

Problem Set 8- Dr. Ted's Last

Due Nov 21, same day as Bruce Walsh's Problem Set 9.

Content: short answer

Yeast STE genes and order of function

1. You have isolated 2 sterile mutants, call them *ste80* and *ste90*. Assume that you have MAT α and MAT α versions of each mutant as well as STE $+$ (i.e. you have both a MAT α *ste80* and MAT α *ste80* mutants, and both versions for *ste90* and STE $+$).
- a. Describe conceptually how you would determine if the mutants are dominant or recessive. What result will tell you if they are dominant or recessive? Comment on whether this is a complementation test or a recombination test.

Mate each to a STE $+$ (wildtype) cell, and determine if diploid can mate. If it can mate, the mutation is recessive, if it cannot mate the mutation is dominant. This is a complementation test. (CORRECTION)

- b. Describe two tests to determine if the mutations are in the same or different genes.

Complementation test; if the "doubly mutant" diploid can mate, the mutations are in different genes. If the "doubly mutant" diploid cannot mate, the mutations are in the same gene, or one is dominant. A recombination test will generate all mutant haploids if the mutations are in the same gene, and $\sim 1/4$ wildtype haploids if the mutations are in different genes.

- c. Discuss in a few sentences why mutants in yeast are easier to identify and to study than are mutant in human cells. *Yeast have a haploid phase allowing direct identification of recessive mutations without having to make both copies of the gene mutant as you would have to do in diploid cells.*
- d. When a yeast mutant is identified (after mutagenesis with EMS and screen for failure to induced FUS1::HIS3, for example), how would you know that the mutant phenotype is due to single nuclear mutation (we did not discuss this in class, but it is in S104 and is within your intellectual grasp!). *Make a *ste/STE+* diploid, allow it to undergo meiosis, and analyze haploids; if mutants to wildtype ratio is 1:1, the original mutant strain had one nuclear mutation.*
- e. A researcher has mated two yeast strains from the wild. He knows that the strains have about 5% sequence heterology. Comment and discuss whether these strains will produce haploid spores after meiosis. *They will not produce any viable haploids because they will not undergo meiotic recombination because the RecA ortholog allows recombination only between sequences that have less than $\sim 3\%$ heterology.*

2. Mutants that cannot mate are identified. To test if *ste#50* is recessive or dominant, a MATa *ste#50* strain is mated to a MATalpha STE50+ strain. The diploid, MATa/MATalpha, *ste#50*/STE50+, is analyzed for mating and it is shown that it cannot mate. What do you conclude? ***The ste#50 mutation is dominant.***

3. *ste#55* is analyzed genetically, contains one mutation, and the phenotypes shown.
ste#60 is analyzed genetically, contains one mutation, and the phenotypes shown.
ste#55 and *ste#60* are unlinked.

Haploids		Diploids	
<u>Genotype</u>	<u>Phenotype</u>	<u>Genotype</u>	<u>Phenotype</u>
MATa STE+	mates normally	$\frac{\text{MATa } \underline{\text{ste#55}}}{\text{MATa } +}$	mates normally
MATa <i>ste#55</i>	never mates		
MATa <i>ste#60</i>	always mates	$\frac{\text{MATa } \underline{\text{ste#60}}}{\text{MATa } +}$	always mates

- Is *ste#55* recessive or dominant? (circle one)
- Is *ste#60* recessive or dominant? (circle one)
- What fraction of haploids formed from the diploid $\frac{\text{MATa } \underline{\text{ste#55}}}{\text{MATalpha } +} \frac{+}{\text{ste#60}}$ should be appropriate to test for order of function (epistasis)? ***1/4th***
- A MATa *ste#55 ste#60* double mutant fails to mate. Given this result, and the recessive and dominant nature of these mutants from parts a and b above, draw the pathway from pheromone to mating. Obviously, your answer must include gene order and bars and arrows.

Pheromone \longrightarrow STE60+ \longrightarrow STE55+ \longrightarrow Mating

- You cannot determine the order of function using the diploid cell $\frac{\text{ste#55 } +}{+ \text{ste#60}}$.
 Give one reason why. Since *ste#55* is recessive, the diploid cell is not phenotypically a double mutant.

5. Another yeast question, slightly different format.

A. A mutant cell that cannot mate (call it *ste#A*) was crossed to a wildtype cell, sporulated, and haploids were analyzed for mating. Of the haploids formed, one mated for every 3 that could not mate (1+:3-). What do you conclude about *ste#A*? (3 pts)

Ste#a mutant actually has 2 mutations, and EACH SINGLE mutant haploids IS mating-defective. CORRECTION

Genotype	Mating Response (e.g. schrr)	
	no pheromone	pheromone
1. WT	-	+
2. <i>ste71</i>	-	-
3. <u><i>ste71</i></u> +	-	+
4. <i>ste72</i>	+	+
5. <u><i>ste72</i></u> +	-	+

B. *ste#71* is analysed genetically and has one mutation. *ste#72* is analyzed genetically and has one mutation. *ste#71* and *ste#72* are unlinked. Phenotypes of haploid and diploid cells are shown.

i. Is *ste#71* recessive or dominant? (circle one)(2pt)

ii. Is *ste#72* recessive or dominant? (circle one)(2pt)

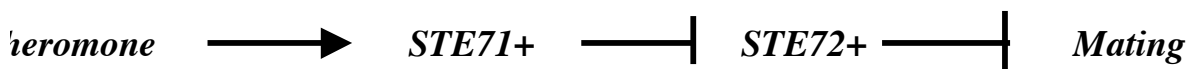
iii. From a diploid $\frac{MATa \ \underline{ste71} \ \underline{ste72}}{MAT\alpha \ ste71 \ +}$

what fraction of the haploids can be used for a mating epistasis test?

1/2

iv. A $MATa \ \underline{ste71} \ \underline{ste72}$ double always mate. Given this result, and the recessive and dominant nature of *ste#71* and *ste#72* you answered in i and ii:

Draw the pathway connecting pheromone, mating (schmooring), *STE71+*, *STE72+* genes, using bars and arrows as discussed in class. (12pts)



C. Two other mutants were identified, call them *ste#81* and *ste#82*. *ste#81* is recessive, and *ste#82* is dominant. Discuss whether a $\frac{MATa \ \underline{ste81} \ \underline{ste82}}{MATa \ + \ +}$ and a $\frac{MATa \ \underline{ste81} \ \underline{ste82}}{MATa \ ste81 \ +}$ cells

can be used to determine order of function.

The first strain cannot be used to determine order of function because the cell is mutant for only ste#82 which is dominant. The second strain can be used to determine order of function because it is homozygous recessive for ste#81 and mutant for the ste#82 dominant if the single mutants have