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Genetics 201

Midterm exam

October 29, 2007

PUT YOUR NAME ON EVERY PAGE.

THERE ARE FIVE MULTI-PART QUESTIONS ON THIS EXAM. THE POINT VALUE FOR EACH PART IS INDICATED.

WE RECOMMEND THAT YOU LOOK THROUGH THE EXAM AND ANSWER THE EASIER QUESTIONS FIRST.

PLEASE TRY TO GIVE SIMPLE AND STRAIGHTFORWARD ANSWERS.

WRITE ALL YOUR ANSWERS IN THE SPACE PROVIDED. WE HAVE OFTEN LEFT MORE SPACE THAN IS NECESSARY FOR YOUR ANSWER. IF YOU NEED EXTRA SPACE FOR ANY ANSWERS, USE THE BACKS OF PAGES.

1. You are studying the ability of *S. cerevisiae* to utilize ethanol as a carbon source. You know from previous work that yeast contain an enzyme, alcohol dehydrogenase (Adh), that is required for cells to grow on medium with ethanol as a carbon source. The *ADH2* gene, which encodes this enzyme, is only expressed when cells are grown on ethanol-containing medium. It is not expressed when cells are grown in glucose medium alone, nor when they are grown in medium containing both ethanol and glucose. Additionally, cells that express *ADH2* will die when grown in the presence of allyl alcohol, an ethanol-like substrate that interferes with Adh activity.

Normal *ADH2* regulation:

Media	<i>ADH2</i> gene
8% Glucose	OFF
3% Ethanol	ON
8% glucose + 3% ethanol	OFF

1a. You want to identify two classes of mutations in genes that regulate *ADH2* expression. You have available medium with and without ethanol, glucose, and allyl alcohol.

1ai. First, briefly describe a strategy to identify mutants in which the *ADH2* gene cannot be activated in ethanol-containing medium. (4 points)

1a.ii. Second, briefly describe a strategy to identify mutants in which the *ADH2* gene is not repressed in the presence of glucose. (4 points)

1b. You identify three mutants in which *ADH2* expression cannot be activated (Adh^- phenotype) and call these *nad* mutants (no alcohol dehydrogenase). You also isolate one mutant in which *ADH2* is always (constitutively) activated (Adh^c phenotype) and call this a *cad* mutant (constitutive alcohol dehydrogenase). These mutants all show 2:2 segregation when crossed to wild-type yeast. Note that wild-type cells express *ADH2* only in the presence of ethanol and absence of glucose (Adh^+ phenotype).

Next, you wish to determine whether the mutant phenotype of each strain is dominant or recessive. You cross each mutant to a wild-type strain and score the phenotype of the diploid.

Mutant	Phenotype of haploid single mutant strain	Phenotype of diploid when mutant is crossed to wild-type yeast
<i>nad1</i>	Adh^-	Adh^+
<i>nad2</i>	Adh^-	Adh^+
<i>nad3</i>	Adh^-	Adh^-
<i>cad1</i>	Adh^c	Adh^+

Which mutations are dominant? Which are recessive? (3 points)

1c. As the *ADH2* gene is not essential for growth, you realize that either the *nad1* or *nad2* mutation could be a loss-of-function mutation in the *ADH2* gene itself, instead of a mutation in a gene regulating *ADH2* expression.

Briefly describe a genetic test to provide evidence whether *nad1* is a mutation in *ADH2* or in a different gene. All standard yeast reagents and strains described in class are available. Clearly note any yeast strains used with their relevant genotypes and give the possible results for either outcome. (5 points)

1d. You focus your studies on two mutant strains you isolated in your screen: *nad2*, in which *ADH2* expression cannot be activated (Adh^-), and *cad1*, in which the *ADH2* gene is always on (Adh^c phenotype). Recall that wild-type cells express *ADH2* only in the presence of ethanol and absence of glucose (Adh^+ phenotype).

You wish to perform epistasis analysis to test the genetic relationship between *nad2* and *cad1*. You cross the two strains together, sporulate the diploid, and analyze spores from 100 tetrads for their *Adh* phenotype.

Your actual cross data is the following:

<u>number of tetrads</u>	<u>spore phenotypes</u>
94	2 Adh^c : 2 Adh^-
6	2 Adh^c : 1 Adh^+ : 1 Adh^-

State the conclusions you can make from these data regarding the epistasis relationship between *nad2* and *cad1* and calculate the linkage between *nad2* and *cad1*. (6 points)

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2. You are studying a newly isolated lambdoid phage called HK201 that infects *E. coli* strain A. Upon infection, HK201 either enters the lytic cycle or establishes lysogeny within the bacterium.

2a. You want to identify phage mutants that can grow lytically on strain A but are unable to establish lysogeny. Given strain A bacteria and an HK201 phage lysate that has been mutagenized, how would you do so? (4 points)

2b. You are also interested in identifying the host factors present in strain A that are required for the lytic cycle of the HK201 phage.

You have available:

λ mini-Tn10(Tet^R) hop phage, which is able to infect strain A
E. coli strain A (Tet^S)
HK201 mutants you isolated in part a, above
Any necessary growth media

Propose a strategy to isolate strain A mutants that are unable to support the growth of HK201 phage. (7 points)

2c. You isolate a number of Tet^R strain A mutants that cannot be infected by HK201 (HK201^R mutants). **For each Tet^R candidate, how will you verify that the mini-Tn10 insertion is responsible for the HK201^R phenotype? You have available phage P1 and any other necessary reagents. (6 points)**

2d. Among your HK201^R bacterial mutants, you would like to distinguish between two potential classes: (i) mutants that are resistant because they lack a cell surface receptor required for the absorption of phage HK201 and (ii) mutants that lack some factor required specifically for lytic development after the phage adsorbs and injects its DNA. You have available wild-type HK201, an HK201 derivative marked with a kanamycin resistance gene that does not affect the ability of the phage to grow either lytically or lysogenically, and the HK201 mutants you isolated in part a.

Briefly describe an experiment to determine whether any of your HK201^R bacterial mutants are class (ii) mutants. (4 points)

3. You are studying the genetics of flower color in the diploid garden pea plant, *Pisum sativum*. You have isolated four different true-breeding mutant strains that produce purple flowers. You also have a true-breeding wild-type strain that produces only white flowers.

3a. For a given purple mutant strain, briefly describe the cross(es) you will perform to determine whether the mutation is dominant or recessive, and how you will interpret the results. (4 points)

3b. You find that all of your purple mutants are recessive. You cross each purple mutant to one another in a pairwise fashion, then score the resulting F1 for their flower color. The flower color observed in each set of F1 cross progeny is listed in the table below.

	Mutant 1	Mutant 2	Mutant 3	Mutant 4
Mutant 1	Purple	White	Purple	Purple
Mutant 2		Purple	White	White
Mutant 3			Purple	Purple
Mutant 4				Purple

What kind of genetic test have you performed? (2 points)

Tentatively, how many different genes have you identified that control flower color? (2 points)

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3c. You cross mutant 1 to mutant 4, intercross the F1 (all purple-flowered), and get the following F2 progeny:

1106 purple flowers
496 white flowers

Provide a genetic explanation for this result, taking into account the data above and the table in part b. Please assign genotypes to each phenotype and define your nomenclature. (6 points)

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You perform pairwise crosses between the *ade1*, *trp1*, *tsa1*, and *tsa2* strains and analyze 100 tetrads for each cross. You collect the following data:

Cross	Number of tetrads		
	PD	NPD	TT
<i>tsa1 x ade1</i>	83	5	12
<i>tsa2 x ade1</i>	90	2	8
<i>tsa1 x trp1</i>	30	32	38
<i>tsa2 x trp1</i>	39	37	24
<i>tsa1 x tsa2</i>	78	8	14

4b. Based on the above data, draw a map that shows the location of the *tsa1*, *tsa2*, *ade1*, and *trp1* mutations. Include the locations of the centromeres and any genetic distances you are able to calculate. (6 points)

4c. You cross *tsa1* to wild-type yeast, sporulate, dissect tetrads, and allow the spores to germinate and form colonies at 30°C. You then test the Ts phenotype by replica plating these colonies to test for growth at both 30°C and 37°C. You notice that one tetrad produces two progeny that grow at both temperatures, one that only grows at 30°C, and a fourth that shows an unusual phenotype. The colony from this fourth spore grows normally at 30°C, but at 37°C only half of the colony grows, looking like a semicircle, suggesting that half the colony is Ts⁺ and the other half is Ts⁻. When you carefully pick cells from each half of the colony at 30°C, purify and retest, you find that the phenotypes are stable.

Identify the genetic event that created this tetrad and briefly explain how it caused the sectored colony phenotype. (4 points)

4d. You take a *tsa1 trp1* double mutant strain and select for Ts⁺ suppressors. You take one Ts⁺ colony, cross it to a wild-type strain, sporulate the diploid, and score tetrads for their Ts phenotype. You find the following:

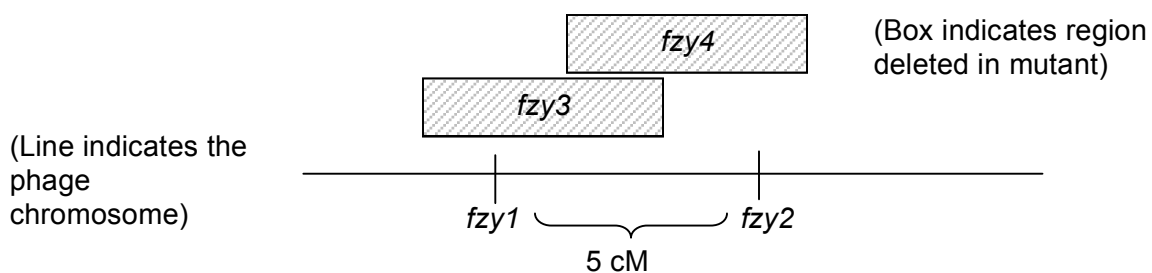
	Number of tetrads seen with each phenotype		
	2 Ts ⁺ : 2 Ts ⁻	0 Ts ⁺ : 4 Ts ⁻	4 Ts ⁺ : 0 Ts ⁻
Ts ⁺ colony x wild-type	66	16	18

Based on the tetrad data above, briefly state your conclusions about the nature of the suppressor mutation in the original Ts⁺ colony. Support your answer by listing the genotypes for each class of tetrad and by explaining the ratios among the different tetrad classes. (6 points)

5. As a new graduate student in a phage lab, you have been assigned the task of characterizing several phage T7 mutants. These mutants cause small fuzzy-edged plaques when grown on *E. coli*, as opposed to the large sharp-edged plaques created by wild-type T7 phage.

5a. You want to determine the genetic distance between *fzy1* and *fzy2*. Briefly describe how you will do this experiment, including a description of how you will use the data you obtain to calculate the map distance. Include all relevant controls. (5 points)

5b. You carry out recombination testing on *fzy1-fzy4* and obtain the map shown below.



You realize that the *fzy3* and *fzy4* deletions may be useful in mapping the remainder of your fuzzy mutants. You take four more fuzzy mutants (*fzy5-8*), cross each separately to *fzy3* and *fzy4*, plate on *E. coli*, and score for the presence of sharp-edged plaques. Note that *fzy5-8* all appear to be point mutations. You find the following:

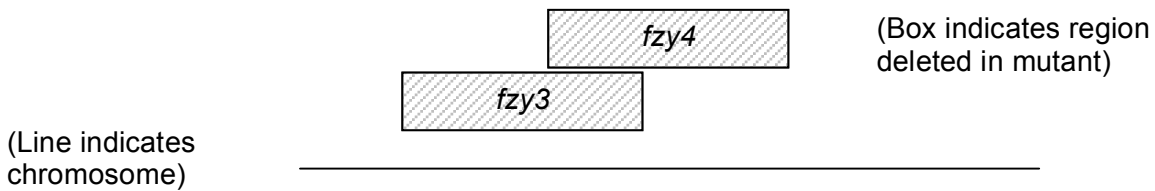
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("+" indicates presence of sharp-edged plaques at a frequency that is significantly above the reversion frequency of *fzy5-8*; "-" indicates no sharp-edged plaques)

	<i>fzy3</i>	<i>fzy4</i>
<i>fzy5</i>	-	-
<i>fzy6</i>	+	-
<i>fzy7</i>	+	+
<i>fzy8</i>	-	+

Place the *fzy5*, 6, 7, and 8 mutations on the map drawn below so that their chromosomal locations are consistent with the data in the table. Indicate any uncertainties. (6 points)



5c. You wish to make a *fzy1 fzy2* double mutant phage (see map in part b). How will you do so? List the crosses you will perform to make such a mutant and to confirm its identity. (5 points)

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5d. You have another fuzzy point mutant, *fzy9*, bearing a mutation that maps very near to the *fzy2* mutation. You perform two crosses in order to determine to which side of *fzy2* the *fzy9* mutation maps. The data you obtain are given below. Note that the genetic distance between *fzy1* and *fzy2* is 5 cM.

	Parent phage	$\frac{\text{\# sharp-edged plaques}}{\text{total \# plaques}}$
Cross 1	<i>fzy1 fzy2</i> (double mutant) x <i>fzy9</i>	2/10,000
Cross 2	<i>fzy2</i> x <i>fzy9</i>	50/10,000

Based on the cross data, indicate the location of *fzy9* on the map below. You do not need to calculate genetic distances. (3 points)

(Line indicates chromosome)

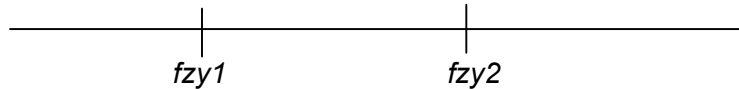


Diagram the crossover(s) that created the sharp-edged plaques seen in cross 1. Please indicate genotypes (mutant or +) at each of the three relevant positions (*fzy1*, *fzy2*, and *fzy9*). (3 points)