

Name \_\_\_\_\_

Genetics 201

Midterm exam

October 31, 2005

PUT YOUR NAME ON EVERY PAGE.

THERE ARE FOUR MULTI-PART QUESTIONS ON THIS EXAM. EACH QUESTION IS WORTH A TOTAL OF 25 POINTS. THE POINT VALUE FOR EACH PART IS INDICATED.

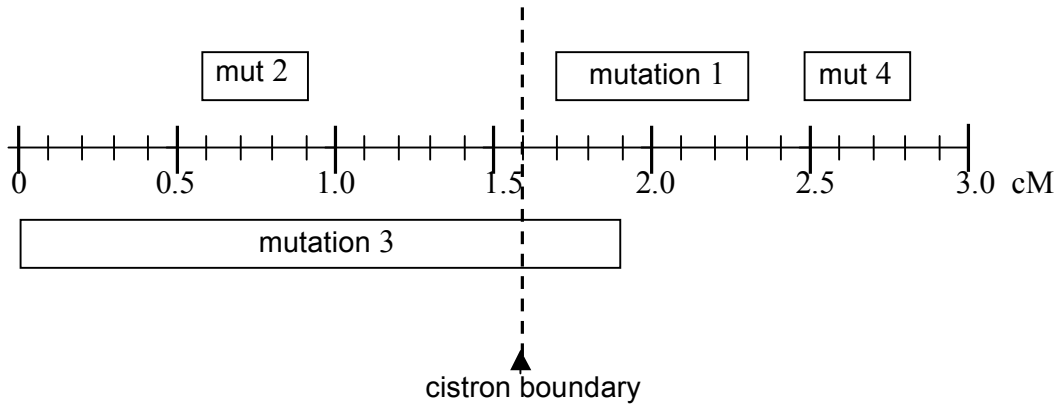
WE RECOMMEND THAT YOU LOOK THROUGH THE EXAM AND ANSWER THE EASIER QUESTIONS FIRST.

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 have four temperature-sensitive mutant isolates of a poorly characterized lysogenic phage called phage 201. Wild-type phage 201 forms turbid plaques on *E. coli* cells at both 37°C and 42°C. In contrast, the mutant isolates that you are studying form clear plaques at 37°C, and form no plaques at 42°C. Another member of your lab has previously determined that all four mutants bear deletions and has established a preliminary map. You now wish to confirm the map positions.

The preliminary map (drawn to scale) is shown below.



You perform the following test: Bacteria are infected pairwise at 37°C at high m.o.i. for one round of infection; the resultant bursts are then titered on *E. coli* at both 37°C and 42°C.

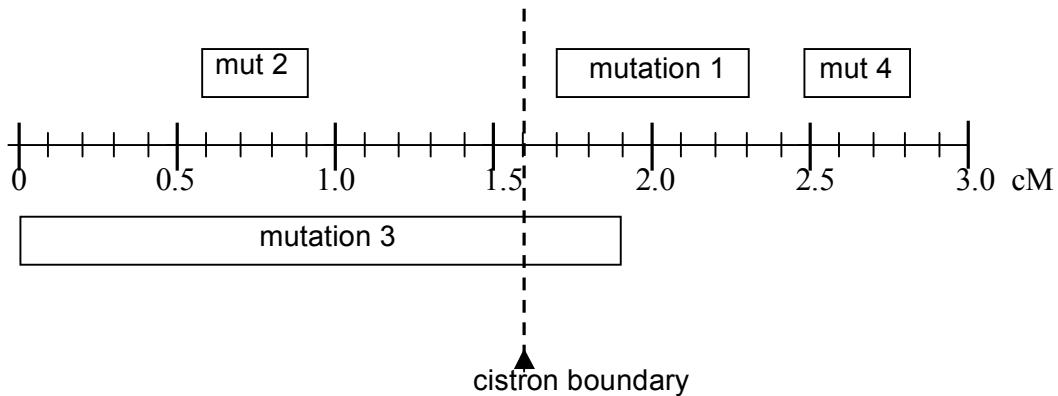
1a. Based on the preliminary map, indicate in the unshaded cells in the table below whether you expect wild-type recombinants to be present in the progeny from a given cross. Please fill in "+" for presence of wild-type phage, and "-" for no wild-type phage detected. (5 points)

	mutant 1	mutant 2	mutant 3	mutant 4
mutant 1				
mutant 2				
mutant 3				
mutant 4				

**1b.** You find that the deletions behave exactly as predicted. You now want to assign a map location to a newly isolated point mutation (mutation 5) that causes the same phenotype as the deletion mutations. You cross this mutant to each of your deletion mutants at 37°C, plate at both 37°C and 42°C, and calculate the ratio of plaques present at the nonpermissive temperature to plaques present at the permissive temperature for each cross. These data are given in the table below.

cross	ratio (plaques at nonpermissive temp./ plaques at permissive temp.)
5 x 1	0
5 x 2	0.0045
5 x 3	0
5 x 4	0.0035
5 x 5	$1 \times 10^{-7}$

**Place mutation 5 on the map below. Also, provide map distances between mutation 5 and the other mutations in the boxes provided. (5 points)**



**5-1 Distance:**

**5-2 Distance:**

**5-3 Distance:**

**5-4 Distance:**

**1c.** You also want to perform complementation tests with mutant 5 and the various deletion mutants in order to confirm the location of mutation 5 with respect to the cistron boundary.

**Describe how you will do such a test, including the two most critical controls. (6 points)**

**1d.** Assuming that the position of the cistron boundary shown in the drawing is correct, fill in the expected results for the complementation data in the unshaded boxes in the table below. Use "+" to indicate complementation and "-" to indicate that two mutants fail to complement one another. (5 points)

	mutant 1	mutant 2	mutant 3	mutant 4	mutant 5
mutant 1					
mutant 2					
mutant 3					
mutant 4					
mutant 5					

**1e.** You then actually perform the complementation tests between mutant 5 and each deletion mutant (1-4). To your surprise, you obtain the following results (again, + indicates complementation and – indicates no complementation):

	mutant 5
wild type	+
mutant 1	-
mutant 2	-
mutant 3	-
mutant 4	-
mutant 5	-

**Which of these results is not as expected? Briefly (in 1-2 sentences) provide one possible explanation for this finding. (4 points)**

2. You are studying mitosis in *S. cerevisiae*. You want to identify genes that, when mutated, cause a temperature-sensitive lethal phenotype at 37°C by blocking the cell cycle at mitosis (viable at 30°C, lethal at 37°C).

**2a. Briefly list the steps you will use to identify such mutants. (4 points)**

**2b.** You identify an interesting mutant, which you name *cdc500-1*. This mutant has the desired phenotype of lethality at mitosis at 37°C (Ts<sup>-</sup> phenotype).

**Briefly state how you will test whether the Ts<sup>-</sup> phenotype is caused by a single mutation or not. (3 points)**

**Assume you find that *cdc500-1* is a single nuclear mutation. Describe how you will test whether the Ts<sup>-</sup> phenotype is recessive or dominant. (3 points)**

Name \_\_\_\_\_

**2c.** After further experiments, you successfully clone the *CDC500* gene. You are excited to find that it encodes a previously unstudied kinesin protein. You now want to identify components of the cytoskeleton that interact with this protein. You decide to begin by isolating suppressors.

**Outline the steps you will carry out to identify unlinked suppressors of *cdc500-1*. Your list of steps should include brief descriptions of how you will:**

- (i) obtain suppressor candidates**
- (ii) test whether each candidate is caused by a single mutation**
- (iii) test whether each candidate is linked or unlinked to *cdc500-1*.**

**Your answer should include the possible outcomes for each test. You have available both wild-type and *cdc500-1* strains in each mating type. (6 points)**

**2d.** Your screen in part c is successful, and you find three unlinked mutations that suppress *cdc500-1*, which you name *sup1*, *sup2*, and *sup3*. Each *sup* mutation is recessive and is caused by a single gene. To further characterize these suppressor mutations, you cross them with each of the following strains:

Strain	Description
<i>cdc500Δ</i>	Deletion of <i>CDC500</i> gene; has Ts <sup>-</sup> phenotype identical to <i>cdc500-1</i>
<i>cdc500-1</i>	Original mutant strain; has a missense mutation in the coding region of the gene.
<i>cdc500-2</i>	A strain with a missense mutation at a different position in the <i>CDC500</i> gene.

For each cross, you sporulate the diploid, collect spores, and identify a haploid double mutant (*cdc500-1 sup1*, for example). You then determine the Ts phenotype of the double mutant by testing its ability to grow at 37°C. Your results are shown in the table below:

Genotype	Phenotype
<i>cdc500Δ sup1</i>	Ts <sup>+</sup>
<i>cdc500-1 sup1</i>	Ts <sup>+</sup>
<i>cdc500-2 sup1</i>	Ts <sup>+</sup>
<i>cdc500Δ sup2</i>	Ts <sup>-</sup>
<i>cdc500-1 sup2</i>	Ts <sup>+</sup>
<i>cdc500-2 sup2</i>	Ts <sup>-</sup>
<i>cdc500Δ sup3</i>	Ts <sup>-</sup>
<i>cdc500-1 sup3</i>	Ts <sup>+</sup>
<i>cdc500-2 sup3</i>	Ts <sup>+</sup>

**Propose a mode of action for each suppressor that is consistent with the data given above. Briefly support your claim. (6 points)**

***sup1*:**

***sup2*:**

***sup3*:**

Name \_\_\_\_\_

**2e. Given your goal of identifying other cytoskeletal components, which suppressor should you attempt to clone? Briefly justify your choice. (3 points)**

3. You have discovered a new strain of *E. coli* (Strain X) living near an oil refinery that can metabolize hydrocarbon waste products as its sole energy source (Oil<sup>+</sup> phenotype). Laboratory *E. coli* strains fail to grow under such conditions (Oil<sup>-</sup> phenotype). You wish to determine whether or not a single chromosomal locus can be identified that confers the Oil<sup>+</sup> phenotype on laboratory strains of *E. coli*.

**3a. To do this, you plan to link the hypothetical *oil* locus in Strain X to a kanamycin resistance gene so that the ability to metabolize hydrocarbon waste products can be transduced into laboratory strain MG1655 (the sequenced strain of *E. coli*). List the steps you will perform to do this (including any tests to confirm linkage), using any of the reagents listed below. (8 points)**

P1 phage

$\lambda$ miniTn5 (Kan<sup>R</sup>)

Strain X

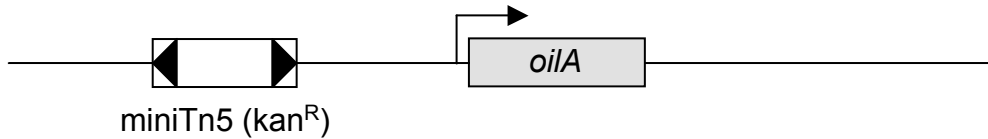
*E. coli* MG1655

Oil-based medium (containing oil as sole carbon source)

Standard LB medium

Kanamycin

**3b.** Assume that your approach was successful and you have constructed an Oil<sup>+</sup> derivative of MG1655 (with a linked kanamycin resistance marker). Using a transductant in which the linkage is ~100%, you sequence the DNA surrounding the Tn5 marker and find that your transductant contains a one-gene insertion (presumably the *oil* locus from strain X), for which there is no corresponding sequence in MG1655. Sequence analysis reveals that this gene, which you name *oilA*, shares homology with a family of genes encoding metabolic enzymes.



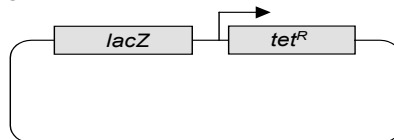
Now you would like to investigate the regulation of *oilA*, which you hypothesize encodes the enzyme responsible for the metabolism of hydrocarbon waste products. For this purpose, you decide to construct a chromosomal fusion such that *lacZ* is under the control of the *oilA* promoter (transcriptional fusion). Note that you wish to construct the fusion in such a way so that expression of the *oilA* gene is not disrupted. List the steps you will perform to construct such a fusion, using any of the following reagents. You may use the above diagram to help illustrate your strategy. Be sure to explain how you will identify the desired fusion. (9 points)

MG1655  $\Delta lacZ$  *oil*<sup>+</sup> Kan<sup>R</sup>: A derivative of MG1655 with a deletion of the chromosomal *lacZ* gene and containing the *oil* locus and the miniTn5 insertion described above.

$\lambda$ Red plasmid: A plasmid with a temperature sensitive origin of replication that directs the synthesis of the  $\lambda$ Red genes under the control of an IPTG-inducible promoter. Note that this plasmid confers resistance to chloramphenicol.

Any necessary primers and PCR reagents

A plasmid containing the *lacZ* gene (without its promoter) together with a downstream tetracycline resistance gene with its own promoter (see diagram below)



Oil-based medium with or without X-gal (cells that express *lacZ* form blue colonies on X-gal-containing indicator medium)

LB medium with or without X-gal

Any necessary antibiotics

IPTG

**(The next page is left blank for your answer.)**

Name \_\_\_\_\_

**3c.** Having successfully constructed the *lacZ* fusion, you find that the fusion strain forms blue colonies on oil-based indicator (X-gal) medium (as expected), but white colonies on standard LB indicator (X-gal) medium. You conclude that the expression of *oilA* is inducible. You now wish to isolate a regulatory mutant that will enable you to identify the gene encoding the regulator that controls the expression of *oilA*. Using nitrosoguanidine, a chemical mutagen, you mutagenize your *lacZ* fusion strain (MG1655 *oilA::lacZ*) and identify a constitutive mutant by looking for a mutant that forms blue colonies on LB + X-gal medium in the absence of oil. As expected, the mutant also forms blue colonies on oil-based medium.

**Assume that you determine that the mutation is unlinked to the *oil* locus. You decide to attempt to clone the regulator. Using any of the reagents listed below, describe a strategy that *might* enable you to clone the regulator (a) if the *oil* locus is under negative control and (b) if the *oil* locus is under positive control. (8 points)**

Reagents

MG1655 *oilA::lacZ*

The constitutive mutant derivative of MG1655 *oilA::lacZ*

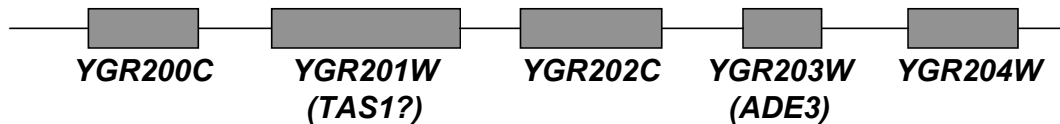
A plasmid vector designed to facilitate construction of an *E. coli* expression library. An expression library is a library in which each plasmid directs the synthesis of a randomly cloned gene from any particular strain of *E. coli*. This vector carries an ampicillin resistance gene as a selectable marker.

LB medium with or without X-gal

Oil-based medium with or without X-gal

Any necessary antibiotics and any necessary reagents for recombinant DNA manipulations

4. Your lab has recently identified an exciting new *S. cerevisiae* gene, *TAS1*. The *tas1* mutation causes the phenotype of telomeres that are shorter than normal (*TAS* = Telomeres Are Shorter), resulting in a decreased life span. For unknown reasons, the *tas1* mutation also causes a bumpy colony morphology. A postdoc in the lab has already tentatively cloned the wild-type *TAS1* gene by complementation of the bumpy colony phenotype. The putative *TAS1* gene is a previously unstudied, nonessential gene (*YGR201W*) on chromosome VII, near the *ADE3* gene, which encodes an enzyme required for adenine biosynthesis. The gene map of this region of chromosome VII is shown below.



4a. Your advisor wants you to confirm that the correct gene has been cloned. You have available the original *tas1* mutant, which is mating type a, and you also have available the entire collection of viable deletions, which are in mating type  $\alpha$ . Recall that in this set of strains, each nonessential gene is deleted and replaced by a gene encoding resistance to the antibiotic G418 (designated as G418<sup>R</sup>).

**Design a cross to test if the cloned gene, *YGR201W*, is linked to the original *tas1* mutation. Your answer should include the relevant genotypes and phenotypes of the parent strains used and the outcome of the experiment if (i) the correct gene was cloned or (ii) an unlinked gene was cloned. (6 points)**

Name \_\_\_\_\_

**4b.** To learn more about *TAS1*, you decide to try to identify other proteins that may function in the same pathway. To do this, you plan to carry out a screen to identify genes that, when overexpressed, cause lethality specifically in strains with a *tas1* deletion (*tas1ΔG418<sup>R</sup>*). You have available the following reagents:

Reagent	Description
<i>tas1ΔG418<sup>R</sup> ura3 leu2</i> strain	A haploid <i>S. cerevisiae</i> strain with chromosomal mutations listed at left
<i>TAS1 URA3 CEN</i> plasmid	A low copy number plasmid carrying the wild-type <i>TAS1</i> gene and <i>URA3</i> as a selectable marker
Recombinant <i>S. cerevisiae</i> library in a <i>LEU2</i> -marked high copy number plasmid	An overexpression plasmid library carrying genomic DNA fragments and <i>LEU2</i> as a selectable marker
5-FOA	Selects against Ura <sup>+</sup> cells when present in the medium

You also have available any selective media you may wish to use.

**List the steps you will perform to identify genes that, when overexpressed in a *tas1Δ* background, cause lethality. Please include the phenotypes for which you will be screening and the specific media you will use at each step. (7 points)**

**4c.** Your screen was successful, and you have recovered a plasmid that, when overexpressed in a *tas1ΔG418<sup>R</sup>* background, causes lethality. This plasmid causes no phenotype in a *TAS1<sup>+</sup>* background. You sequence the plasmid inserts, perform subcloning experiments, and identify the responsible gene, which you name *AGE1*. *AGE1* is not essential for growth.

You now wish to characterize the phenotype of an *age1* null mutant strain (as opposed to the overexpression phenotype you have worked with until this point). When you characterize an *age1ΔG418<sup>R</sup>* mutant, you find that it has longer-than-normal telomeres. (Recall that the *tas1Δ* strain has telomeres that are shorter than normal.)

**Design an experiment to determine the epistasis relationship between the *age1ΔG418<sup>R</sup>* and the *tas1ΔG418<sup>R</sup>* mutations. Your answer should include the genotypes of any strains used, the methods used to construct and identify any new strains, the possible results, and their interpretations. (6 points)**

**4d.** Additional analysis of *AGE1* reveals that *S. cerevisiae* has a second gene whose sequence is closely related to that of *AGE1*. You name this gene *AGE2*. In order to construct an *age1Δ age2Δ* double mutant, you perform the following cross, using strains derived from crosses with deletion set strains:

*MATa age1ΔG418<sup>R</sup>* x *MATα age2Δ G418<sup>R</sup> trp1* (recall that *trp1* is very tightly centromere-linked)

After dissecting and analyzing 100 tetrads, you analyze segregation of the markers and get the following results:

*age1ΔG418<sup>R</sup>, age2Δ G418<sup>R</sup>* segregation – 81 PD, 1 NPD, 18 TT

*age2Δ, trp1* segregation – 17 PD, 16 NPD, 67 TT

**Based on the tetrad data, provide as much information as possible about the map positions of these three genes, including calculations of distances and possible centromere linkage. You may draw a map as part of your answer, but it is not necessary. (6 points)**