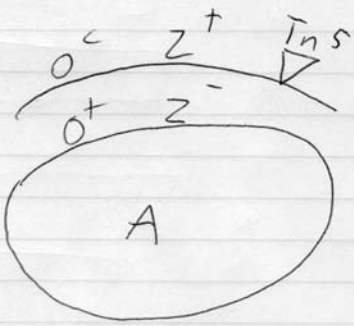
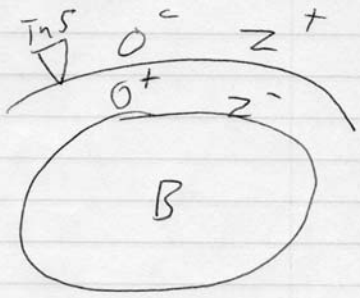
Problem Set 4 Solutions

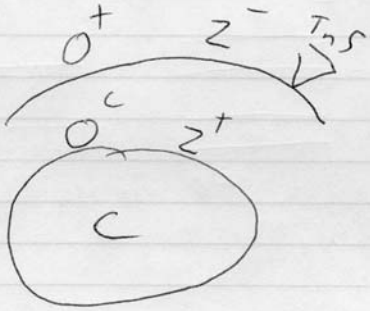
① a)

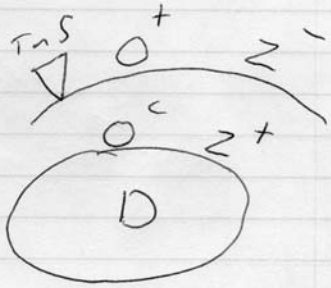
Cross I





Cross II





	Recombinant Class	# crossovers	Phenotype
For A:	$O^c Z^+$	DCO	Constit.
	$O^c Z^-$	QCO	Uninducible
	$O^+ Z^-$	DCO	Uninducible
	$O^+ Z^+$	DCO	Normal

For B:		
	$O^c Z^+$	DCO
	$O^c Z^-$	DCO
	$O^+ Z^-$	DCO
	$O^+ Z^+$	QCO

For C:		
	$O^c Z^+$	DCO
	$O^c Z^-$	DCO
	$O^+ Z^-$	DCO
	$O^+ Z^+$	QCO

For D:		
	$O^c Z^+$	DCO
	$O^c Z^-$	QCO
	$O^+ Z^-$	DCO
	$O^+ Z^+$	DCO

1. a) O+ Z+ is required for normal B-Gal regulation. This is achieved only in cross number 1. In orientation A, O+ Z+ is the result of a double cross over, while in B it would be the result of a quadruple crossover. Therefore, orientation A (and C) are correct. We do not see O+ Z+ recombinants in cross 2 because they would have to results from a quadruple cross over.

b) Distance from Tn5 to LacO

In A, Oc is transduced with Tn5 36/100 times (constitutive recombinants).

Cotransduction freq. = 36 +- sqrt (36)% = 36+/- 6%

Distance from Tn5 to LacZ

In C, Z- is transduced with Tn5 65/100 times (uninducible recombinants).

Cotransduction freq. = 65 +- sqrt (65) %= 65+/- 8%

In A, Z- is transduced with Tn5 40/100 times (constitutive + regulated recombinants).

Cotransduction freq. = 40+- sqrt (40) % = 40 +/- 6.2%

Taking the mean of C and A cotransduction frequencies would also count for full credit.

2. a) A "super-activator" allele of Reg1 ($Reg1^{SA}$)

i. **Uninducible**

Reg1 is constitutively active and always activates transcription of Reg2. Enz1 expression is always inhibited by Reg2. Enz1 is never produced.

ii. **Dominant**

$Reg1^{SA}$ $Enz1^{+}$/ $Reg1^{+}$ $Enz1^{+}$	Uninducible	SA allele dominant to WT

iii. **Trans-acting**: merodiploids have the dominant SA phenotype in both cis and trans.

Cis-test	**$Reg1^{SA}$ $Reg2^{+}$ $Enz1^{+}$/F' $Reg1^{+}$ $Reg2^{+}$ $Enz1^{-}$**	Uninducible
Trans-test	**$Reg1^{SA}$ $Reg2^{+}$ $Enz1^{-}$/F' $Reg1^{+}$ $Reg2^{+}$ $Enz1^{+}$**	Uninducible

b) A loss of function allele of Reg1 ($Reg1^{-}$)

i. **Constitutive**

Reg1 function is abolished, and will never activate transcription of Reg2. Enz1 expression can never be inhibited by Reg2. Enz1 is always produced.

ii. **Recessive**

$Reg1^{-}$ $Enz1^{+}$/ $Reg1^{+}$ $Enz1^{+}$	Regulated	LOF allele recessive to WT

iii. **Trans-acting**: merodiploids have the dominant WT phenotype in both cis and trans.

Cis-test	**$Reg1^{-}$ $Reg2^{+}$ $Enz1^{-}$/F' $Reg1^{+}$ $Reg2^{+}$ $Enz1^{+}$**	Regulated
Trans-test	**$Reg1^{+}$ $Reg2^{+}$ $Enz1^{-}$/F' $Reg1^{-}$ $Reg2^{+}$ $Enz1^{+}$**	Regulated

*Note that we are testing the dominant phenotype, which is wildtype.

c) A dominant negative allele of Reg1 ($Reg1^{d-}$)

i. **Constitutive**

Same as in b)

ii. **Dominant**

$Reg1^{d-}$ $Enz1^{+}$/ $Reg1^{+}$ $Enz1^{+}$	Regulated	DN allele dominant to WT

iii. **Trans-acting**: merodiploids have the dominant DN phenotype in both cis and trans.

Cis-test	**$Reg1^{d-}$ $Reg2^{+}$ $Enz1^{-}$/F' $Reg1^{+}$ $Reg2^{+}$ $Enz1^{+}$**	Constitutive
Trans-test	**$Reg1^{+}$ $Reg2^{+}$ $Enz1^{-}$/F' $Reg1^{d-}$ $Reg2^{+}$ $Enz1^{+}$**	Constitutive

d) A loss of function allele of Reg2 ($Reg2^{-}$)

i. Constitutive

Reg2 function is abolished, and can never inhibit expression of Enz1. Enz1 is always produced.

ii. **Recessive**

$Reg2^{-}$ $Enz1^{+}$/ $Reg2^{+}$ $Enz1^{+}$	Regulated	LOF allele recessive to WT

iii. **Trans-acting**: merodiploids have the dominant WT phenotype in both cis and trans.

Cis-test	**$Reg1^{+}$ $Reg2^{-}$ $Enz1^{-}$/F' $Reg1^{+}$ $Reg2^{+}$ $Enz1^{+}$**	Regulated
Trans-test	**$Reg1^{+}$ $Reg2^{+}$ $Enz1^{-}$/F' $Reg1^{+}$ $Reg2^{-}$ $Enz1^{+}$**	Regulated

*Note that we are testing the dominant phenotype, which is wildtype.

e) A dominant negative allele of Reg2 ($Reg2^{d-}$)

i. **Constitutive**

Same as in d)

ii. **Dominant**

$Reg2^{-}$ $Enz1^{+}$/ $Reg2^{+}$ $Enz1^{+}$	Regulated	DN allele dominant to WT

iii. **Trans-acting**: merodiploids have the dominant DN phenotype in both cis and trans.

Cis-test	**$Reg1^{+}$ $Reg2^{+}$ $Enz1^{-}$/F' $Reg1^{+}$ $Reg2^{d-}$ $Enz1^{+}$**	Constitutive
Trans-test	**$Reg1^{+}$ $Reg2^{d-}$ $Enz1^{-}$/F' $Reg1^{+}$ $Reg2^{+}$ $Enz1^{+}$**	Constitutive

f) A mutation in I-Reg that prevents Reg1 binding ($I\text{-}Reg^{-}$)

i. **Constitutive**

Reg1 can no longer bind and activate Reg2 expression. Reg2 is no longer produced and can never inhibit Enz1 expression. Enz1 is always produced.

ii. **Recessive**

$I\text{-}Reg^{-}$ $Enz1^{+}$/ $I\text{-}Reg^{+}$ $Enz1^{+}$	Regulated	I-reg mutation recessive to WT

iii. **Trans-acting**: merodiploids have the dominant WT phenotype in both cis and trans.

Cis-test	**$I\text{-}Reg^{-}$ $Reg2^{+}$ $Enz1^{-}$/F' $I\text{-}Reg^{+}$ $Reg2^{+}$ $Enz1^{+}$**	Regulated
Trans-test	**$I\text{-}Reg^{+}$ $Reg2^{+}$ $Enz1^{-}$/F' $I\text{-}Reg^{-}$ $Reg2^{+}$ $Enz1^{+}$**	Regulated

*Note that we are testing the dominant phenotype, which is wildtype.

**Also, though I-Reg is a promoter element, it functions in trans to Enz1.

g) A mutation in O-enz that prevents Reg2 binding (O-enz$^-$)

i. **Constitutive**

Reg2 can no longer bind and inhibit Enz1 expression. Enz1 is always produced.

ii. **Dominant**

O-enz$^-$ Enz1$^+$/ O-enz$^+$ Enz1$^+$	Constitutive	O-enz mutation dominant to WT

iii. **Cis-acting**: merodiploids have the dominant mutant phenotype **only in cis**.

Cis-test	O-enz$^+$ Enz1$^-$/F' O-enz$^-$ Enz1$^+$	Constitutive
Trans-test	O-enz$^-$ Enz1$^-$/F' O-enz$^+$ Enz1$^+$	Regulated

3. We can assume that mutant Suc2- is a loss of function mutation, since a Tn5 insertion in a gene would abolish gene function.

a) Because all Tn5::Suc2- mutants still show the Suc1- phenotype, Suc1 and Suc2 are never co-transduced and we can assume that they are unlinked and represent mutations in different genes. Since the Suc1-, Suc2- double mutants all exhibit the Suc1- phenotype, we can conclude that Suc2 acts upstream of Suc1 in the sucrase regulatory pathway:

Suc2 --| Suc1

b) Because all Tn5::Suc3- mutants still show the Suc2 phenotype, Suc2 and Suc3 are never co-transduced and we can assume that they are unlinked and represent mutations in different genes. Since the Suc2-, Suc3- double mutants all exhibit the Suc2- phenotype, we can conclude that Suc3 acts upstream of Suc2 in the sucrase regulatory pathway:

Glucose --| Suc3 --| Suc2 --| Suc1

4. **<u>Dominance and cis-trans test</u>**

Mate each uninducible mutant (all MATa) to Rep strain (wildtype, MATα). Grow diploid on X-Gal media and X-Gal+Mal media:

Strain	**X-Gal**	**X-Gal + Mal**	**Phenotype**	**Conclusion**
Mut1xRep	White	Blue	Inducible	Recessive
Mut2xRep	White	Blue	Inducible	Recessive
Mut3xRep	White	Blue	Inducible	Recessive

All mutant diploids were maltose-inducible, which is the wildtype phenotype. They are all recessive.

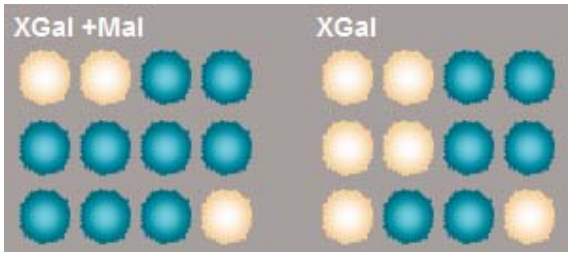
To test cis/trans, mate the mutant to the MATα WT (no reporter) strain. All colonies are blue on X-Gal + Mal, showing Mut1,Mut2, and Mut3 all act in <u>trans</u>.

<u>Linkage analysis and Epistasis test</u>

A) Rev1 vs Mut1

The revertant was isolated in a Mut1 background, so its genotype is Mut1$^-$, Rev1$^-$ (wildtype is Mut1$^+$, Rev1$^+$). The revertant causes constitutive enzyme expression, which is the opposite phenotype to the Mut1 phenotype. This suggests that the revertant mutation occurs in the same gene as Mut1 (maybe as a gain of function), or in a gene downstream of Mut1. To determine this, we must look at the linkage relationship between Rev1 and Mut1 genes.

Cross the double mutant (Rev1) to wildtype reporter strain (Rep α) and sporulate. Grow tetrads on X-Gal media and X-Gal + Mal media.



	Genotype and phenotype of spores			
NPD	1$^-$, R$^+$ uninducible	1$^-$, R$^+$ uninducible	1$^+$, R$^-$ constitutive	1$^+$, R$^-$ constitutive
PD	1$^+$, R$^+$ inducible	1$^+$, R$^+$ inducible	1$^-$, R$^-$ constitutive	1$^-$, R$^-$ constitutive
TT	1$^+$, R$^+$ inducible	1$^+$, R$^-$ constitutive	1$^-$, R$^-$ constitutive	1$^-$, R$^+$ uninducible

PD	:	TT	:	NPD	Total
8	:	31	:	11	50 tetrads

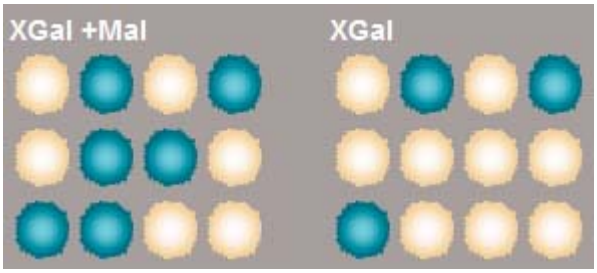
Since this is a 1:4:1 ratio, we can assume that Mut1 and Rev1 are unlinked. Therefore Rev1 must be a mutation in a gene downstream of Mut1 in the pathway.

B) Rev1 vs Mut2

To analyze the relationship between Rev1 and Mut2 or Mut3, we must first isolate a spore from the cross above which is Rev1-, but Mut1+. Any of the constitutive expressors from the NPD category fits these criteria (Notice that you can't distinguish the Rev1$^-$, Mut1$^+$ spores from the Rev1$^-$, Mut1$^-$ spores in the TT tetrads because both are constitutive). Mate this to MATa on

-lys,-leu media to ensure you have a MATa mating type, then cross this spore (Rev1α) to Mut2. To determine if Mut2 and Rev1 are in the same gene, we analyze the linkage relationship between them:

	Genotype and phenotype of spores			
PD	2$^-$, R$^+$ uninducible	2$^-$, R$^+$ uninducible	2$^+$, R$^-$ constitutive	2$^+$, R$^-$ constitutive
NPD	2$^+$, R$^+$ inducible	2$^+$, R$^+$ inducible	2$^-$, R$^-$ unknown	2$^-$, R$^-$ unknown
TT	2$^+$, R$^+$ inducible	2$^+$, R$^-$ constitutive	2$^-$, R$^-$ unknown	2$^-$, R$^+$ uninducible



PD	:	TT	:	NPD	Total
8	:	37	:	5	50

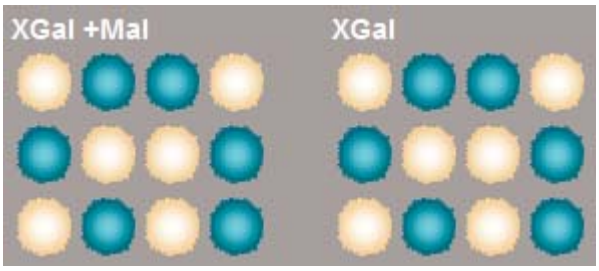
From the linkage analysis, we again see a 1:4:1 ratio, so we can conclude that Mut2 and Rev1 are unlinked.

Additionally, we see that phenotype of the double mutant is uninducible. This is an epistasis test between Mut2 and Rev1. Mut2 is therefore epistatic to Rev1.

C) Rev1 vs Mut3

To determine if Mut3 and Rev1 are in the same gene, we analyze the linkage relationship between them:

	Genotype and phenotype of spores			
PD	$3^-, R^+$ uninducible	$3^-, R^+$ uninducible	$3^+, R^-$ constitutive	$3^+, R^-$ constitutive
NPD	$3^+, R^+$ inducible	$3^+, R^+$ inducible	$3^-, R^-$ unknown	$3^-, R^-$ unknown
TT	$3^+, R^+$ inducible	$3^+, R^-$ constitutive	$3^-, R^-$ unknown	$3^-, R^+$ uninducible



PD	:	TT	:	NPD	Total
50	:	0	:	0	50

In this cross, all tetrads are PDs. Mut3 and Rev1 are therefore tightly linked mutations, with a maximum genetic distance of (1+6(0)/2(50))x100 = 1cM between them. 1cM<3cM, so we cannot rule out the possibility that Rev1 and Mut3 are mutations in the same gene. Rev1 could be a gain of function mutation in the gene in which Mut3 is a loss of function mutation.

Conclusions

1. Mut1 is upstream of Rev1, inhibits enzyme expression.
2. Mut2 is downstream of Rev1, inhibits enzyme expression.
3. Mut3 is in the same gene as Rev1, inhibits enzyme expression.

Since maltose is an inducer of enzyme expression, we can clonclude that the regulatory pathway is as follows:

Mal—> Mut1—> Mut3—> Mut2—> enzyme