7.003 Spring 2022
Day 4 In-Lab Questions

1) When performing a forward genetic screen, it is important to know when your screen is saturated.

A) What does it mean when a mutagenesis screen has been saturated? Why is it important to know whether your screen is saturated or not?

B) Which one of the following factors below would best allow you to determine whether or not your screen is saturated? Explain why.

- Total number of mutagenized yeast colonies screened
- Total number of mutagenized yeast colonies with your desired mutant phenotype
- Percentage of mutagenized yeast colonies screened that have your desired mutant phenotype
- Total number of different genes identified from your screen
- Number of times each gene identified from your screen was found

C) If you perform a mutagenesis screen and determine the screen is saturated, does that necessarily mean you have successfully identified all genes involved in your process of interest? Why or why not?

2) To celebrate the Super Bowl, you wish to perform a mutagenesis screen with PPY295 yeast to look for yeast mutants that form football-shaped colonies.

A) You predict that the type of mutations you will be looking for are likely to be gain-of-function (GOF) mutations. Which type of mutagenesis should you use for your screen, transposon mutagenesis or chemical mutagenesis? Explain your choice.

B) From your screen, you isolate a yeast mutant (Mut1) that has football-shaped colonies. Describe a simple experiment you could perform to test whether the football-shaped colony mutant phenotype in Mut1 is a recessive or dominant phenotype.

Testing your YAPD media: To check if the YAPD media you made last lab session works properly, you will set up a test overnight (O/N) culture today.

1) If you have not already added the glucose and adenine to your YEP media (see Day 3 protocols), do so now.

2) Using lab tape, label the blue caps of two glass test tubes with your bench number. Label one tube with "Test O/N" and the other tube with "Blank."

3) Using sterile technique, pipet 3 mL YAPD media into both tubes.

4) Continuing to use sterile technique, use an inoculating stick to pick a single colony from one of your SC-Leu replica-plated plates and inoculate the "Test O/N" tube with that colony (dip the stick with the cells on it into the YAPD media in the tube and wiggle it around to transfer some cells into the liquid media). Note that you are <u>not</u> adding any yeast colonies to the "Blank" tube – the only thing added to the "Blank" tube should be plain YAPD liquid media.

5) Place your "Test O/N" and "Blank" tubes on the roller drum in the 30^{o}C room to grow overnight. Always balance your tubes when placing them on the roller drum – wherever

one tube is on the roller drum, a second tube has to be in a matching position directly across from the first tube on the opposite side of the roller drum.

6) What do you expect to happen/see in your ‘Test O/N” and “Blank” tube after letting them grow overnight? What was the purpose of each tube?

7) The 7.003 teaching staff will check your tubes tomorrow – if your YAPD media ends up being bad, they will provide you with fresh back-up YAPD media for the rest of the semester.

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