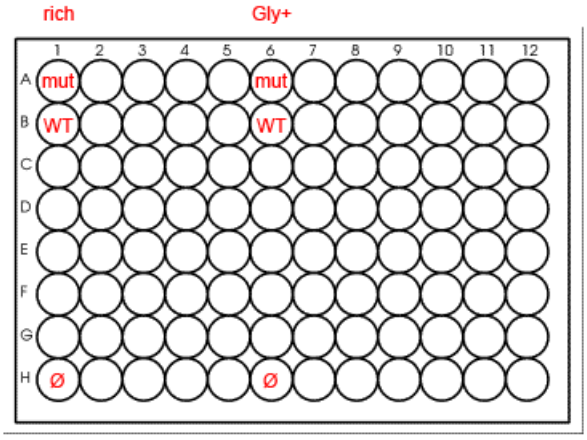
In part 1 of Yeast Showdown, a candidate human autism gene replaced the homologue in yeast, and we compared how the human wildtype and mutant alleles differed in gene expression by imaging a fluorescent reporter. However, we mentioned that when the effect of the mutant allele is strong, we can simply compare the growth rate of the human mutant to that of the human wildtype instead of doing microscopy.

Here you will analyze the data from such a growth experiment. Our hypothesis is that the mutant allele will be unable to properly metabolize glycine and use it as a source of nitrogen. Therefore, the mutant will grow more slowly than the wildtype in media where glycine is the sole source of nitrogen available to the cell; we will refer to this media as Gly+ media. On the other hand, rich media provides all necessary nutrients to the cell in a ready-to-use form, including nitrogen.

Typically, this type of experiment is done on a 96-well plate (shown below), which allows multiple strains to be grown in multiple media conditions, along with replicates and controls, all at the same time. For this experiment we inoculate the wild-type and mutant strains into separate wells containing media and monitor their optical densities (OD) over time using an automated plate reader. OD will be proportional to cell density at low ODs where these measurements are taken. We want to know whether the mutant allele conveys a growth rate disadvantage to the yeast, and specifically whether this disadvantage is due to improper glycine metabolism.

> *If we do find a growth disadvantage in the mutant, is this experiment sufficient to conclude that it is related to glycine metabolism? If yes, why? If no, what control(s) do we need so that we can make this conclusion?(The figure below should provide a hint.)*

Figure of experimental layout. 'mut' and 'WT' indicate wells containing mutant and wildtype yeast strains respectively. 'Gly+' and 'rich' denote the type of media. Ø denotes media control (no inoculum).



You prepare a 96-well plate with rich media in column 1 and Gly+ media in column 6 (see above). You inoculate row A with the mutant strain and row B with the wildtype strain. Row H contains no inoculum, and therefore serves as a control for contamination in your media. The rich media contains nitrogen sources other than glycine, so you can see if the mutant gene is causing a growth defect unrelated to glycine metabolism. The other columns and rows contain additional strains and medias, but you will ignore them as they are not relevant for this experiment.

The data is contained in '**growth_curve_data.mat**.' When you load this into MATLAB it will give you two arrays:

> **`all_data`**, a 2x50x96 array containing OD measurements and whose dimensions correspond to 2 replicate plates, 50 time points, and 96 wells on each plate, respectively. The 96 wells have been collapsed into a single dimension columns-first, i.e. it's ordered A1, B1, …, H1, A2, … H2, etc.
> **`all_times`**, a 2x50 array which contains the time (in minutes) when each time point was taken for each plate.

Exercises:

1. Explore the data by plotting growth curves (OD versus time).

a. Warm up with one well. Before visualizing all the data, you probably want to start to play with data for one single well (for example, A1). How does OD change versus time? Do you think it's more informative to plot OD on linear or logarithmic axes? Convince yourself that there is an exponential growth phase. Try to overlay data from two replicate plates, and check how reproducible they are.

b. To get a sense of what the data looks like, plot growth curves for all the data, including the wells that aren't specific to this experiment. In other words, make a grid of sub-plots in the same layout as the 96-well plate, and plot both replicate curves in each sub-plot. Label each well with its position (for example, 'A1'). Which wells are the ones we want? How well do the replicates agree with each other? Do all the wells on the 2 plates appear to contain growing cells?

c. Now plot growth curves only for the wells that you are concerned with for the current experiment, including ones for media control. Compare the growth rates of the strains *qualitatively*. What do you see? What do your media control "growth curves" tell you?

2. Compare the growth rates quantitatively. Which region do you use to get growth rates? Does the mutant have a lower growth rate than the wildtype? Do you think this is due to a defect in glycine metabolism?

3. (BONUS) Distinguish between technical and biological explanations for the 'lag' phase. For the growth curves you plot, you might find that they have an 'S' shape, i.e. there is a 'lag' phase before exponential growth. This could be an interesting observation, but we need to make sure that the observation is due to the actual behavior of the cells and not an artifact of our measurement technique or data analysis.

a. Is the lag phase an artifact of how you are scaling the OD axis (linear or logarithmic)? If not, what are possible explanations for this lag phase?
b. Try to think of one biological reason and one technical reason that we might observe a S-shaped plot of log(OD) versus time.
c. Using the given data and controls, think of a way to rule out or correct for one possible technical explanation for the lag phase. Perform this correction, and plot the growth curves. Do you think the lag phase is due to technical or biological factors?
d. Now recalculate the growth rates of each of the cultures. Does correcting for technical artifacts affect your conclusion from part 2?

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