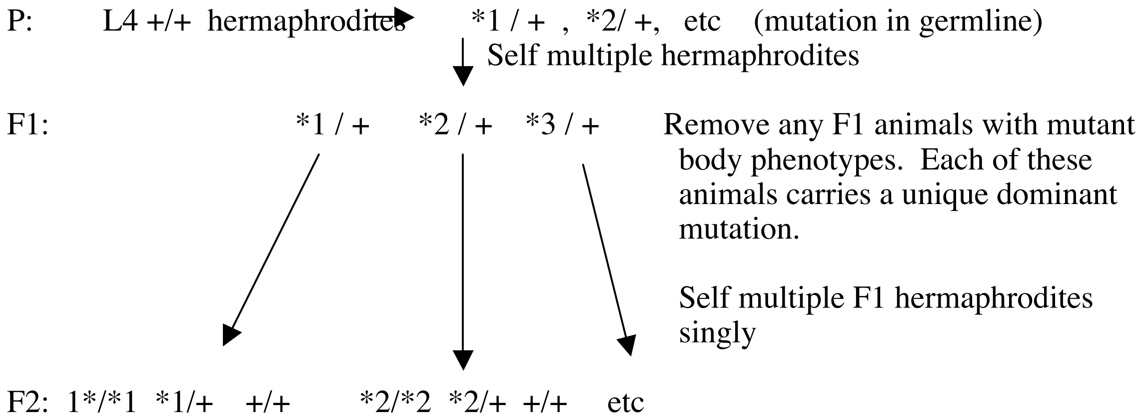
Answers to Genetics 201 final – December, 2005

1a).

EMS



Look for F2 plates that contain animals with mutant body phenotypes. These animals carry recessive mutations. Each plate contains a unique mutation.

1b). i. The two genes will segregate in a Mendelian pattern.
9/16 nonPlp nonUn
3/16 nonPlp Unc
3/16 Plp nonUnc
1/16 Plp Unc

ii. The ratio will not change.

iii. In the absence of recombination, the Plp and Unc phenotypes will segregate opposite one another. Recombination levels between the two genes will be low, as they are closely linked.
1/2 nonPlp nonUnc
1/4 Plp nonUnc
1/4 nonPlp Unc

1c). i. Yes - if you detected stop or nonsense mutation in *plp-1(xx1)* animals.
ii. No - would indicate where *plp-1* gene product is required, not identity of gene.
iii. Yes - if injected animals had Plp phenotype.
iv. Yes - if transgenic animals had nonPlp phenotype (rescue by addition of ORF-1).

1d). The phenotypically wild-type hermaphrodite contains a suppressor of the *plp-1* mutation. This suppressor has a dominant phenotype. Homozygosity for the suppressor mutation results in lethality. (You cannot determine whether the suppressor is linked or unlinked to *plp-1* – not required for full credit).

Phen. wild-type hermaphrodite: *plp-1* / *plp-1* ; *Sup* / +

↓ Self

	Genotype	Phenotype
1/4	*plp-1 ; + /+*	Plp
1/2	*plp-1 ; Sup/ +*	NonPlp
1/4	*plp-1; Sup/Sup*	Dead

Therefore, 1/3 of the living progeny are Plp, and the remaining 2/3 are nonPlp (wild-type).

1e). The suppressor mutation does not suppress the Plumpy phenotype in nematodes that are homozygous for the *plp-1(xx2)* mutation. However, it does suppress it in *plp-1(xx1)* / *plp-1(xx2)* nematodes. Based on the data, you can now determine that the suppressor mutation is unlinked to the *plp-1* gene.

Cross genotypes:

plp-1(xx2) ; +/+ hermaphrodite X *plp-1(xx1); Sup*/+ male

↓

Genotype	Phenotype
plp-1(xx1) / plp-1(xx2) ; Sup/+	nonPlp
plp-1(xx1) / plp-1(xx2) ; +/+	Plp

Cross *plp-1(xx1) / plp-1 (xx2) ; Sup/+* hermaphrodites X *plp-1(xx2) / plp-1(xx2) ; +/+* males

↓

Genotype	Phenotype
1/4 *xx1 / xx2 ; +/+*	Plp
1/4 *xx1 / xx2 ; Sup/+*	nonPlp
1/4 *xx2 / xx2 ; +/+*	Plp
1/4 *xx2 / xx2; Sup/+*	Plp

2a). Phase is known.
Each backcross animal contains 1 informative chromosome; therefore, there are a total of 100 informative chromosomes present.

2b). D2Mit20 is most closely linked.
NR = 81, R = 19, θ = 0.1

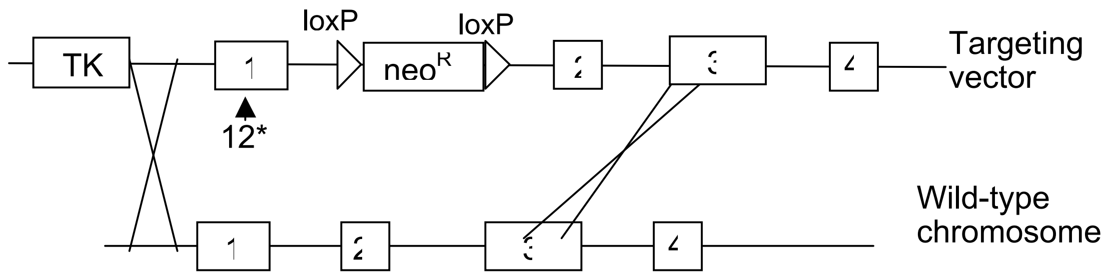
$$\begin{aligned} \text{LOD (phase known)} &= R\log\theta + NR\log(1-\theta) + (R+NR)\log 2 \\ &= 19\log(0.1) + 81\log(0.9) + 100\log 2 \\ &= -19 + (-3.706) + 30.103 \\ &= 7.397 \end{aligned}$$

LOD = 7.397; this does provide significant evidence for linkage.

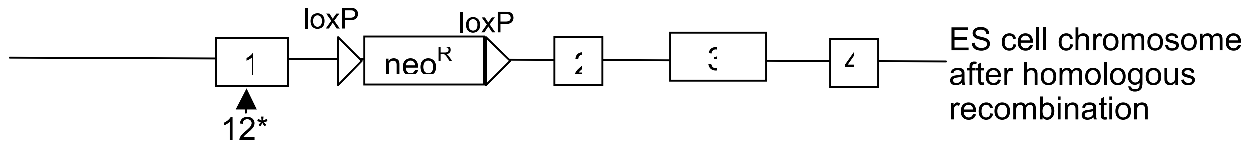
2c). A significant LOD is 3.3 or greater. θ is defined as = 0.01. You now need to solve for the number of informative chromosomes (R+NR). The best evidence for linkage between the short tail allele and the marker is if little or no recombination occurs between the gene and marker; this will produce a higher LOD score. So, you can set R=0, and then calculate the number of NR chromosomes needed.

LOD (phase known) $= R\log\theta + NR\log(1-\theta) + (R+NR)\log 2$
$3.3 = 0 + NR\log(0.99) + NR\log 2$
$3.3 = -.00436(NR) + .301(NR)$
$3.3 = .297(NR)$
$NR = 11.11$
You will need a minimum of 12 informative, nonrecombinant chromosomes to get a significant LOD. Since each mouse carries one informative chromosome, you must genotype at least 12 mice.

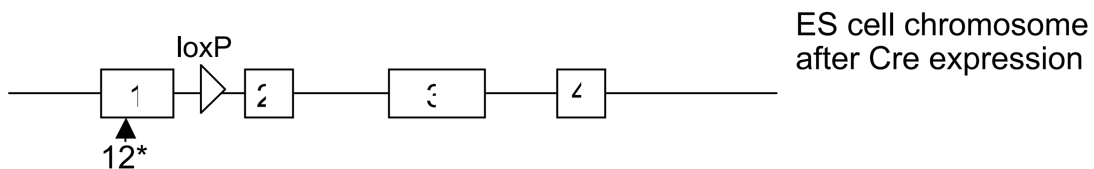
2d).



Electroporate into wild type ES cells
Select with G418 and gancyclovir to obtain homologous recombinants.



Next, electroporate a Cre-expressing plasmid into these ES cells, and use PCR to identify clones in which excision of the neo resistance gene has occurred. The resulting chromosome will be marked with a single loxP site as shown below:



Finally, inject ES cells containing the codon 12 allele into blastocysts. Implant the blastocysts into pseudopregnant females, identify chimeric pups, and establish lines that carry the codon 12 mutation.

Alternatively, instead of using Cre in the ES cells, you can use the same targeting vector, to make ES cells, then generate germline chimeras bearing the neo gene flanked by LoxP sites. Next, you cross a male chimera to a transgenic female expressing Cre in early development. The neo marker gets removed, and the animals have a clean mutation, which is then transmitted to their progeny. This answer gets credit as long as it is specified that the Cre is expressed either in early development or in the germline (so that the clean allele gets transmitted to the next generation).

3a). If X-linked dominant: Let Wh = new mutation on X.

Cross is $X^{Wh}Y$ x XX

Genotype: $X^{Wh}X$ and XY

Phenotype: white eye female; red eye male

All females will have white eyes; all males will have red (wild-type) eyes.

3b). Note, the best scheme is described below. There are other possibilities that also received full credit. However, if at step 2 you mated the X^{Wh} / X females back to the single X^{Wh} /Y male, 2 points were taken off, since it would be difficult to use that single male again.

1. Cross white eye male to X/FM7 female:

X^{Wh} /Y male x X / FM7 female

↓

X^{Wh} / X females X^{Wh} / FM7 females X/Ymales FM7 / Y males

2. Select X^{Wh} / FM7 females and mate to FM7/Y males:

X^{Wh} / FM7 females x FM7/Y males

↓

X^{Wh} / FM7 females FM7/FM7 females X^{Wh} / Y males FM7/Y males

3. Select X^{Wh} / FM7 females and cross by X^{Wh} /Y males:

X^{Wh} / FM7 females x X^{Wh} /Y males

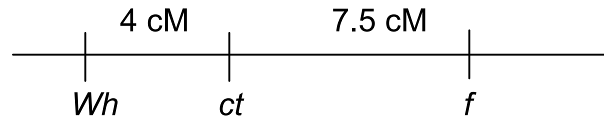
↓

X^{Wh} / FM7 females X^{Wh} / X^{Wh} females X^{Wh} / Y males FM7/Y males

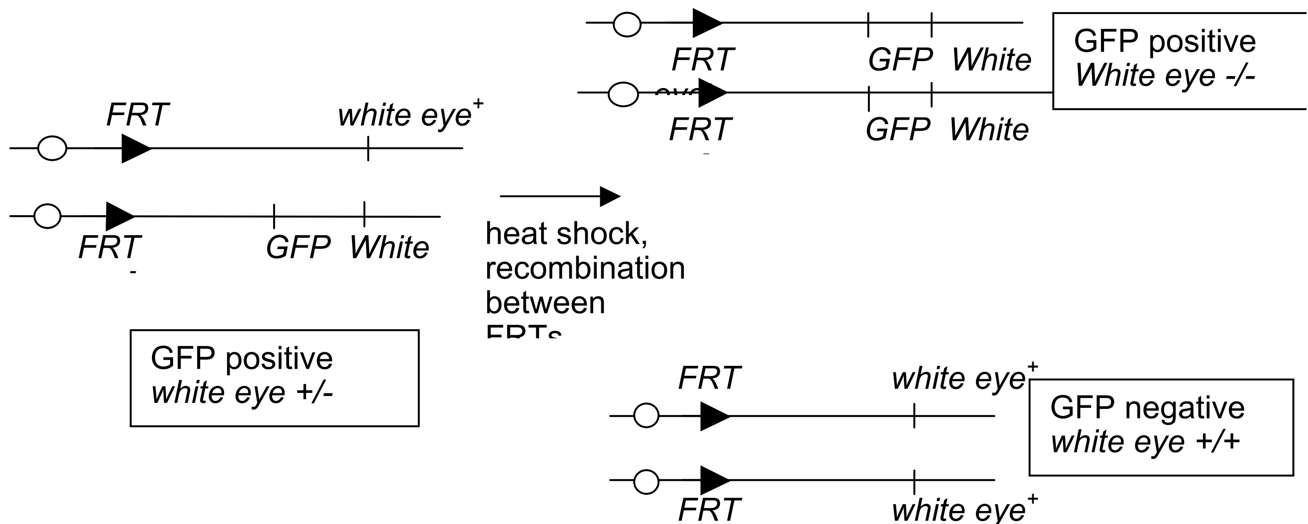
4. Select X^{Wh} / X^{Wh} females and cross by X^{Wh} /Y males for homozygous stock.

3c). The male F2 flies provide all the data needed for the calculations. Using those data give the answer shown below. The female flies can be used only to calculate the distance between the cut wing and forked bristle genes since they will be heterozygous for some recessive markers.

Map:



3d). i. In order to distinguish between cells that carry the dominant *Wh* mutation and those that do not, you will need to place the GFP marker on the chromosome bearing the mutant *White eye* allele. (Note: some gave answers using Gal4 and Gal80. These could also be correct, if they were configured correctly.)

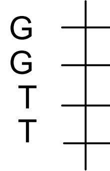


3d. ii. Cells that are genotype *white eye* +/- (most of the fly) will carry the *Wh* mutation and will be GFP positive. The clones generated following FLPase activity are: *white eye* +/+ , phenotype GFP negative ; and *Wh*/*Wh*, phenotype GFP positive.
If your hypothesis is correct, then the only R7 cells that develop will be GFP negative. If your hypothesis is incorrect, GFP positive R7 cells will also be present.

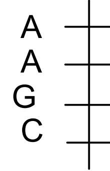
The white eye +/+ would presumably also give you a red clone in addition to being GFP negative. So people could also incorporate that in their answer (optional).

4a). 1. Autosomal dominant: not consistent with data because unaffecteds give rise to affected children (Granger family).
2. X-linked dominant: not consistent because either Mr. or Mrs. Granger would have to be affected.
3. X-linked recessive: not consistent because Mr. Granger would have to carry trait allele (and therefore should be affected).
4. Y-linked dominant; not consistent because it would only appear in males.
5. Cytoplasmic inheritance.

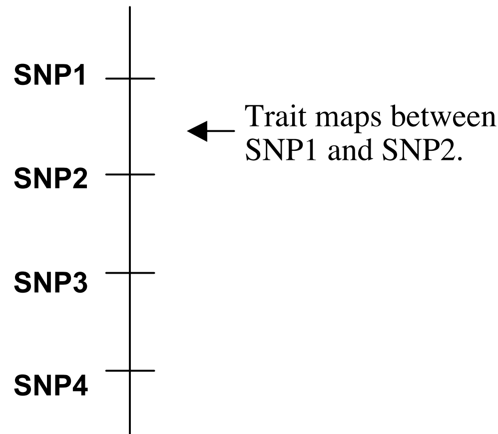
4b). Mr. Granger:



Mrs. Granger:



4c). Minerva and Aura provide the closest recombinational breakpoints.



4d). Yes, she is a carrier. She received a nonrecombinant wild-type chromosome from her father (haplotype TCGA). She received a recombinant chromosome from her mother that carries the wizardry trait allele. Therefor, her genotype at the wizardry locus is +/w, and she is a carrier for the trait.