PROBLEM SET 3 – COMPLEMENTATION AND PATHWAY ANALYSIS
(covering the lecture from week 4)

1. Describe the series of steps that you would perform to isolate arginine-requiring mutants from a wild-type haploid yeast strain.

2. From problem 1 you identified 8 arginine-requiring mutants (*arg*) from an a mating type and 8 *arg* mutants from an α mating type. All of the *arg* mutations are recessive. You cross all the a mating type mutants to all of the α mating type mutants and analyze the resulting diploids for growth on plates lacking arginine. The results are shown below, where "+" means growth without arginine and "-" means no growth without arginine.

	α 9	α 10	α 11	α 12	α 13	α 14	α 15	α 16
a 1	+	+	-	-	+	+	+	+
a 2	-	-	+	+	+	-	-	+
a 3	+	+	+	+	+	+	+	+
a 4	-	-	+	+	+	-	-	+
a 5	+	+	-	-	+	+	+	+
a 6	+	+	+	+	+	+	+	-
a 7	+	+	+	+	-	+	+	+
a 8	+	+	+	+	-	+	+	+

2a. How many different genes did you find among your mutations?

2b. Which mutations affect which genes? (come up with a naming scheme for the genes and assign the numbered alleles to them)

3. You are studying aging in fruit flies and have generated a number of homozygous long-lived fly mutants. You now wish to determine how many genes these six mutants represent and you perform pairwise crosses with all of the homozygous mutants. Results of this analysis are shown in the table below (where the intersection represents the phenotype of the offspring resulting from a particular cross):

	Mut 1	Mut 2	Mut 3	Mut 4	Mut 5	Mut 6	WT
Mut 1	-	+	-	-	+	+	+
Mut 2		-	+	+	+	-	+
Mut 3			-	-	+	+	+
Mut 4				-	+	+	+
Mut 5					-	+	+
Mut 6						-	+

+ indicates all offspring have normal lifespan.
- indicates all offspring are long-lived.
WT = a wild type strain of flies.

3a. Are these mutations dominant or recessive? How do you know?

3b. How many complementation groups do these mutations represent?

3c. Describe which mutations fall into each complementation group.

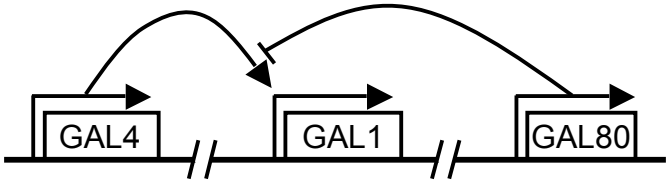
In addition to the long-lived fly mutants described above, you have also isolated two additional long-lived mutants (Mut 7 and Mut 8) that exhibit an unusual complementation pattern with respect to your other long-lived mutants, as shown below:

	Mut 1	Mut 2	Mut 3	Mut 4	Mut 5	Mut 6	WT
Mut 7	-	-	-	-	-	-	-
Mut 8	+	-	+	+	-	-	+

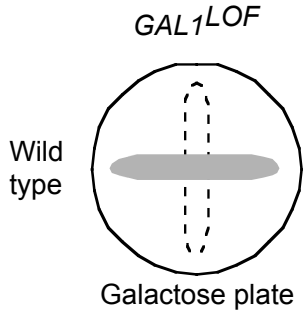
+ indicates all offspring have normal lifespan.
- indicates all offspring are long-lived.
WT = a wild type strain of flies.

3d. What can you conclude from the results of crosses with Mut 7 and Mut 8?

4. You are studying three yeast genes, *GAL1, GAL4* and *GAL80* that influence galactose metabolism. The *GAL1* gene encodes an enzyme that is required for growth in media containing galactose as the sole carbon source. The *GAL4* gene encodes a protein that is required for the transcription of the *GAL1* gene, and the *GAL80* gene encodes a protein that prevents GAL4 from promoting transcription of *GAL1*. *GAL80* is normally only transcribed when yeast cells are grown on media lacking galactose. Models describing this regulation are shown below.

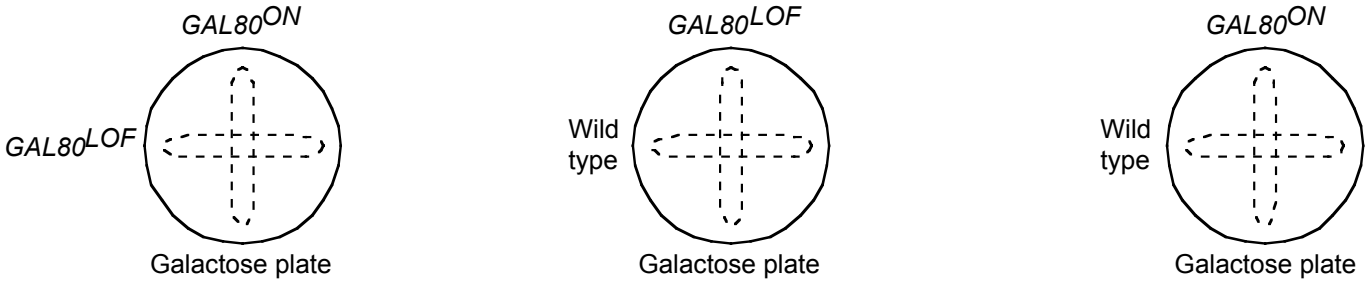


4a. You have identified a mutation that creates a stop codon early in the coding sequence of the *GAL1* gene (designated $GAL1^{LOF}$). The haploid $GAL1^{LOF}$ mutant is cross stamped to a wild type haploid yeast strain with the results shown below (shaded regions indicate cell growth; dotted lines indicate no growth). Is this *GAL1* mutation dominant or recessive to the wild type *GAL1* gene? Briefly explain.

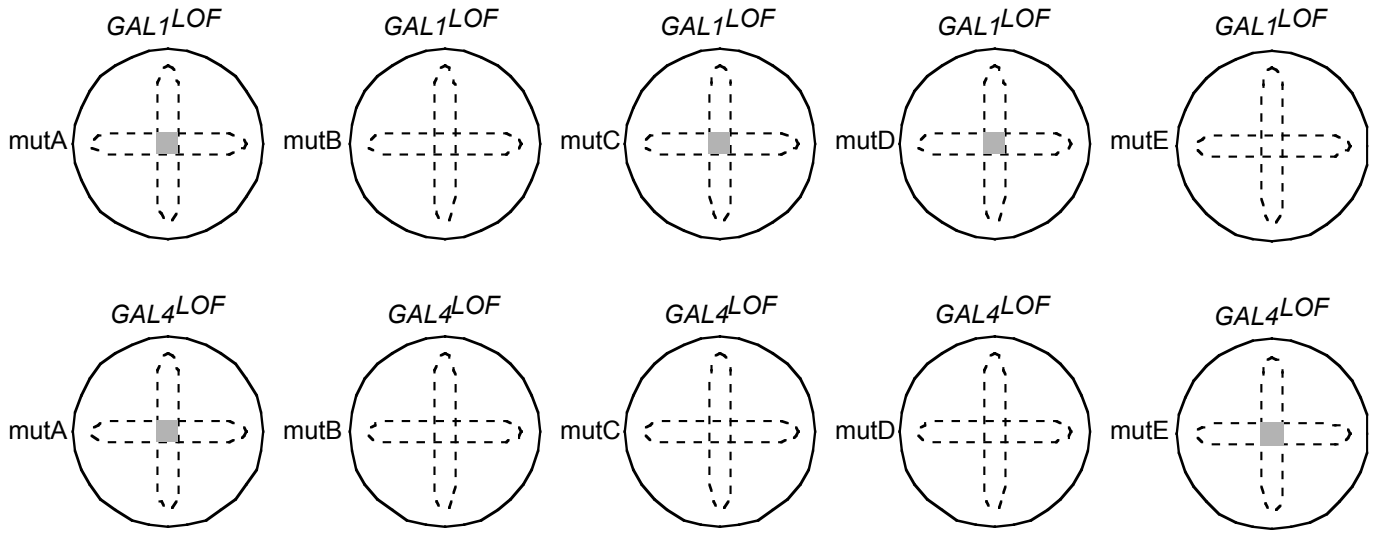


4b. You identify the following two mutations affecting the *GAL80* gene:
***GAL80^{LOF}*:** A mutation that deletes the entire protein coding sequence of the *GAL80* gene.
***GAL80^{ON}*:** A mutation in the *GAL80* promoter that makes the promoter active on media containing galactose.

Show the results expected from crossing these two mutants to one another and to a wild type yeast strain by shading to indicate cell growth.



4c. You have identified 5 new mutations (designated mutA-mutE) that prevent growth on plates containing galactose. The results of crossing these 5 new mutations to a haploid yeast strain containing the *GAL1^{LOF}* mutation and a haploid yeast strain with a mutation that deletes the entire coding sequence of the *GAL4* gene (designated *GAL4^{LOF}*) on plates containing galactose are shown below.



Describe which of these mutations (mutA-mutE) represent:

New alleles of *GAL1*:

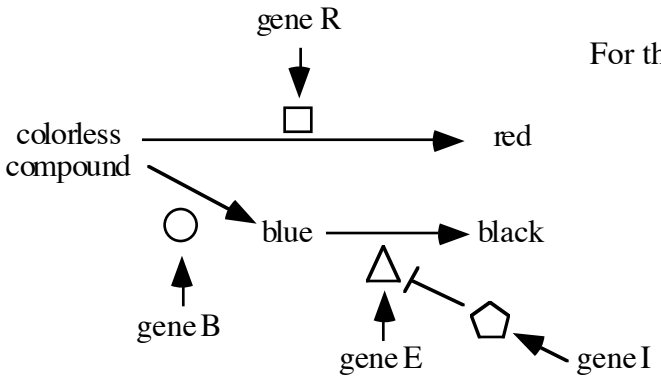
New alleles of *GAL4*:

New alleles of *GAL80*:

New genes affecting Galactose metabolism:

Can't tell:

5. In the flower petals of a certain plant, a colorless compound is converted to colored pigments by a branching pathway shown below. The products of genes R, B, and E are enzymes; the product of gene I is an inhibitor of the E enzyme. Gene I is ubiquitously expressed (i.e., it is made everywhere in the plant all of the time).

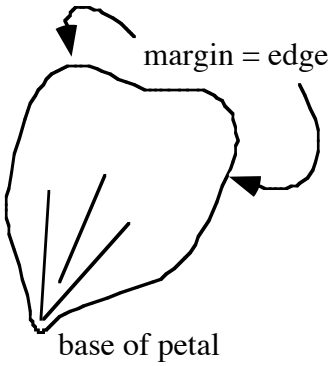


For those with no art background:

Red + Blue = purple
Red + Black = maroon
Blue + Black = navy

5a. What loss-of-function mutations would give the following flower colors? Consider both single and double mutations. There may be more than one possibility for some colors, but just give one. If one of these colors is the wild type color, indicate that choice with "wt".

color:	mutant gene(s):
Red	
Blue	
Purple	
Maroon	
White	
Black	



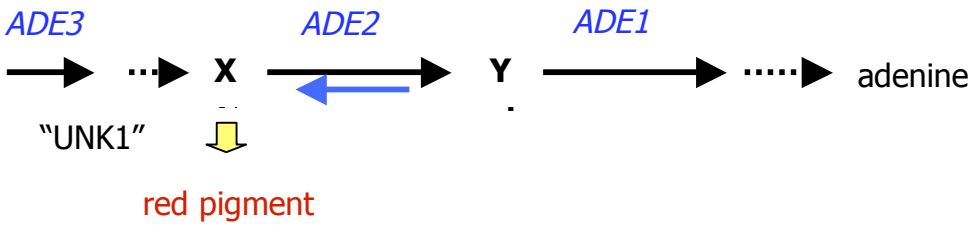
5b. You discover a mutation in gene I that causes gene I to be expressed in just the cells along the margin of the petal. What would this petal look like?

5c. Would the mutation in part (B) be dominant or recessive? Why?

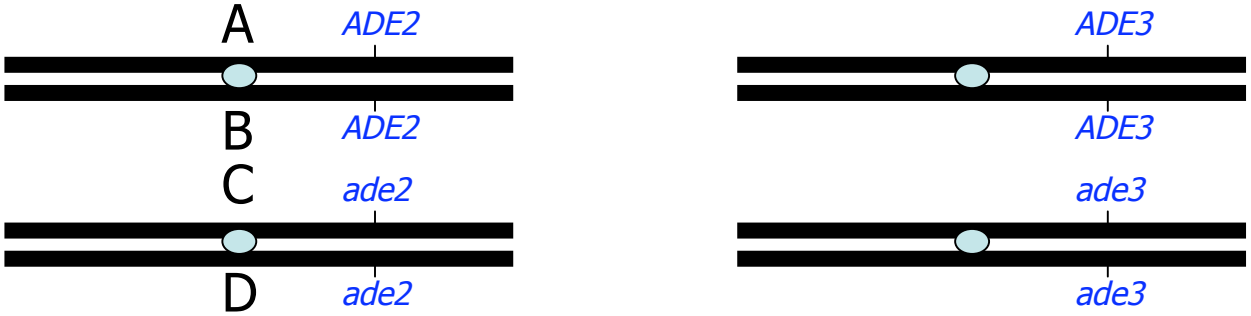
5d. A partial loss-of-function mutation in the I gene is discovered (I^P). What flower color would be expected in a plant that is r/r B/B/ E/E, and homozygous for I^P? Explain.

5e. What is the expected dominant/recessive relationship of I^P to the alleles I and i?

6. (this question derives from one presented in lecture 7 and relates to the yeast adenine biosynthetic pathway, a portion of which is shown below).



6a. A *MATa ade2 ADE3* mutant was mated to a *MATα ADE2 ade3* mutant to create a diploid. *ADE2* and *ADE3* assort independently. On one of the chromosomes shown below, place a crossover on the chromosome arm that is opposite to that of the *Adenine* gene. On the other chromosome, place a crossover BETWEEN the centromere and the *Adenine* gene.

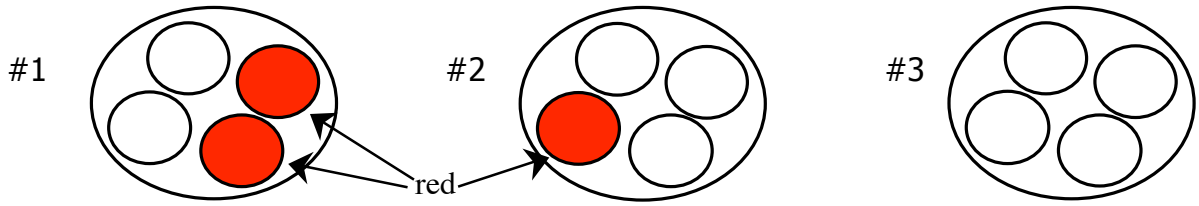


6b. In the table below predict the genotypes and growth properties of each spore resulting from this meiosis.

Spore	complete genotype?	grow without adenine?
A		
B		
C		
D		

6c. Which tetrad best fits the meiosis you just drew? Letter the spores below to match the genotypes in your table.

Tetrad on complete plates



7. Epistasis refers to a situation in which alleles of one gene mask the phenotypes conferred by alleles of another gene. In many cases of epistasis involving genes that act in a common metabolic pathway, the epistatic gene (the one that masks alleles of the other gene) is the one that acts earlier in the pathway. For example, homozygous recessive alleles of the C gene mask alleles of all of the downstream genes in the cat coat color pathway. The lac operon of *E. coli* is an example of a negative regulatory pathway that is involved in lactose metabolism. One component of the lac operon, the *lacZ* gene, encodes an enzyme required for growth on plates that contain lactose as the sole carbon source. Another component of this pathway, the *lacI* gene, encodes a protein that inhibits the transcription of *lacZ* in the absence of lactose.

7a. Which of the two lac operon genes (*lacZ* or *lacI*) acts upstream in this pathway?

7b. What would you predict from the phenotypes of double mutants bearing loss-of-function alleles of *lacZ* and *lacI*?

7c. What do the answers to the questions above tell you about epistasis involving negative regulatory factors?

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1.
i. mutagenize yeast cells.
ii. plate out mutagenized yeast cells on complete plates.
iii. replica plate colonies onto plates lacking arginine.
iiii. Select colonies that grow on complete plates but not on arginine-deficient plates.

2a. 5

2b. 1, 5, 11, 12 affect the ARG1 gene; 2, 4, 9, 10, 14, 15 affect the ARG2 gene; 3 affects the ARG3 gene; 6, 16 affect the ARG4 gene; 7, 8, 13 affect the ARG5 gene.

3a. They are all recessive to WT because they have a WT phenotype when heterozygous with the WT allele.

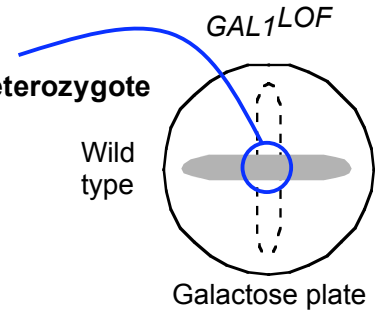
3b. Three

3c. Complementation group 1: 1, 3, 4; group 2: 2, 6; group 3: 5.

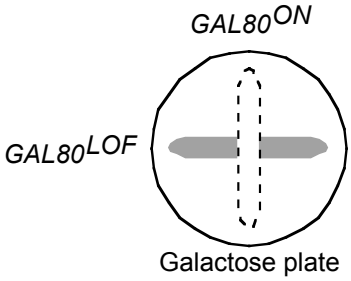
3d. Mut 7 is a dominant mutation; Mut 8 has two mutations, one in group 2 (Mut 2 & Mut 6) and one in group 3 (Mut 5).

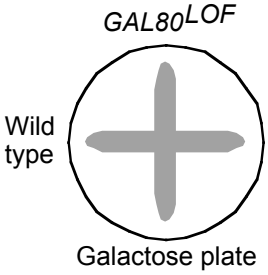
4a.

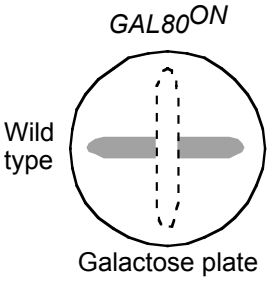
This mutation is recessive because the diploid *GAL1*^{WT}/*GAL1*^{LOF} heterozygote has exactly the same phenotype as the haploid *GAL1*^{WT} strain.



4b.







4c.
New alleles of *GAL1*: **mutE**

New alleles of *GAL4*: **mutC, MutD**

New alleles of *GAL80*: **(mutB could be an allele of GAL80, but because mutB is dominant we cannot use the complementation test to address this matter)**

New genes affecting Galactose metabolism: **mutA**

Can't tell: **mutB**

5a.

Color	Mutant gene(s):
Red	B
Blue	R
Purple	This is the WT color
Maroon	I
White	B and R (both mutations are necessary)
Black	I and R (both mutations are necessary)

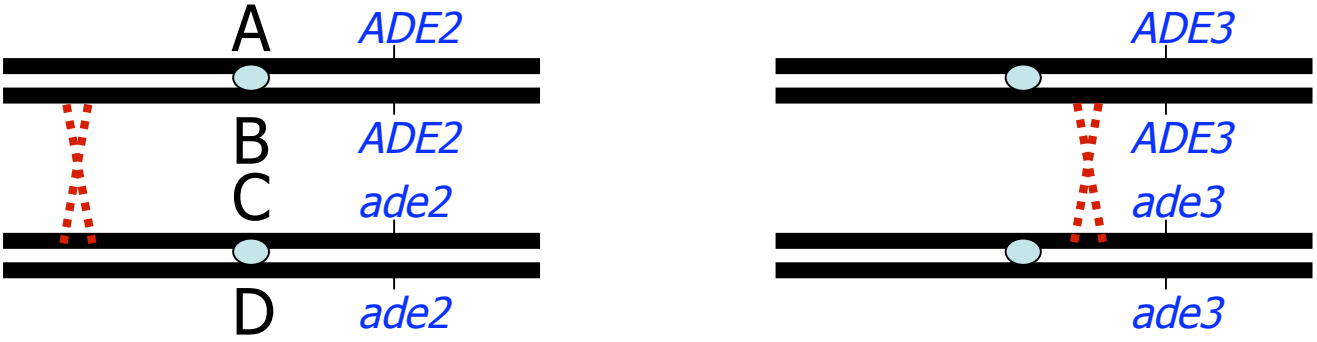
5b. The petals would be maroon with purple coloration along the margin.

5c. Recessive, because a heterozygote with this mutation and the WT allele would have the WT phenotype.

5d. Navy, because only some of the blue pigment would be converted to black and there would be a mixture of blue + black pigment. No red pigment would be made because the recessive loss-of-function allele of r is homozygous.

5e. I dominant to I^P and i and I^P dominant to i.

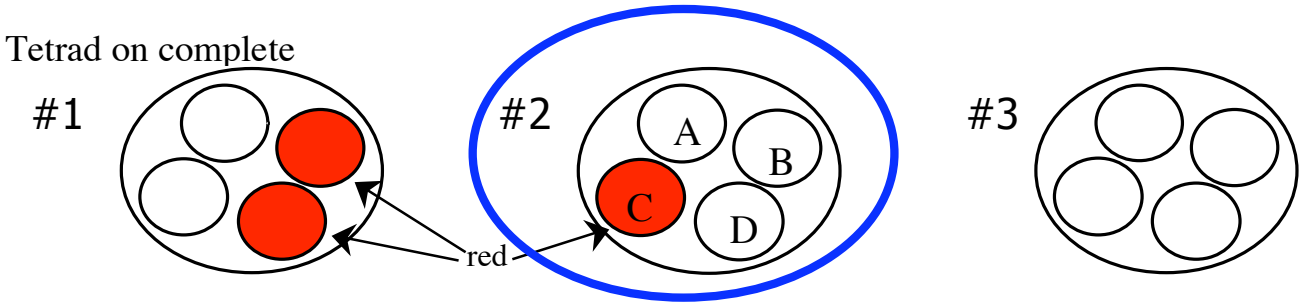
6a.



6b.

Spore	Complete genotype?	Grow without adenine?
A	*ADE2 ADE3*	Yes
B	*ADE2 ade3*	No
C	*ade2 ADE3*	No
D	*ade2 ade3*	No

6c.



7a. Lac I acts upstream in this pathway.

7b. The double mutants would fail to produce the lacZ gene product. The lacZ mutation would be epistatic to the LacI mutation.

7c. If one of the two factors in a pathway acts as a negative regulatory factor, the epistatic gene is the one that acts downstream in the pathway.