

Growth curve experiments

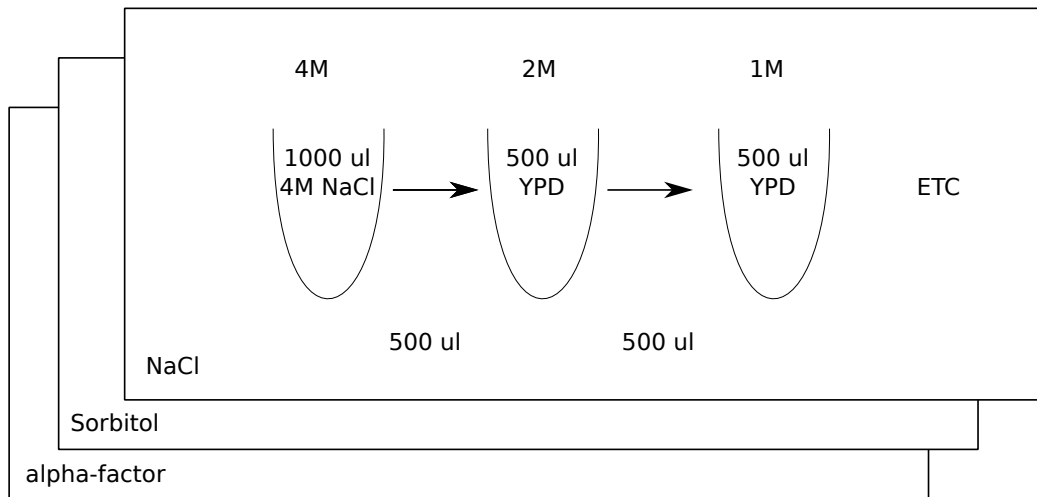
Purpose: By measuring the concentration of cells in solution over time we can figure out the maximum growth rate of cells in solution, how long they take to adapt to their new conditions, and the maximum density that they can reach given the resources available to them. This experiment will test the response of two yeast strains to growing in various concentrations of three different stressors: *alpha* mating factor, sorbitol, and salt (NaCl). The cells will be seeded at a low concentration into wells of a 96-well plate, then the plate will be measured in a machine that will record how dense the cells are every 5 minutes for about 48 hours.

Solutions: Stock solutions of growth media (YPD) supplemented with 4M NaCl, 1M Sorbitol, and 6.25mM *alpha*-factor have been prepared. Two cell lines have also been grown overnight to $\sim 1 \times 10^8$ cells/ml. You will seed these cells to $\sim 1 \times 10^6$ cells/ml for the experiment (a 1:100 dilution).

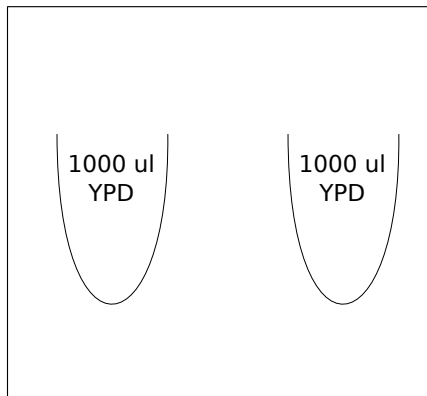
Protocol:

- 1) Prepare 500ul of 7 1:2 serial dilutions of each stressor by diluting into un-supplemented YPD in 1.5ml eppendorf tubes. (For example, for NaCl prepare the following: 4M, 2M, 1M, 0.5M, 0.25M, 0.125M, 0.0612M.)
- 2) Prepare 2 tubes with 1000ul of un-supplemented YPD.
- 3) Split each of these tubes in half into new eppendorf tubes.
- 4) For each cell line, add 1ul of yeast culture per 100ul of solution to these tubes. Mix well on the vortexer.
- 5) Add 100ul of the mixed culture to the proper well of the 96 well plate (see Colin for the plate map).

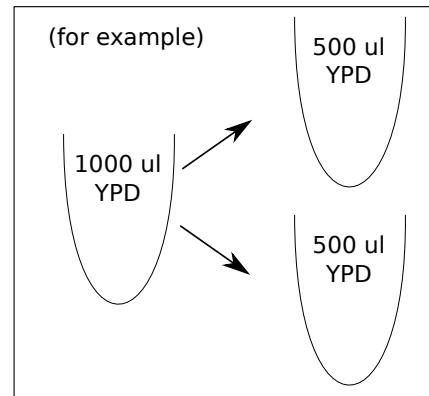
1



2



3



4

