

## Genetics 201 Extra Phage and Bacteria Problems

These problems are NOT required, and are provided to help those students who wish additional problem solving practice.

Please note that as the content of the course lectures changes from year to year, these problems may not exactly correlate with what is covered in class. You are responsible solely for material that is covered in lecture and on the regular problem sets.

1. You are studying genetic recombination in the vicinity of the *ADE2* locus of *S. cerevisiae*. You are provided with the two strains listed below.

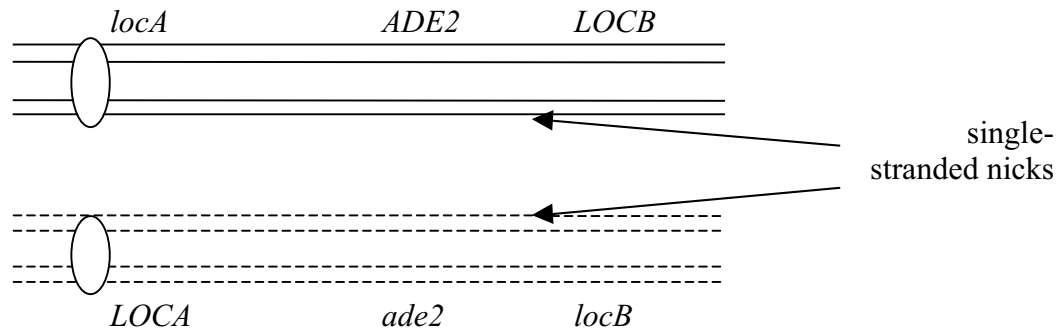
(i) *MATa locA ADE2 LOCB*

(ii) *MAT $\alpha$  LOCA ade2 locB*

The markers *LOCA*, *ADE2*, and *LOCB* are linked in the order shown. When grown on media lacking adenine, *ADE2* strains form white colonies and grow well, whereas *ade2* mutants form red colonies and grow poorly.

You cross strains (i) and (ii), sporulate the diploid, and analyze the resulting spores for their Ade phenotype. You observe that the tetrads usually show a segregation pattern of 2 Ade<sup>+</sup>: 2 Ade<sup>-</sup> (also referred to as 4:4 segregation). However, at a frequency of approximately 2%, you see tetrads showing a pattern of 3:1 (6:2), or gene conversion.

- a.
  - i. Describe the Ade phenotype you would expect to observe for the spores from a tetrad that exhibits a 3 Ade<sup>+</sup>: 1 Ade<sup>-</sup> segregation pattern.
  - ii. What other data from your cross would convince you that these gene conversion events are associated with normal meiotic recombination? (Hint - consider the *LOCA* and *LOCB* genes).
- b. Diagram how the Holliday model accounts for 3:1 segregation of Ade<sup>+</sup> to Ade<sup>-</sup>. The first step is drawn for you below (in this diagram, each line represents one strand in double-stranded DNA). In your diagram, you only need to show the particular chromatids involved in the recombination event, but be sure to
  - (i) show all relevant intermediates
  - (ii) indicate clearly which strands are from which parent  
(You only need to show one possible resolution, but be sure to indicate which strands are cut to produce that product.)



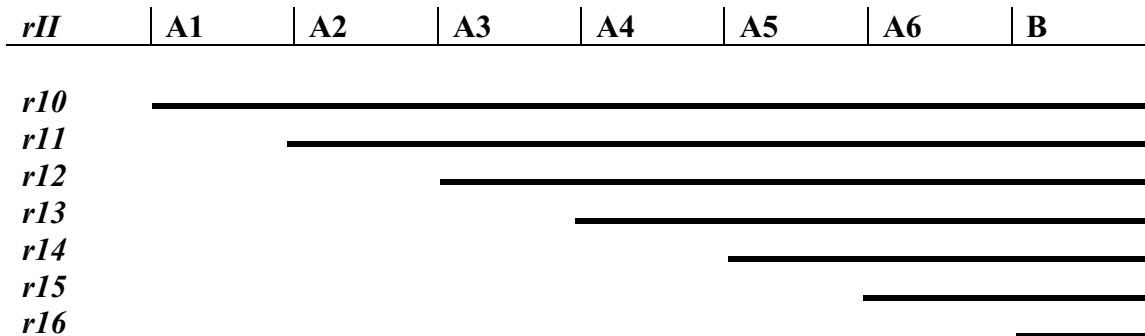
1c. You have available a mutant strain that is completely unable to carry out mismatch repair (the mutant allele is designated *mmr1*). You construct a diploid with the genotype *mmr1 locA ADE2 LOCB / mmr1 LOCA ade2 locB*, sporulate it, and look for tetrads that show abnormal segregation patterns at the *ADE* locus.

- i. According to the Holliday model, what types of aberrant segregation patterns (6:2, 5:3, etc) should be detectable in the absence of mismatch repair? Why?
  - ii. When you sporulate the diploid and perform tetrad analysis, you see tetrads showing a 3:1 segregation pattern for Ade<sup>+</sup>:Ade<sup>-</sup> at a frequency of approximately 2%. With which of the models for genetic recombination discussed in class (Holliday or double-strand-break repair) is this result consistent? Why?
- 1d. In the *MMR1*<sup>+</sup> background, you fail to detect any tetrads showing a 5:3 segregation pattern for Ade<sup>+</sup>:Ade<sup>-</sup>. However, in the *mmr1* mutant background, you detect such tetrads at a very low frequency (0.01%).
- i. Describe the Ade phenotype you would expect to observe for the spores from a tetrad that exhibits a 5 Ade<sup>+</sup>: 3 Ade<sup>-</sup> segregation pattern.
  - ii. Which model (Holliday or double-strand-break repair) better explains the observation that the 5:3 pattern is observed only in the *mmr1* mutant background? Why?
2. You have been given 5 phage T4 stocks, each of which carries an independently-derived mutation in the A cistron of the *rII* locus. You call them *rIIa* - *rIIe*. You have been told that *rIIa*, *rIIb*, *rIIc*, and *rIId* are all mutations which can be reverted, but that *rIIe* cannot be reverted. Your task is to begin to map these mutations by recombination using methods similar to those of Benzer. With this system, you can detect as little as one wild type phage in 10<sup>8</sup>.

Remember: wild type T4 and *rII* mutants both grow on *E. coli* B but with different plaque morphologies. Only wild type T4 will grow on *E. coli* K(λ).

- a. Outline an experiment to determine the recombination frequency between two of your *rII* mutants. Include any control experiments necessary for its interpretation.
- b. You decide to determine whether your mutations can form wild type recombinants with seven known *rII* deletion mutants, named *r10-r16*. Your deletion stocks are as follows:

**rII region**



(Note: Horizontal lines indicate regions of *rII* that are **missing** from each stock.)

You look for the formation of wild type recombinants between each of your mutants and each deletion and obtain the following:

- + = wild type recombinants recovered  
 - = no wild type recombinants recovered

	<i>r10</i>	<i>r11</i>	<i>r12</i>	<i>r13</i>	<i>r14</i>	<i>r15</i>	<i>r16</i>
<i>rIIa</i>	-	-	-	+	+	+	+
<i>rIIb</i>	-	-	-	-	+	+	+
<i>rIIc</i>	-	-	-	-	-	-	+
<i>rIId</i>	-	-	-	-	+	+	+
<i>rIIe</i>	-	-	-	-	-	+	+

You perform the same experiment with all combinations of your mutations and obtain the following:

	<i>rIIa</i>	<i>rIIb</i>	<i>rIIc</i>	<i>rIId</i>	<i>rIIe</i>
<i>rIIa</i>	-	+	+	+	-
<i>rIIb</i>	+	-	+	-	-
<i>rIIc</i>	+	+	-	+	+
<i>rIId</i>	+	-	+	-	-
<i>rIIe</i>	-	-	+	-	-

Using all of the information available, explain these results, indicating the nature of each mutation and its location (insofar as possible from the data given).

2c. You are given another *r* mutant T4 phage by a collaborator. You wish to determine whether it also carries a mutation in the A cistron of the *rII* locus. Describe how you would perform a complementation test with your new mutant, including any relevant controls. Be sure to specify appropriate strains when necessary.

3. You are given two conditional lethal phage  $\lambda$  mutants; one has the genotype  $N^{am} cI^{ts} cro^+$  and the other,  $N^+ cI^+ cro^{am}$ . The  $cI^{ts}$  allele encodes a thermolabile repressor that is functional at 30°C, but nonfunctional at 42°C.

(Note:  $N$  encodes an essential early lytic gene.

Phage with the  $cro^{am}$  mutation fail to form plaques on a nonpermissive host.

The  $N$ ,  $cI$ , and  $cro$  genes are linked in the order given:  $N -- cI -- cro$ ).

You wish to obtain  $N^+ cro^+$  recombinant progeny, so you coinfect a bacterial host and prepare a phage lysate.

a. For obtaining  $N^+ cro^+$  recombinant progeny, you coinfect a bacterial host which is permissive for amber mutations ( $su^+$ ). Does it matter at what temperature you perform the coinfection? Why or why not?

b. You plate your phage lysate from part a onto a nonpermissive ( $su^-$ ) host. What genotypes of phage progeny will produce plaques on this host?

c. Describe the expected plaque morphologies if you plate the phage lysate onto a  $su^-$  host (i) at 30°C, and (ii) at 42°C.

d. You notice an unusual plaque morphology from some of your recombinant phage progeny. These plaques contain both turbid and clear areas, creating a mottled effect. Explain the origin of the mottled plaques. Would the mottled plaques be present at 30°C, 42°C, or at both temperatures?

e. Describe a strategy that you could use to isolate an  $N^{am} cro^{am}$  recombinant. Also, describe how you could confirm that you had indeed isolated such a recombinant. (Note: The  $N$ ,  $cI$ , and  $cro$  genes are located relatively close to one another on the phage genome).

4. You are rotating in a bacterial genetics lab that studies kalomycin, an antibiotic that is very effective against a number of human pathogens, including *E. coli*. For your rotation project, you isolate kalomycin resistant mutants. In order to get such mutants, it was necessary to mutagenize heavily. Therefore, you are concerned that more than one mutation may be responsible for the mutant phenotype.

- a.
  - i. Describe how you would transduce the mutation conferring kalomycin resistance to a clean background (i.e, an unmutagenized wild-type strain). You may use any tools of bacterial genetics that were discussed in lecture.
  - ii. Assume you are successful in transducing the mutant phenotype to a clean strain background. Have you shown that the kalomycin resistance ( $Kal^R$ ) phenotype is due to a mutation at a single locus? Why or why not?

Further analysis reveals that a single mutation is responsible for the  $Kal^R$  phenotype. This mutation is in an ORF of unknown function named *yacX*. You also note that *yacX* is tightly linked to the *leu* operon.

b. You would like to know whether the  $Kal^R$  phenotype is dominant or recessive. Given the strains listed below, provide an experiment that would let you address this question. Include in your answer the predicted results you would obtain if the phenotype were dominant or recessive.

Strain	Genotype	Description
1	$F^- yacX^+ (Kal^S) strep^R kan^S$	Wild-type
2	$F^- yacX^* (Kal^R) strep^R kan^S$	$Kal^R$ mutant strain
3	$F' leuA::kan^R yacX^+ / strep^S$	Contains $F'$ carrying a <i>leuA</i> gene disruption marked with a kanamycin resistance gene ( $kan^R$ ) and the wild-type <i>yacX</i> gene

c. From the previous experiment, you determine that the  $Kal^R$  phenotype is dominant. You decide that you would like to generate a *yacX* knockout mutant for further experiments.

- i. List the steps you would take to create and isolate a knockout in the *yacX* gene. You may use a  $\lambda$  mini-Tn10( $tet^R$ ) hop phage and any bacterial strains that were listed and/or generated in part b, above.
- ii. Would your strategy work if *yacX* were an essential gene? If not, describe a modified strategy that would allow you to obtain a *yacX* knockout allele. Again,

you may use a  $\lambda$  mini-Tn10( $tet^R$ ) hop phage and any bacterial strains that were listed and/or generated in part b.

4d. Another student in the lab challenges you to design a genetic experiment to show that your putative *yacX* null mutant actually bears the mini-Tn10 insertion at the *yacX* locus. In particular, she points out that you have not ruled out the possibility that the mini-Tn10 has inserted at another locus to cause the loss of kalomycin resistance. Outline a genetic experiment that would allow you to show that the mini-Tn10 insertion is linked to the *leuA* gene, and therefore likely to be at the *yacX* locus. Assume that the co-transduction frequency of *leuA* and *yacX* is 50%. In addition to the strains listed (or generated) above, you have available strain 4 with the following genotype:  $F^- leuA^- yacX^+ strep^R$ .

Note: Design your strategy assuming that *yacX* is an essential gene.