

Genetics 201 2008
Problem Set 1 Solutions

1a. The F1 phenotypic ratio is 1 albino: 2 gray: 1 black, suggesting that only one gene is involved, the two parents are heterozygotes, and the gray fur color is a result of incomplete dominance. Using the allele names *A/a*, assign *A* = black allele and *a* = white allele. The original parents are both *Aa*, and the F1 animals are *AA* (black), *Aa* (gray), and *aa* (albino).

One simple way to test this would be to mate the albino x gray mice (*aa* x *Aa*). This cross would be predicted to yield ½ albino mice and ½ gray mice (*aa* and *Aa* genotypes, respectively). One could also do a similar cross with black x gray mice (*AA* x *Aa*), which would be predicted to yield ½ black mice (*AA*) and ½ gray mice (*Aa*).

An alternative way to test this would be to mate the albino and black F1 mice (*AA* x *aa*). If both are homozygotes, the F2 animals should all be heterozygous and gray (*Aa*).

1b. There are two autosomal genes that regulate the body mass phenotype, both of which produce a homozygous recessive mutant phenotype. For the runty gene, let *r* = mutant allele and *R* = corresponding wild-type allele. Similarly, for the puny gene, let *p* = mutant allele and *P* = corresponding wild-type allele.

Parental strain genotypes:

rrPP : *runty* mutant strain (80% of the normal body mass)

RRpp: *puny* mutant strain (80% of the normal body mass)

F1 genotype:

RrPp results in normal body mass (wild type)

F2 genotypes:

	RP	Rp	rP	rp
RP	<i>RRPP</i> Wild type	<i>RRPp</i> Wild type	<i>RrPP</i> Wild type	<i>RrPp</i> Wild type
Rp	<i>RRPp</i> Wild type	<i>RRpp</i> 80% body mass	<i>RrPp</i> Wild type	<i>Rrpp</i> 80% body mass
rP	<i>RrPP</i> Wild type	<i>RrPp</i> Wild type	<i>rrPP</i> 80% body mass	<i>rrPp</i> 80% body mass
rp	<i>RrPp</i> Wild type	<i>Rrpp</i> 80% body mass	<i>rrPp</i> 80% body mass	<i>rrpp</i> 50% body mass

The phenotypic ratio is 9:6:1 (9 wild type: 6 80% body mass: 1 50% body mass), which is a variation of 9:3:3:1. This ratio suggests that the genes are unlinked.

The *runty* and *puny* mutations produce the same phenotype (80% body mass), while the *rrpp* genotype results in a more severe double mutant phenotype (50% body mass).

1c. There are two separate genes that control fur color and body size. The major phenotypes in the F2 generation show an atypical ratio of 2:1:1, suggesting that the fur color and body size genes are linked.

As in part a, for fur color gene, let A = black allele and a= albino allele.
AA = black, Aa = gray, and aa= albino phenotype.

P: *AArr* X *aaRR*
 black mice albino mice
 80% body mass normal body mass

F1: *AaRr*
 gray mice, normal body mass

Because the two genes are located close together on the same chromosome, the majority of the gametes produced by the F1 animals are nonrecombinant (*Ar* and *aR*).

F2 genotypes :

	<i>Ar</i> (nonrecombinant gamete)	<i>aR</i> (nonrecombinant gamete)	<i>AR</i> (recombinant gamete)	<i>ar</i> (recombinant gamete)
<i>Ar</i> (nonrecombinant gamete)	<i>AArr</i> Black, 80% body mass	<i>AaRr</i> Gray, normal body mass	<i>AARr</i> Black, normal body mass	<i>Aarr</i> Gray, 80% body mass
<i>aR</i> (nonrecombinant gamete)	<i>AaRr</i> Gray, normal body mass	<i>aaRR</i> Albino, normal body mass	<i>AaRR</i> Gray, normal body mass	<i>aaRr</i> Albino, normal body mass
<i>AR</i> (recombinant gamete)	<i>AARr</i> Black, normal body mass	<i>AaRR</i> Gray, normal body mass	<i>AARR</i> Black, normal body mass	<i>AaRr</i> Gray, normal body mass
<i>ar</i> (recombinant gamete)	<i>Aarr</i> Gray, 80% body mass	<i>aaRr</i> Albino, normal body mass	<i>AaRr</i> Gray, normal body mass	<i>aarr</i> Albino, 80% body mass

The progeny produced from the union of two nonrecombinant gametes are shown in bold. The phenotypic ratio of these progeny is 2 gray with normal body mass: 1 albino with normal body mass: 1 black with 80% body mass.

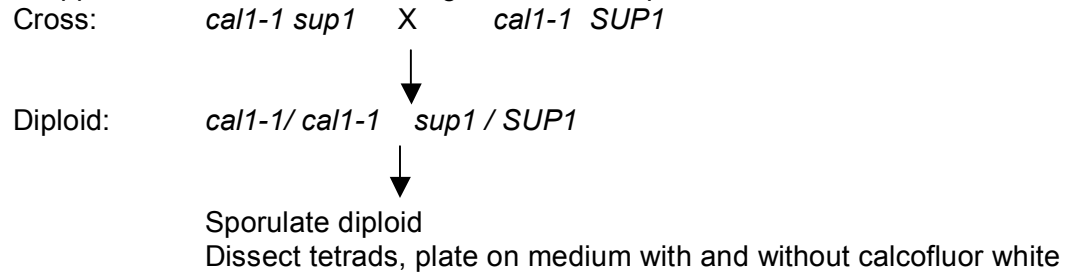
Occasionally, recombination occurs between the coat color and mass loci, producing a recombinant gamete (*ar* or *AR*). When this recombinant gamete combines with a nonrecombinant gamete from the other parent, mice with the genotypes *Aa rr* (gray, 80% mass) and *AA Rr* (black, normal mass) are observed.

Albino mice of reduced mass will only be produced from the union of two recombinant gametes. Based on the low frequency of recombination between the two genes, this event will be even

rarer (its frequency should be the square of the recombination frequency). Thus, this phenotype is not observed with this number of progeny.

2a. Cross each suppressor strain (for example, *cal1-1 sup1*) back to the original *cal1-1* strain in order to confirm that the suppressor mutation segregates 2:2.

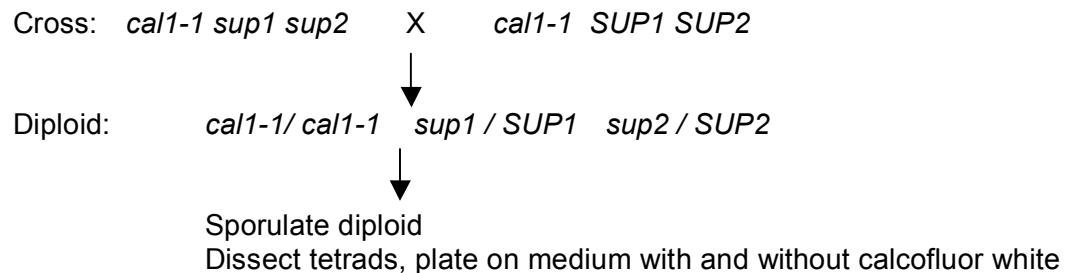
i. Assuming the suppressor strain contains a single mutation, *sup1*:



<u>Spore genotype</u>	<u>Phenotype</u>
<i>cal1-1 sup1</i>	Cal ^R
<i>cal1-1 sup1</i>	Cal ^R
<i>cal1-1 SUP1</i>	Cal ^S
<i>cal1-1 SUP1</i>	Cal ^S

If the Cal^R phenotype is caused by a single mutation, then you will observe only tetrads that contain 2 Cal^R : 2 Cal^S spores (PD class tetrads).

ii. Assume the suppressor strain contains two mutations (*sup1* and *sup2*), both of which are required in order to suppress calcofluor white sensitivity.



One type of tetrad that can be recovered from this cross is the tetratype tetrad:

<u>Spore genotype</u>	<u>Phenotype</u>
<i>cal1-1 sup1 sup2</i>	Cal ^R
<i>cal1-1 sup1 SUP2</i>	Cal ^S
<i>cal1-1 SUP1 sup2</i>	Cal ^S
<i>cal1-1 SUP1 SUP2</i>	Cal ^S

Tetratype tetrads will contain 1 Cal^R : 3 Cal^S spores. NPD and PD tetrads will also be recovered; drawing them and determining their phenotypes would be useful practice. In short, if the Cal^R phenotype is caused by two or more mutations, then you will recover all three classes of tetrads from the cross, instead of just PD tetrads.

2b. Cross each *cal1-1 sup* strain back to a wild type *CAL1* strain.
 If the *sup1* mutation is a revertant, then the cross can be written as follows:

CAL1 (revertant) X *CAL1*



Diploid: *CAL1 / CAL1*



Sporulate, dissect tetrads, score for calcofluor white phenotype

All spores will be calcofluor white resistant, so only the PD classes of tetrads (4 Cal^R : 0 Cal^S) will be observed.

If the *sup1* mutation is extragenic, the cross can be written as follows:

cal1-1 sup1 X *CAL1 SUP1*



Diploid: *cal1-1 / CAL1 sup1 / SUP1*



Sporulate, dissect tetrads, score for calcofluor white phenotype

The *sup1* mutation should segregate independently of the *cal1-1* mutation, and all three classes of tetrads (PD, NPD, and TT) should be observed, including some Cal^S spores. Try drawing the genotypes and phenotypes of the tetrads for practice.

2c. A complementation test can only be performed with mutations that cause recessive phenotypes. In this cross, a mutation is dominant if the diploids resulting from a cross back to the *cal1-1* strain are Cal^R. Therefore, *sup4* is dominant (and cannot be placed into a complementation group), and the remaining mutations are recessive.

Mutations that complement result in a Cal^S phenotype in the diploid.

Mutations that fail to complement will cause a Cal^R phenotype in the diploid.

Complementation groups 1,3,5
 2,7
 6

2d. The classes of tetrads are PD (4 Cal^R: 0 Cal^S) ; NPD (2 Cal^R: 2 Cal^S) ; and TT (3 Cal^R: 1 Cal^S).

Because PD=NPD, *sup1* and *sup5* appear to be unlinked. As *sup1* and *sup5* also fail to complement one another, this is a case of unlinked non-complementation. A possible explanation for this is that the two gene products function in the same pathway or reside in the same complex, and the cell cannot tolerate simultaneous mutations in both, leading to loss of suppression.

2e. The genes contained on the insert are: *SEF1* (partial sequence), *PRX1*, *KIP1*, *SKT5*, and *YEL1* (partial sequence).

The most likely candidate is *SKT5* (also known as *CAL2*). As described under the mutant phenotypes section, some mutant alleles of this gene can confer decreased resistance to calcofluor white.

To test whether *SKT5* is the same gene that is mutated in the *cal1-1* strain, you would obtain the *skt5Δ::G418^R* strain from the deletion set. Mate the *skt5Δ::G418^R* strain to a *cal1-1* strain of opposite mating type, induce sporulation of the diploid, and dissect tetrads. Score spores for their calcofluor white resistance. If a mutation in *SKT5* is indeed responsible for the calcofluor white sensitive phenotype of *cal1-1*, then the only tetrads present will contain 2 *Cal^S* spores and 2 *G418^R* spores. If *cal1-1* is in a different gene than *SKT5*, then other segregation patterns will also be present.

3a. Parental Ditypes: 2 *Cyh^S Can^R* : 2 *Cyh^R Can^S*

Nonparental Ditypes: 2 *Cyh^S Can^S* : 2 *Cyh^R Can^R*

Tetatype: 1 *Cyh^S Can^S* : 1 *Cyh^S Can^R* : 1 *Cyh^R Can^S* : 1 *Cyh^R Can^R*

TT contain 1 double mutant (*Cyh^R Can^R*) spore, and NPD contain two double mutant spores. Based on the observation that PD >> NPD, you can conclude that the *CYH1* and *CAN2* genes are linked. You can calculate the distance between them using the following formula:

Map Distance in cM = $\frac{1}{2} [(TT+6NPD) / (PD+NPD+TT)] \times 100$

Distance (cM) = $\frac{1}{2}[12 + 6(1)] / 32 = 28.125$

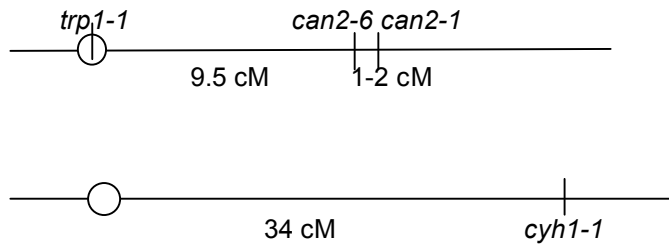
The two genes are 28cM apart.

3b. Diploid phenotype: By crossing each mutant to wild-type, you demonstrate that the *can2-1* and *can2-6* phenotypes are both recessive to wild-type and can be tested for complementation. By crossing the two *can* mutant strains together and looking at the diploid phenotype, you have performed a complementation test. Because the diploid has the *Can^S* phenotype, *can2-1* and *can2-6* complement and therefore are probably in different genes. The possible exception to this interpretation is that *can2-1* and *can2-6* are in the same gene but exhibit intragenic complementation.

Tetrad data: You observe 98 PD tetrads (0 *Can^S* : 4 *Can^R*) and 2 other tetrads. Based on the large number of PD tetrads, *can2-1* and *can2-6* must be tightly linked. Therefore, these two rare tetrads must be TT class. (Remember that NPD tetrads are only generated when double crossovers occur, which will be rare between two tightly linked genes). The unexpected part is that the TT tetrads contain 2 *Can^S* : 2 *Can^R* spores, not 1 *Can^S* : 3 *Can^R* spores as might be predicted. The most logical explanation for this is that the *can2-1 can2-6* spores have a *Can^S* phenotype.

You cannot definitively determine whether *can2-1* and *can2-6* are in the same gene and display intragenic complementation or are in two closely linked genes. The combination of the complementation and linkage studies do not provide a conclusive answer to your question. In this case, DNA sequence analysis to determine the location of the mutations with respect to open reading frames and will provide the final verdict.

3c.



cyh1 – CEN distance can be calculated using the following formula:

$$\text{Dist (cM)} = \frac{1}{2} \text{TT} / \text{PD} + \text{NPD} + \text{TT}$$

$$\text{Dist (cM)} = \frac{1}{2}(68) / 100 = 34 \text{ cM}$$

Note that this formula only works for calculating gene-CEN distance when the other gene in the cross is known to be tightly linked to its centromere, as is the case with *trp1-1*.

- 4a. PD: Class III
NPD: Class II
TT: Class I

The *ste1-1* mutation is *MAT α* specific. In tetrad class II (NPD), all four spores mate normally. In this tetrad, the two spores that have the *ste1* mutation are mating type α and do not have a temperature sensitive sterile phenotype; the remaining two spores are wild-type at the *STE* locus and are mating type **a**. Similarly, in tetrad class I, one spore that has the *ste1* mutation and is mating type α is not sterile. Thus, the mutation is specific to cells that are *MAT α* .

You can also tell that the mutation is *MAT α* specific by looking at all three tetrad classes and noting that each class contains two *MAT α* spores that can mate normally. Therefore, the presence or absence of the *ste1-1* mutation does not affect the mating ability of a *MAT α* spore.

4b. To perform epistasis analysis, you need to construct a *phe1 Δ ::G418^R ste1 Δ ::G418^R* double mutant strain, then observe whether its pheromone and mating phenotypes resemble those of either of the single mutants.

1). Cross *MAT α phe1 Δ ::G418^R* x *MAT α ste1 Δ ::G418^R*, sporulate the diploid, and analyze tetrads.

(Note: You must use a *MAT α ste1 Δ* strain as the *MAT α ste1 Δ* strain cannot mate.)

2). Obtain haploid strains that are *MAT α phe1 Δ ::G418^R ste1 Δ ::G418^R* and *MAT α phe1 Δ ::G418^R ste1 Δ ::G418^R*. First, to identify the *phe1 Δ ::G418^R ste1 Δ ::G418^R* double mutants, find NPD tetrads for these markers, where G418R segregates 2:2. The two G418^R spores will contain both deletions. To identify those double mutants that are also *MAT α* , test mating type by PCR. Note that you do not want to make any assumptions about the mating phenotype of the double

mutant as that is unknown; therefore, assaying the *MAT* allele by PCR is essential. Once the mating types have been determined, each one can be tested for mating and for pheromone production.

3). Analyze pheromone production phenotype of *MATa ste1Δ phe1Δ* strain by measuring levels of mating pheromone produced.

Analyze mating phenotype of *MATa ste1Δ phe1Δ* strain by testing if it is able to mate with a wild-type *MATα* strain.

{Note: You must use a *MATa* strain for the actual epistasis analysis, as the *ste1* phenotype is *MATa* specific. You must use a haploid strain, as mating ability is a haploid-specific phenotype (diploid yeasts do not mate)}.

Possible results:

If the *MATa ste1 phe1* strain produces mating pheromone constitutively, then *phe1* is epistatic to *ste1* with respect to the pheromone production phenotype. If the strain can mate, then *phe1* is also epistatic to *ste1* with respect to the mating phenotype.

Alternatively, if the *MATa ste1 phe1* strain produces no mating pheromone, then *ste1* is epistatic to *phe1* with respect to the pheromone production phenotype. If the strain cannot mate, then *ste1* is also epistatic to *phe1* with respect to the mating phenotype.

- 5a. To generate a targeted deletion of *RCG1*, one would perform the following steps:
1. Based on the DNA sequence, order PCR primers that will amplify a selectable marker, such as *G418^R*, and whose ends will have about 40 bases of homology to the sequences immediately flanking the *RCG1* coding region. When used in a PCR reaction with a *G418* gene as a template, these primers will amplify your targeting construct.
 2. Use the PCR product to transform a diploid yeast strain to *G418^R* using standard methods. You must use diploid yeast in case the deletion results in lethality. The homology on the ends of the fragment will target the gene replacement event to *RCG1*.

To confirm that you have successfully deleted *RCG1*, you can perform a Southern blot or use PCR to check the genomic DNA in the region of the gene.

To determine the phenotype of the *RCG1* deletion, you will take the *G418^R* diploid colonies generated above, sporulate, dissect tetrads, and score the segregants for their *G418^R* phenotype. If haploid yeast bearing *rcg1Δ* are viable, then you should get 4 viable spores, two of which are *G418^R*. If the deletion is lethal, then you should get 2 viable spores and 2 dead spores; the viable spores should be *G418^S*.

Alternatively, one could make the deletion in a haploid strain. In case the deletion results in lethality, the strain would also have to carry a *CEN* plasmid that contains *RCG1*. However, this approach is not as straightforward, as the deletion could then occur either on the plasmid or in the genome. You would need to use Southern hybridization analysis to determine where the deletion occurred. One would also need to use a plasmid that contains *URA3*, to enable to select for cells that have lost it by growing on 5-FOA medium.

- 5b. 1. Pick the *Sect⁻* colony that contains the candidate synthetic lethal mutation, purify it, and grow a liquid culture.
2. Transform candidate with a genomic *CEN* plasmid library marked with a selectable marker (*LEU2*), plate at low density, and grow up colonies.
3. To identify clone candidates, replica the transformants to a 5-FOA plate. Transformants that contain *SLR1* on the plasmid will allow loss of the *URA3 RCG1* plasmid, thereby allowing growth on 5-FOA.
4. Isolate the plasmid from any *Sect⁺* colonies and sequence the insert to identify *SLR1*. If multiple genes are present on the insert, subclone and test each gene individually to determine which one causes the *Sect⁺* phenotype.

(Another strategy would be at step 3 above, to plate *Leu⁺* transformants on normal medium (not 5-FOA) and screen for colonies that now had regained their ability to sector. While this approach could work, it would require much more work than selecting on 5-FOA as described above.)

5c. Starting strain: Use the strain with the two plasmids.
Mutagenize the *pSLR1, LEU2* plasmid and use to transform the starting strain.
Select *Leu⁺* transformants and test them for growth on three types of plates: plates with 5-FOA, plates with 5-FAA, and plates with both 5-FOA and 5-FAA. The desired class of mutant will fail to grow on plates with both 5-FOA and 5-FAA, but will grow on the other two. For further details, see below, including the table of phenotypes on the next page.

Synthetic lethal mutation in *SLR1* (desired class of mutant):

When grown on medium containing both 5-FOA and 5-FAA, the cells will lose both the *pSLR1, TRP1* and the *pRCG1, URA3* plasmids. If the remaining plasmid, *pSLR1 LEU2*, contains a mutation in the *SLR1* gene that is synthetic lethal in combination with the *rcg1Δ* deletion, then cells will fail to grow on this type of medium.

When grown on medium containing only 5-FOA, the cells will lose only the *pRCG1, URA3* plasmid. The remaining strain will be null at the *RCG* locus; however, this is not lethal. It will also carry two copies of the *SLR1* gene, so even if one copy has acquired a mutation, the other copy is most likely still functional. Thus, the desired class of mutant would be expected to grow on 5-FOA medium.

When grown on medium containing only 5-FAA, the cells will lose only the *pSLR1 TRP1* plasmid. The strain thus created will still contain the mutagenized *pSLR1 LEU2* plasmid. Any synthetic lethal phenotype will not be observed on 5-FAA, as there is still a copy of *RCG1* present. Again, the desired class of mutant would be expected to grow on this medium.

Wild-type *SLR1*:

If the mutagenized *pSLR1 LEU2* plasmid still carries a wild-type copy of *SLR1*, then the strain should grow on all three types of media (5-FAA, 5-FOA, and both). Loss of the *pSLR1 TRP1* plasmid, the *pRCG1 URA3* plasmid, or both plasmids will not affect viability. Specifically, *RCG1* is not required for viability. *SLR1* is required for viability; however, a functional copy of *SLR1* is still present on the remaining *pSLR1 LEU2* plasmid.

Null *slr1* mutation:

If the mutagenized p*SLR1 LEU2* plasmid carries a null mutation in *slr1*, then the strain will not grow on plates containing 5-FAA or on plates containing both 5-FOA and 5-FAA. Any conditions that cause loss of the p*SLR1, TRP1* plasmid will result in lethality, as *SLR1* is required for viability. However, this strain should still grow on plates with 5-FOA, as loss of the p*RCG1 URA3* plasmid will not affect viability.

<i>slr1</i> allele on p <i>SLR1 LEU2</i>	growth on		
	5-FOA	5-FAA	both
<i>SLR1</i> ⁺	+	+	+
<i>slr1</i> null	+	-	-
<i>slr1</i> syn. let.	+	+	-