7.003 Spring 2022
Day 5 In-Lab Questions

1) Today in lab you performed the Shmoo Assay.

   A) Before you treat the yeast cultures with α-Factor, the overnight yeast cultures are first diluted in media and allowed to grow for several hours. Why do you think we include that dilution/grow-out step (i.e. why didn't we just directly treat the overnight yeast cultures with the α-Factor)?

   B) When observing your positive control samples, you notice that some cells are shmooing, but many cells are still budding. Assuming everything in the assay was set up properly, what might be a reason why all the cells in the positive control sample are not shmooing?

2) Today, you set up a Halo Assay with your α-factor-resistant mutants, using melted top agar to add the yeast cells to the plates. What would be an alternative way to add the yeast cells to the plates? Why might the top agar method be preferred?

3) Today, you set up a Mating Assay with your α-factor-resistant mutants.

   A) Suppose the 7.003 lab has run out of SC-His plates. What other kind of plates could you successfully use instead for your Mating Assay? List all plates that would apply and explain your reasoning.

B) Your instructor says that the auxotrophic markers in PPY295 and PPY144 can have either a recessive mutant phenotype or a dominant mutant phenotype for the Mating Assay to work properly. Do you agree? Explain why or why not.

4) Today you set up a sporulation assay with six α-factor-resistant mutants.

A) Briefly describe how a *S. cerevisiae* yeast cell can switch mating types. How do we prevent yeast strains used in research labs (including the 7.003 lab) from switching mating types?

B) How might diploid yeast arise and be selected for in our mutagenic screen in 7.003? Why is this undesirable? How can we identify any potential diploid false positive mutants?

C) What are the ingredients of the sporulation plate and how do they trigger sporulation?

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