

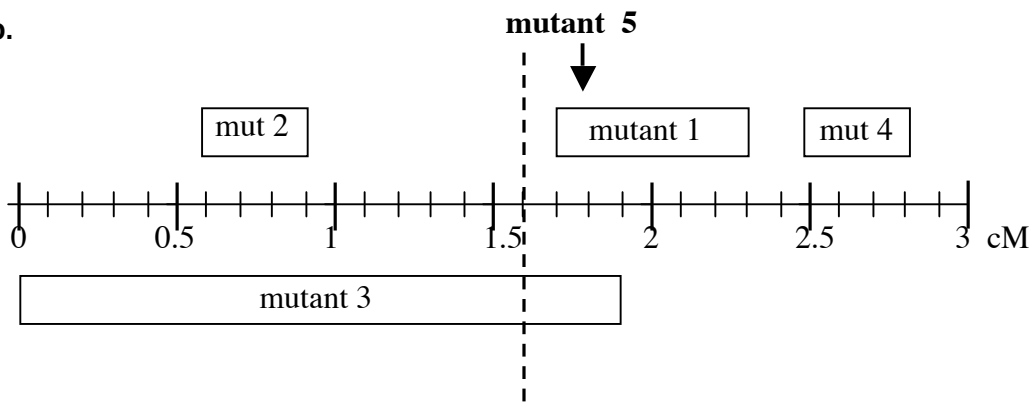
Genetics 201, Midterm answers, 10/31/05

Question 1

1a.

	mutant 1	mutant 2	mutant 3	mutant 4
mutant 1	-	+	-	+
mutant 2		-	-	+
mutant 3			-	+
mutant 4				-

1b.



5-1 Distance: 0 cM

5-2 Distance: 0.9 cM

5-3 Distance: 0

5-4 Distance: 0.7 cM

1c. Infect *E. coli* at high m.o.i. with each possible pair of mutants at the restrictive temperature (42°C) for one round of infection. Plate at 37°C to detect whether or not progeny phage were produced (a productive burst).

Critical controls:

1. Make sure progeny phage are still mutant: Perform test as above, but titer at 42°C. Almost all progeny phage should fail to grow.

2. Make sure each mutation is recessive. To do this, coinfect wild-type phage 201 with each 201 mutant at high m.o.i. at 42°C, then titer on *E. coli* at 37°C to determine the burst size. One should detect a burst comparable in size to the burst released following infection with wild-type 201 alone, indicating that each mutation is recessive. Note: while formally, a double mutant

would be tested instead of a single mutant, in practice, single mutants are used to avoid having to construct each double mutant.

1d. Expected results:

	1	2	3	4	5
1	-	+	-	-	-
2		-	-	+	+
3			-	-	-
4				-	-
5					-

1e. Possible explanations:

Mutation 2 and mutation 5 are in separate cistrons but show unlinked noncomplementation.

Question 2

2a. Mutagenize *S. cerevisiae* and grow up colonies. Replica plate to two plates with rich medium. Incubate one plate to the restrictive temperature (37°C) and one at the permissive temperature (30°C) to look for colonies that are temperature sensitive (fail to grow at 37°C). Retrieve these colonies from the plate growing at 30°C and screen to identify those in which lethality occurs specifically at mitosis by looking at the bud size.

2b. To test whether *cdc500-1* is a single mutation, cross *cdc500-1* to wild-type yeast, sporulate the diploid, dissect tetrads, and analyze spores for their Ts phenotype. If it is a single mutation all tetrads will be 2 Ts⁺: 2 Ts⁻ (PD). If the Ts⁻ phenotype is caused by two or more mutations different segregation patterns will occur.

To test for dominant vs. recessive phenotype, cross the *cdc500-1* mutant to wild-type yeast to generate a diploid. Test the diploid for growth at 37°C. If cells die at mitosis, then *cdc500-1* is dominant; if they grow like wild-type, then *cdc500-1* is recessive.

2c.

i. Mutagenize the *cdc500-1* strain and plate it at restrictive temperature to select for rare mutants that can now grow at 37°C. Any colonies which now grow are Ts⁺ and may contain the desired suppressor mutations.

ii. To determine if a candidate is caused by a single mutation, it should be crossed back to a *cdc500-1* mutant. If it is a single mutation, Ts⁺:Ts⁻ will segregate 2:2. Note: this is true whether or not the event is a true revertant (linked) or an unlinked, extragenic suppressor.

iii. To test if a candidate is linked or unlinked to *cdc500-1*, cross it to a wild-type haploid strain of the opposite mating type. Sporulate the diploid and score the tetrads for growth at 37°C. If the Ts⁺ phenotype is due to reversion of the original *cdc500-1* mutation, all spores recovered will be Ts⁺, and all the tetrads will be PD (4 Ts⁺ : 0 Ts⁻). If the Ts⁺ phenotype is due to an extragenic suppressor mutation, then some spores will be Ts⁻, and some of the tetrads will be TT (3 Ts⁺ : 1 Ts⁻) or NPD (2 Ts⁺ : 2 Ts⁻), assuming the suppressor mutation does not have an independent phenotype.

If the suppressor mutation is extragenic, but linked to *cdc500-1*, then the number of PD tetrads (4 Ts⁺: 0 Ts⁻) will be greater than the number of NPD tetrads (2 Ts⁺ : 2 Ts⁻). Some TT tetrads will also be present.

If the suppressor mutation is unlinked to *cdc500-1*, then you will recover equal numbers of PD and NPD tetrads. (The number of TT tetrads recovered will depend on the locations of the *cdc500* and *sup* genes relative to their centromeres).

2d.

sup1: bypass suppressor. The mutation suppresses all 3 *cdc500* mutations, including the null. Likely creates neomorphic mutation in another gene that can substitute for the Cdc500 product.

sup2: allele-specific suppressor. This mutation only suppresses the *cdc500-1* mutation, not the null or the other point mutation. It may be in a gene encoding a protein that interacts with Cdc500.

sup3: overproduction suppressor. This mutation suppresses both *cdc500-1* and *cdc500-2* mutant phenotypes, but it cannot suppress the null. Overproduction of the Cdc500 mutant product suppresses defect.

2e. You should attempt to clone *sup2*, as it is most likely to encode a protein that interacts with the Cdc500 gene product.

Question 3

3a.

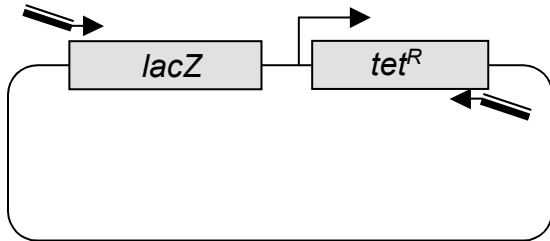
1. Infect Strain X with \square miniTn5 and select for kanamycin resistant clones on LB medium containing kanamycin.
2. Pool several hundred Kan^R colonies, infect with PI, and prepare a lysate.
3. Infect MG1655 cells with the P1 lysate and select for Kan^R transductants on oil-based medium containing kanamycin.
4. Infect individual Kan^R/Oil⁺ candidate clones with PI and make lysates.
5. Use these lysates to infect MG1655 cells and select for Kan^R clones on LB medium containing kanamycin. For each lysate, determine what fraction of the Kan^R clones are also Oil⁺. If the percentage is greater than 50%, you have successfully linked the Kan^R gene to the oil locus.

3b.

Use \square Red-mediated recombination to introduce the *lacZ* gene just downstream of *oilA*.

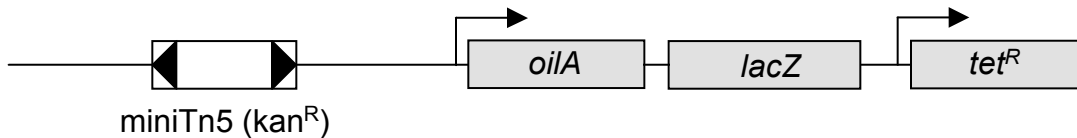
1. Introduce the λ Red plasmid into MG1655 $\Delta lacZ$ oil^+ Kan^R by selection for chloramphenicol resistance. (Plate transformants at the permissive temperature so that the λ Red plasmid can be maintained.)

2. PCR amplify the *lacZ* and *tet^R* genes together using primers, each of which carries about 50 nucleotides of sequence that is homologous to the DNA just downstream of *oilA*. In the diagram below is shown the template and primers. The heavy lines on the primers represent regions homologous to sequences 3' of the *oilA* gene.



3. Electroporate the linear PCR product into cells already containing the λ Red plasmid. Select for tet^R transformants. (These transformants are plated at the nonpermissive temperature to ensure loss of the λ Red plasmid.)

4. Sequence chromosomal DNA downstream of *oilA* to confirm presence of *lacZ* at the desired location. The final construct will look as diagrammed below.



3c.

(a) If the locus is under negative control, then your constitutive mutant likely contains a recessive (inactivating) mutation in the gene encoding the repressor. Therefore, you may be able to clone the repressor gene by making an expression library from strain MG1655 *oilA::lacZ*. Transform the constitutive mutant strain with the expression library and screen for transformants that once again express *lacZ* only in the presence of oil in the medium.

(b) If the locus is under positive control, then your constitutive mutant likely contains a dominant mutation in the activator gene such that the mutant activator turns on expression of *oilA* regardless of the presence of oil in the medium. Therefore, you may be able to clone the activator gene by making an expression library from the mutant (constitutive) strain. Transform the wild type parent strain with this expression library and screen for transformants that express *lacZ* constitutively.

Question 4.

4a. Cross the *tas1* mutant by the strain deleted for *YGR201W*. If *TAS1 = YGR201W*, then all tetrads will be PDs. The analysis of the cross depends upon the phenotype of the *ygr201w* deletion. If it also causes bumpy colonies, then all four spores in each tetrad will be bumpy. If *TAS1* is unlinked to *YGR201W*, then there will be wild-type spores and, therefore, smooth colonies. If the *ygr201w* deletion does not cause bumpy colonies, then the PDs will have two bumpy colonies that are G418^S and two smooth colonies that are G418^R. If the genes are unlinked, then there will be bumpy colonies that are G418^R (in TT and NPD tetrads).

4b. Screen for high copy number plasmids that cause lethality in a *tas1*Δ background:

1. Take the *tas1*Δ *ura3 leu2* yeast strain, transform it with the *TAS1 URA3* plasmid. Select transformants on Ura⁻ medium to obtain cells that have the plasmid.
2. Transform the newly created strain from step 1 with the *LEU2*- marked overexpression library. Select Leu⁺ transformants by growing on medium lacking leucine.
3. Replica plate colonies onto medium containing 5-FOA to select for cells that have lost the *TAS1 URA3* plasmid. Look for colonies that fail to grow as compared to the master plate from step 2. Such colonies may contain a high copy number plasmid that, when overexpressed in a *tas1*Δ background, results in a lethal phenotype.
4. Recover plasmid into *E. coli*, isolate the plasmid, sequence the ends, subclone individual genes into the high copy number plasmid, transform with each into the same *tas1*Δ strain to determine which gene causes the lethality.

4c.

1. Cross *tas1*Δ X *age1*Δ. Sporulate the diploid and analyze the tetrads.
2. Obtain double mutant spores from NPD tetrads. The NPD tetrads will have a phenotype of 2 G418^S and 2 G418^R spores. The two that are resistant should each be the double mutant. Score colony morphology and telomere length.

Predicted results: If *age1*Δ is epistatic to *tas1*Δ, then the double mutant spores will have the *age1*Δ phenotype (long telomeres). If *tas1*Δ is epistatic to *age1*Δ, then the double mutant spores will have the *tas1*Δ phenotype (short telomeres).

4d. *age1* and *age2* are linked, with a map distance of 12 cM. Neither is centromere linked. *age2* is unlinked to *trp1*, so *age1* is likely also unlinked to *trp1*.