Genetics 201
2007 Midterm solutions

1ai. Mutagenize haploid cells and plate onto glucose medium at low density. After colonies grow, replica plate onto ethanol medium and look for colonies that fail to grow. These colonies are unable to metabolize ethanol.

1aii. Mutagenize haploid cells and plate on glucose medium. Replica plate onto medium with glucose + allyl alcohol and look for colonies that fail to grow. These colonies are expressing *ADH2* when glucose is present.

1b. *nad3* is dominant; *nad1*, *nad2*, and *cad1* are recessive.

1c. There are two possible correct answers for this question, doing a linkage test or doing a complementation test.

Test 1: Test for linkage between *nad1* and *adh2* using tetrad analysis.
Obtain the *adh2ΔG418*^{R} deletion strain from the yeast deletion set. The phenotype of this strain will be Adh$^-$, like the *nad1* mutant.

Cross the deletion strain to *nad1*, sporulate the diploid, and analyze the tetrads for the ability to metabolize ethanol (Adh phenotype).

If *nad1* is a mutation in *ADH2*, then all tetrads will be 2 G418^R Adh$^-$: 2 G418^S Adh$^-$ (PD).
If *nad1* is a mutation in a gene other than *ADH2*, then other tetrad classes will be present that contain Adh$^+$ spores.

Even if the *NAD1* and *ADH2* genes are closely linked, given the high level of meiotic recombination in yeast, we would expect to observe Adh+ spores.

Test 2: Perform a complementation test between *nad1* and *adh2*.
Again, use the *adh2ΔG418*^{R} deletion strain. Cross to *nad1* and analyze the phenotype of the diploid. If the diploid is able to metabolize ethanol, then complementation has occurred, and *nad1* is probably a mutation in a gene other than *ADH2*. If the diploid is unable to metabolize ethanol, then nad1 is probably a mutation in *ADH2* itself.

1d. 94 PD, 6 TT; therefore, *nad2* and *cad1* are linked by 3 cM.
In the TT tetrad, the double mutant spore is Adh^c (*cad1* phenotype); therefore, *cad1* is epistatic to *nad2*.

Problem 2

2a. Infect strain A with mutagenized HK201 at low moi and plate out to examine a large number of single plaqes. Look for plaques that are clear (not turbid). These plaques contain phage mutants (so-called clear mutants) that cannot lysogenize strain A.

2b. Take strain A and infect with λ mini-Tn10 (Tet^R). Isolate Tet^R colonies. Pool as many colonies as possible; this is your mutant library. Plate the library of Tet^R cells on medium seeded with an excess of the HK201 clear plaque mutant isolated in part a. Look for clones that are not killed by the clear mutant phage. These colonies contain mutant bacteria that are resistant to infection by HK201. (Individual surviving colonies could be tested by the cross streak assay to confirm that they are in fact resistant to the HK201 clear mutant phage).

2c. Grow P1 on each Tet^R mutant. Collect lysate and use to infect parental strain A; select for Tet^R colonies. Screen individual Tet^R colonies for their HK201 phenotype by performing a cross-streak assay with the HK201 clear plaque mutant. If the mini-Tn10 insertion is responsible for the $HK201^R$ phenotype, then 100% of the Tet^R colonies will also be $HK201^R$. (Alternatively, one could screen individual Tet^R colonies by growing each in liquid culture, then infecting with HK201 clear plaque mutant phage at low moi and plating the mixture in top agar to determine whether any plaques formed.)

2d. Infect each $HK201^R$ mutant with HK201[kan^R] and determine whether or not Kan^R lysogens can be formed. (This can be done by spotting an HK201[kan^R] lysate on a lawns of each bacterial mutant. After incubating the plates, use a toothpick to remove cells from the center of each spot and streak for colonies on medium containing kanamycin.)

Problem 3

3a. Cross purple x wild-type. Look at F1. If F1 have purple flowers, mutation is dominant; if F1 has white flowers, mutation is recessive.

3b. Complementation test; 2 genes

3c. Mutant 1 and mutant 4 are mutations in two unlinked genes. The mutations show unlinked noncomplementation. Let a = mutant allele in 1st purple gene, and b = mutant allele in 2nd purple gene.

P: aaBB x AAbb
↓
F1: AaBb x AaBb (purple)
↓
F2: see Punnett square

	AB	Ab	aB	ab
AB	AABB White	AABb White	AaBB White	AaBb Purple
Ab	AABb White	AAbb Purple	AaBb Purple	Aabb Purple
aB	AaBB White	AaBb Purple	aaBB Purple	aaBb Purple
ab	AaBb Purple	Aabb Purple	aaBb Purple	aabb Purple

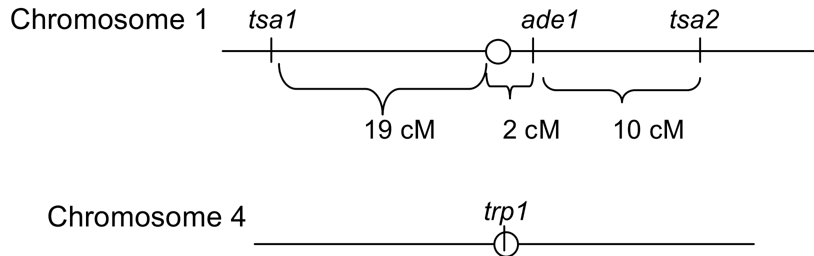
An approximate ratio of 11 purple : 5 white-flowered progeny is seen in the F2.

Any progeny that is homozygous wild-type at either gene (AA or BB) is white. However, any progeny that are either heterozygous at both genes or homozygous mutant at either gene is purple.

Problem 4

4a. *tsa1, 2*, and *3* each contain a single mutation that confers temperature sensitivity. The *tsa4* strain contains 2 unlinked mutations, either of which is sufficient to confer temperature sensitivity. The mutations are both centromere linked.

4b.



4c. The tetrad shows 5:3 segregation. It was produced by unrepaired heteroduplex DNA that spanned the *tsa1* gene during a crossover event. One strand of the DNA is *TSA1* and produces a sector that can grow at both temperatures, while the other strand of DNA is *tsa1* and produces a sector that can only grow at 30°C.

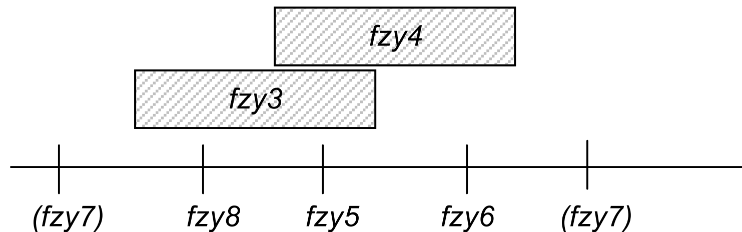
4d. PD = 4 Ts^+ : 0 Ts^- ; NPD = 0 Ts^+ : 4 Ts^- ; TT = 2 Ts^+ : 2 Ts^-

The Ts^+ colony contains a suppressor mutation. When present singly, this mutation has a Ts^- phenotype. The mutation is unlinked to *tsa1* (PD = NPD). It is also unlinked to its centromere (1:1:4; *tsa1* known to be centromere linked).

Problem 5

5a. Coinfect *E. coli* with *fzy1* x *fzy2* at a high multiplicity of infection. Harvest the phage lysate and plate out serial dilutions at low moi. Count the number of small fuzzy plaques and the number of large sharp plaques.
The recombination frequency (cM) = # large sharp plaques x 2 / total # plaques x 100
Controls: Infect *E. coli* singly with *fzy1* and *fzy2* and titer, to verify that the reversion frequency is much lower than the recombination frequency.

5b.

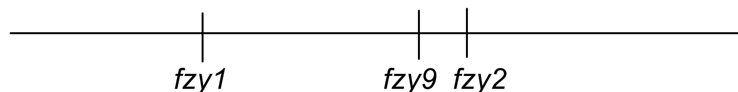


fzy7 could be located to the left or the right of the two deletions.

5c. To make a double mutant, coinfect *E. coli* with *fzy1* and *fzy2* single mutant phage at high moi. Plate the resulting phage lysate at low moi onto *E. coli* and look for fuzzy plaques; these plaques will contain *fzy1, fzy2,* or *fzy1 fzy2* mutant phage.

To determine the genotypes, screen individual progeny phage from fuzzy plaques by crossing back (separately) to both the *fzy1* and the *fzy2* parent and scoring the progeny for the ability to produce large sharp-edged plaques. The *fzy1* and *fzy2* single mutant phage will each produce wild-type (sharp-edged) progeny when crossed to the parent of the opposite genotype. The *fzy1 fzy2* double mutant will not produce large sharp-edged progeny when crossed to either parent phage.

5d.
The correct order is:



A double crossover event occurred between *fzy1* and *fzy2*.