Bio 111 Last Lab   
Competition Assay Protocol

As the finale to our semester-long CURE, your yeast populations in your section will compete   
to determine which has the highest fitness in the presence of acetic acid. As a complementary experiment, we will perform a competition in growth media without acetic acid at neutral pH.

**Part 1:**

Measuring the relative cell densities of the liquid yeast cultures.  
Materials:  
 stock of YPD (yeast peptone dextrose growth media) media  
 students’ yeast cultures   
 1 spectrophotometer cuvette for each yeast culture  
 1 cuvette for use as a blank  
 spectrophotometer set to measure OD600  
 micropipettes/pipette tips  
  
Method:  
1. Add 500 µL of the stock liquid media to a spectrophotometer cuvette.  
2. Agitate your yeast culture vigorously by vortexing. If your yeast shows “clumpy” flocculent   
 growth, try to break up the suspension as much as possible.  
3. Add 500 µL of your liquid yeast culture to the same spectrophotometer cuvette.  
4. Mix the two liquids well in the cuvette by pipetting up and down.  
5. Blank the spectrophotometer using stock liquid media.  
6. Measure and record the absorbance of your yeast culture at a wavelength of 600 nm.  
7. Write your culture’s absorbance on the whiteboard. You’ll call this number **Ai** (when all groups are accounted for, we’ll proceed to part 2)

**Part 2:**Starting the competition assay culture  
Materials:  
 2 competition flasks  
 one flask filled with YPD liquid media  
 one flask filled with YPD media and acetic acid  
 one liquid yeast culture from each team  
 micropipettors/pipette/tips  
  
Method:  
Please use good sterile technique during this exercise.

1. Calculate the volume of your yeast strain to enter the culture.   
  
Your strain’s absorbance = **Ai** The lowest absorbance of all strains = **Ao** Divide the lowest absorbance by your absorbance and then multiply that number by 250.   
Here’s the formula:  
(Ao / Ai) x 250 = \_\_\_\_\_  
the product = the volume of your groups yeast stock that you’ll add to the flask.  
Call me over to confirm your calculations!  
2. Add the volume of your yeast culture you’ve calculated to the YPD media stock bottle.  
3. Add the volume of your yeast culture you’ve calculated to the YPD with Acetic acid stock bottle.