

## Genetics 201 Extra Yeast 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). An eager first-year graduate student has started a rotation in a yeast lab, where he is assigned a cutting-edge project that is sure to result in a Cell paper. However, after waiting several days for the postdoc he is working with to show up, he decides to fill his time learning the basics of tetrad analysis instead. He obtains two strains, with the following genotypes:

*MAT* $\square$  *HIS9*<sup>+</sup> *LYS6*<sup>+</sup> *GEN2*<sup>+</sup> *SPT1*<sup>+</sup> *THR4*<sup>+</sup>

*MAT**a* *his9*<sup>-</sup> *lys6*<sup>-</sup> *gen2*<sup>-</sup> *spt1*<sup>-</sup> *thr4*<sup>-</sup>

The only piece of information he has ahead of time is that *GEN2* is very tightly linked to its centromere. He dissects and analyzes 167 tetrads and finds that when he examines each individual marker, it segregates 2:2 in every tetrad. He then looks at the tetrad segregation pattern for pairs of markers and compiles some of his data in the following table:

Relevant markers	PD	NPD	TT
<i>his9, lys6</i>	167	0	0
<i>his9, spt1</i>	51	54	62
<i>his9, thr4</i>	75	2	90
<i>his9, gen2</i>	141	0	26
<i>gen2, spt1</i>	73	77	17
<i>gen2, thr4</i>	76	4	87

- a). For each gene pair, list if they are linked to each other. For any that are linked to each other, calculate the distance between them in centimorgans (cM).
- b). Determine which genes are centromere linked, and, for any that are centromere linked, calculate their distance from the centromere in cM.
- c). Taking into account all of the data presented, what is the most consistent genetic map for all of these markers with respect to each other and to their centromeres?
- d). Which calculated map distance is the least reliable and why?

2). You will find the following web sites useful for this problem:  
*Saccharomyces* Genome Database (SGD): <http://www.yeastgenome.org>

MIPS: <http://www.mips.gsf.de/genre/proj/yeast/index.jsp>

In yeast, there are several ways to perform mutagenesis screens. Among the more common mutagens used are EMS, UV radiation, and transposon insertions. You decide that you are going to use UV mutagenesis to study inositol biosynthesis in yeast, starting with the following two yeast strains:

*MATa his3 leu2* (does not grow unless supplemented with histidine and leucine)

*MAT $\alpha$  trp1 leu2* (does not grow unless supplemented with tryptophan and leucine)

You screen through 20,000 yeast colonies and find 8 mutants that are unable to grow on media lacking inositol (*ino*<sup>-</sup>). Four of the mutants are mating type *a*, which you name *ino1*, *ino2*, *ino3*, and *ino4*. The other four mutants are mating type *alpha* and you name them *ino5*, *ino6*, *ino7*, and *ino8*. You do complementation analysis by crossing the mutants to each other and scoring the growth of the diploid on media lacking inositol. You obtain the following results:

	WT <i>alph</i> <i>a</i>	<i>ino5</i>	<i>ino6</i>	<i>ino7</i>	<i>ino8</i>
WT <i>a</i>	+	+	-	+	+
<i>ino1</i>	+	-	-	+	+
<i>ino2</i>	+	+	-	-	+
<i>ino3</i>	+	+	-	+	+
<i>ino4</i>	+	+	-	-	+

+ = signifies that the diploid grows on media lacking inositol.

- = signifies that the diploid shows no growth on media lacking inositol.

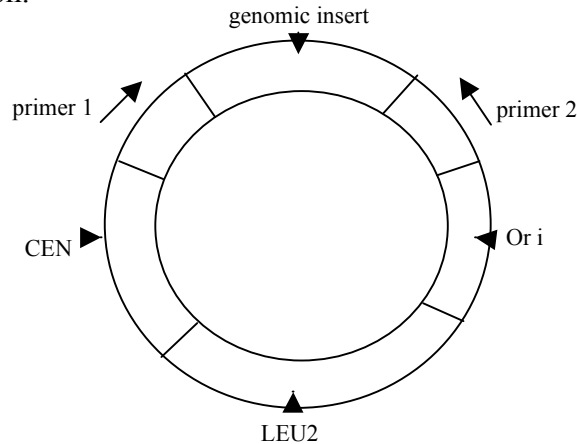
a) Provide the number of complementation groups and the members in each group. Are there any mutants that you cannot place in a group? Why not?

b) In your next set of experiments, you cross each of your *ino*<sup>-</sup> mutants to a WT strain. From each cross, you dissect 10 tetrads. For each mutant, you see a 2:2 segregation of the *ino*<sup>-</sup>:WT phenotypes, except for *ino8*, for which you observe tetrads segregating 2:2, 0:4, and 1:3 for the *ino*<sup>-</sup>:WT phenotypes. What can you conclude from this data about the nature of the different *ino*<sup>-</sup> mutants?

c) What genetic experiment could you do to show that the mutations in each complementation group map to the same location, and are therefore most likely alleles of the same gene? Briefly describe the results you would expect if the mutations are allelic, as well as the results expected if the mutations are nonallelic.

d) After characterizing your mutants further, you decide you want to clone *ino3* first. In your lab, you possess a wild-type yeast genomic library in LEU2-marked CEN plasmids (plasmids also contain a bacterial *ori* and ampicillin resistance cassette).

Please explain the strategy you would use in order to clone the gene responsible for the *ino3* mutation.



e) You try to clone the gene responsible for the *ino6* mutation using the same cloning strategy as for *ino3*. Why is this approach unsuccessful? How would you go about cloning the gene responsible for the *ino6* mutation?

f) A competing lab is also interested in studying inositol biosynthesis. Instead of using UV mutagenesis, they decide to do a Snyder mutagenesis because they believe it will be faster. A Snyder mutagenesis uses transposon insertions to cause mutations (details of how a Snyder mutagenesis works are not necessary here). Since the sequence of the transposon inserted into the yeast's genome is known, a primer is made to the end of the transposon and the sequence next to the transposon insertion site can be sequenced. The following sequence is returned from one of their mutants that fails to grow on media lacking inositol:

```
5' - TTCTACT CAATAAGGAG GACAAGGATA TATCTGATTT TTCAAAGACT
ACCGCAGGCA AGTCTGCTAA GAAGAATAGC AGAGAGAGAG TTGCCGATGT
GGCGCCACC AGAGTGCTAG ATAAGAAACA AGCGTATCTA - 3'
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Use the BLAST algorithm in the SGD database to match the above sequence to the *S. cerevisiae* genome. Then, click the "ORF Map" link from the BLAST results page to see the chromosomal features for that region.

-What gene did the transposon insert into? Where in the protein product is this insertion (C-term, N-term, *etc.*; can even give the amino acid codon it inserted into if you are feeling confident). What type of allele is this insertion likely to be and why?

-Using resources available to you on the World Wide Web, briefly list some possible biochemical functions of this gene. What other yeast proteins does this protein share homology with? Where is this protein localized in the cell?

3). You are moonlighting in a yeast genetics lab when the power suddenly goes out. You look down at your plates and observe that one of your yeast colonies is glowing in the dark. The next day, you decide to switch the focus of your project from transcriptional regulation to yeast bioluminescence.

a). Describe a simple experiment you could conduct to determine whether the glow-in-the-dark phenotype (Glo-) is caused by a mutation in one gene or by mutations in different genes. Assume your original Glo- mutant arose in a haploid strain.

What results and phenotypic ratios would you predict if the Glo- phenotype is caused by a single gene?

What results and phenotypic ratios would you predict if the Glo- phenotype is caused by two unlinked genes, both of which are required to cause the Glo- phenotype? Be as specific as possible.

b). You decide to screen for additional mutants with a Glo- phenotype. After spending many hours in the darkroom looking at plates of yeast, you identify four new Glo- mutants, which you name them *glo2* through *glo5*. You determine that the Glo- phenotype of each of your new mutants is caused by a single mutation. You then perform pairwise complementation tests on all of your mutants and determine that they fall into the following complementation groups:

Complementation Groups

**Group A:** *glo 1, 2*

**Group B:** *glo 3*

**Group C:** *glo 4, 5*

To map your *glo* mutants, you sporulate the diploids you obtained from complementation testing and conduct tetrad analysis. You dissect 100 tetrads from each cross. In your preliminary data, you find that *glo4* maps directly to a centromere. Some of the other results you obtain are shown below.

Cross	Tetrad Phenotypic Ratios		
	0 Glo+ : 4 Glo -	1 Glo+ : 3 Glo -	2 Glo+ : 2 Glo -
<i>glo1</i> x <i>glo2</i>	100	0	0
<i>glo1</i> x <i>glo3</i>	80	18	2
<i>glo1</i> x <i>glo4</i>	45	10	45
<i>glo2</i> x <i>glo5</i>	33	34	33
<i>glo3</i> x <i>glo4</i>	30	40	30
<i>glo4</i> x <i>glo5</i>	38	24	38

Which phenotypic ratios (0:4, 1:3, or 2:2) represent...  
-parental ditype (PD) tetrads?

- nonparental ditype (NPD) tetrads?
- tetratype tetrads (TT)?

Draw a complete genetic linkage map of *glo1* - *glo5*. Include distances between linked alleles and distances between each allele and its respective centromere whenever possible. Please show your work.

c). Considering both complementation and mapping data, how many different genes are represented by your *glo* mutants?

Provide a brief description and genetic explanation for the behavior of the *glo4* and *glo5* mutants.

d). In order to test whether the glow-in-the dark phenotype depends on the genetic background of the wild-type strain used in your lab, you cross your *glo1* mutant strain to another wild-type strain obtained from your collaborator (WT2).

You obtain the following results after dissecting 100 tetrads:

Cross	4:0 (Glo <sup>+</sup> : Glo <sup>-</sup> )	3:1(Glo <sup>+</sup> : Glo <sup>-</sup> )	2:2 (Glo <sup>+</sup> : Glo <sup>-</sup> )
<i>glo1</i> x WT2	1	46	53

You notice that these results differ significantly from those obtained when you crossed *glo1* to your own wild-type strain (you may wish to refer back to your answer in part a).

Provide a hypothesis that explains the results obtained. Your answer should explain each class of tetrad and the frequencies with which they occur, including all relevant genotypes and phenotypes.

4). A new postdoc in a yeast lab isolates several mutants that are resistant to high levels of zinc. All of the mutations that confer this resistance are dominant to the wild type allele. Assume that all the types of vectors and strains discussed in class are available for this person's research.

i). Starting with a wild-type yeast strain, briefly describe the best way to isolate this class of mutants.

ii). How should this postdoc determine how many genes have been identified?

iii). Assume that the mutations identify a single gene. Describe the steps the postdoc should follow to clone the gene.

iv). What cross should he set up to prove that he has cloned the correct gene? What result will he obtain if the correct gene is cloned? What result will he obtain if the correct gene is not cloned?

4b). Another new postdoc in the same lab isolates a series of yeast mutants that are super-sensitive to normal levels of zinc. She demonstrates that these mutations are recessive to wild type.

i). Starting with a wild-type yeast strain, briefly describe the best way to isolate this class of mutants.

ii). By standard procedures, this postdoc demonstrates that two genes are identified by these recessive mutations. Describe a cross to construct a double mutant containing mutations in both of these genes. Remember that each confers the same phenotype. How can one verify that a putative double mutant really contains both mutations?

iii). For each class of recessive mutation, what cross(es) should be done to determine if any of these mutations are allelic to the dominant zinc resistant class described in part a. What results are expected if they are allelic or if they are not allelic?

iv). Assuming that at least one class of the super-sensitive mutations are not allelic to the resistant mutation, how would one determine the epistasis relationship between these two mutations?