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Genetics 320 Final. December 13, 2005.

1. Lac Operon (25 pt) _____

2. Mitotic Recombination (25pt) _____

3. T/F short answer (25 pt) _____

4. Yeast Mating Pathway (25pt) _____

TOTAL _____

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1. Lac Operon (25 points)

A. Given the genotypes, fill in the appropriate phenotypes for LacZ and LacY. The inducer is lactose or IPTG.

Genotype	Phenotypes				10 pt, 1 ea
	LacZ expression		LacY expression		
	no inducer	inducer	no inducer	inducer	
1 I ⁺ O ⁺ Z ⁺ Y ⁺	-	+	-	+	
2 I ^S O ⁺ Z ⁺ Y ⁺ / F' I ^{O^C} Z ⁻ Y ⁺	-	-	+	+	
3 I ^S O ⁺ Z ⁺ Y ⁺ / F' I ⁺ O ⁺ Z ⁺ Y ⁻	-	-	-	-	
4 I ⁺ O ^C Z ⁻ Y ⁺ / F' I ⁺ O ⁺ Z ⁺ Y ⁻	-	+	+	+	

B. Name one strain that indicates that O^C acts downstream (is epistatic to) I^S?
And what data tells you that? Strain 2, Y expression

2pt

C. One new gene, called Gene X, was identified that regulates the Lac operon.
 An allele Gene X* was analyzed.
 Gene X⁺ acts in the glucose, ADC1, CRP pathway somewhere between glucose and lacZ.
 (The assays are done in an I- cell.)
 Adc1⁻ and crp⁻ are both **recessive** mutations.

2pt

- i. Is the Gene X* mutation recessive or **dominant** (circle one)?
- ii. Draw the most likely **New pathway**, with gene order and sign of regulation (bar or arrow) **8pt**

Old pathway glucose —|ADC1⁺—> CRP⁺—> LacZ⁺

New pathway

glucose —> Gene X⁺ —|ADC1⁺—> CRP⁺—> LacZ⁺

Strain	LacZ expression
1. Gene X ⁺	normal
2. Gene X [*]	always ON
3. Gene X [*] / Gene X ⁺	normal
4. crp ⁻	always OFF
6. Gene X [*] crp ⁻	always OFF
7. adc1 ⁻	always OFF
8. Gene X [*] , adc1 ⁻	always OFF

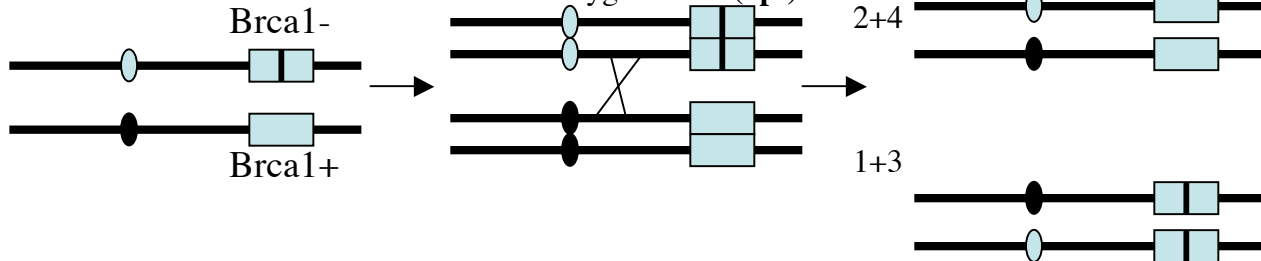
(Note: crp⁻ and adc1⁻ are recessive mutations)

- iii. The phenotype of a crp⁻adc1⁻ double mutant is not informative with respect to the order of CRP and ADC1. Is this because both mutations are recessive? Explain. **3pt**

No, it is not informative because both single mutants have the same phenotype.

2. Mitotic Recombination (25 points)

A. The diploid shown below undergoes DNA replication and cell division. Sometimes homozygous mutant cells are generated following DNA damage (that generates a DNA break). DRAW CLEARLY how a crossover event in this cell can form a homozygous cell. (6pt)



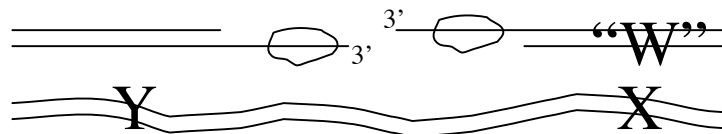
B. Explain BRIEFLY (less than ~ 5 sentences) why the *ccr5*Δ32 mutation leads to resistance to AIDS, Then explain how we can make a CCR5+ cell *ccr5*Δ32. (7 pt)

The CCR5 protein is required to allow the AIDS virus to enter the cell.

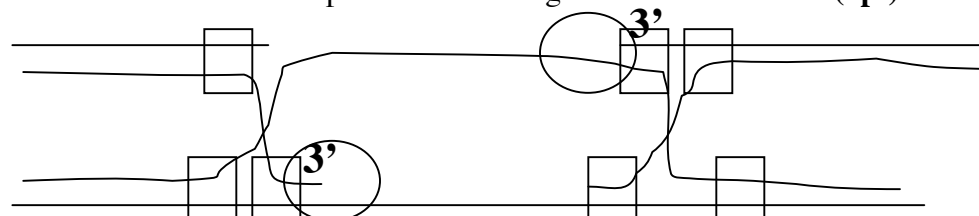
*A *ccr5* mutant cell has no receptor, and so the AIDS virus cannot infect the cell.*

*To make the *ccr5* Δ 32 mutation, we insert both a gene that makes the zinc finger nuclease that can make a DNA break in the CCR5 gene, and a piece of DNA with the *ccr5*Δ32 mutation. The nuclease makes the DNA break, and that break is repaired using the *ccr5*Δ32 mutant DNA fragment such that the mutation is introduced into the chromosome copies of CCR5.*

C. On DNA molecules shown below, indicate with a circle(s) where the RecA protein will bind initially; indicate with a “X” sequence(s) that are allelic to the sequence marked “W”; and indicate with a “Y” a sequence that is NOT allelic to “W”. (6pt)



D. Draw the structure after pairing has occurred. Label 3' free ends of DNA with 3". Put a circle where DNA polymerase will synthesize DNA. Put a square on one region that either is heteroduplex in your version, or would become heteroduplex if branch migration were to occur. (6pt)



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3. True/False short answer. (25 points)

Write “True” or “False” then provide one statement that is relevant to the question. Credit for T/F is given only if you provide a relevant comment. Do not restate question in your comment.

- A. A yeast mutant that cannot mate has mutations in 2 genes. The intact genes act redundantly in mating. Genetic analysis cannot allow you to determine that the mutant has two mutations. ***False. If you cross this mutant to wildtype and generate haploids (by meiosis), mating segregates 3+ to 1-.***
- B. The Philadelphia chromosome is an excellent example of the perils of allelic recombination. ***False. It is an example of non-allelic recombination, between chr22 and chr9***
- C. Consider the Cancer Genetics Pathway shown below. The order of function of Rb and p21 can be determined by using homozygous recessive mutations in both genes.

DNA damage ► p53+ ► p21+ ─| CDK4-cyclinD ─| Rb+ ─| E2F+ ─► Start cell cycle

False, because both homozygous single mutants have the same phenotype- they both let the cell cycle start.

- D. RecA is critical for complementation tests. ***False...complementation does not require recombination.***

- E. The meiotic cell cycle is designed to increase allelic variation in the progeny cells, while the mitotic cell cycle seeks to maintain the genetic content of the cell. ***True- Spo11 makes DNA breaks that lead to recombination and mixture of alleles in meiosis, while in mitosis Spo11 is not made and DNA undergoes recombination only after error.***

1. Yeast Mating Pathways (25 points)

A. Given in the table are mutants in mating pathway. Each mutant has only one mutation. Ste70* and ste80* are two mutants of the STE90+ and STE100+ genes, respectively. STE90+ and STE100+ are not linked.

i. Is ste90* recessive or dominant (circle one)? **2pt.**

ii. Is ste100* recessive or dominant (circle one)? **2pt.**

iii. Consider a ste90* ste100* diploid cell.

STE90+ ste100*

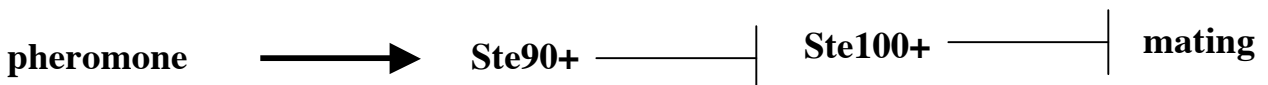
Is the mating phenotype of this cell informative for determining the order of function of STE90+ and STE100+ genes.

EXPLAIN BRIEFLY YOUR ANSWER. **4pt**

	<u>Genotype</u>	<u>Phenotype</u>
1.	Ste+	mates normally
2.	ste90*	always mates
3.	ste100*	never mates
4.	<u>ste90*</u> STE90+	always mates
5.	<u>ste100*</u> STE100+	never mates
6.	ste100* ste90*	never mates

Yes its informative because the cell is a double mutant because ste90 is dominant.*

iv. Draw the pathway of gene function in mating of STE90+ and STE100+ genes. **(10pt)**



B. Consider another gene called STE101+. You have two alleles; ste101-1 always mates and is dominant, and ste101-2 never mates and is recessive.

i. What will the phenotype be of a diploid cell that has both alleles?. **(2 pt)**

always mates (because dominant allele “predominates”)

ii. Explain briefly how you would determine where in the mating pathway STE101+ acts. **(5pt)**. You should be able to say this in just 1-2 sentences.

Mate ste101-1 to ste100 and isolate double mutants, (“make” ste101-1 ste100* double) and mate ste101-2 to ste90* (“make” ste101-2 ste90*), and test their mating phenotypes.*