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7.03 - Genetics - Fall 2004
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Name: _____

1. Drawn below is part of a wild-type gene. The DNA sequence shown encodes the last amino acids of a protein that is normally 380 amino acids long. The bracketed codon indicates the correct reading frame of this gene. The lower strand of the gene is used as the template during the transcription of mRNA from this gene.

┌
...GCTAAGTATTGCTCAAGATTAGGATGATAAAATAACTGG-3'
...CGATTCATAACGAGTTCTAATCCTACTATTTATTGACC-5'

(a, 6 pts) In the copy of the sequence drawn below, circle one base pair that you could change to make a mutant form of the gene that produces a protein that is now 381 amino acids long. Indicate the identity of one new base pair that could take its place.

...GCTAAGTATTGCTCAAGATTAGGATGATAAAATAACTGG-3'
...CGATTCATAACGAGTTCTAATCCTACTATTTATTGACC-5'

(b, 6 pts) In the copy of the sequence drawn below, draw a slash between two base pairs where you could add one extra base pair in order to make a single mutant form of the gene that produces a protein that is 373 amino acids long.

...GCTAAGTATTGCTCAAGATTAGGATGATAAAATAACTGG-3'
...CGATTCATAACGAGTTCTAATCCTACTATTTATTGACC-5'

(c, 9 pts) Multiple mutant suppressor tRNAs could suppress the early termination defect in part **(b)** by allowing a longer protein to be produced from that mutant form of the gene. Make a list of **all** of the tRNA genes that could produce such mutant suppressor tRNAs if each tRNA gene contained a **single base** substitution. (Use the notation: "ala-tRNA.")

Name: _____

2. You are studying the regulation of a bacterial gene called *nytT*, which is expressed only when the bacterial strain is grown in the dark. You isolate two mutations, *nytA1*⁻ and *nytB1*⁻, which affect the regulation of *nytT*.

	Genotype	Is <i>nytT</i> expressed in the dark?	Is <i>nytT</i> expressed in the light?
Strain 1	<i>nytA</i> ⁺ <i>nytB</i> ⁺ <i>nytT</i> ⁺ (wild type)	yes	no
Strain 2	<i>nytA1</i> ⁻ <i>nytB</i> ⁺ <i>nytT</i> ⁺	no	no
Strain 3	<i>nytA</i> ⁺ <i>nytB1</i> ⁻ <i>nytT</i> ⁺	no	no
Strain 4	<i>nytA</i> ⁺ <i>nytB</i> ⁺ <i>nytT</i> ⁺ / F' <i>nytA1</i> ⁻	yes	no
Strain 5	<i>nytA</i> ⁺ <i>nytB</i> ⁺ <i>nytT</i> ⁺ / F' <i>nytB1</i> ⁻	yes	no

You grow P1 phage on an otherwise wild-type strain that contains a transposon insertion carrying a gene that confers tetracycline resistance. The transposon insertion in this strain is linked to the *nytT* locus with a cotransduction frequency of 85%, and this insertion does not alter normal *nytT* regulation. You use the resulting lysate to infect a *nytA1*⁻ strain, and select for tetracycline resistance. None of the 30 Tet^r cotransductants you examine express the *nytT* gene under any conditions. You obtain the same results when you use the same P1 lysate to infect a *nytB1*⁻ recipient strain.

(a, 5 pts) Can you conclude if *nytA1*⁻ is constitutive or uninducible? **If so**, state whether *nytA1*⁻ is constitutive or uninducible, and state what was the most important piece of information (for example, which strain in the table) you used to reach your conclusion.

(b, 5 pts) Can you conclude if *nytA1*⁻ is dominant or recessive? **If so**, state whether *nytA1*⁻ is dominant or recessive, and state what was the most important piece of information (for example, which strain in the table) you used to reach your conclusion.

Name: _____

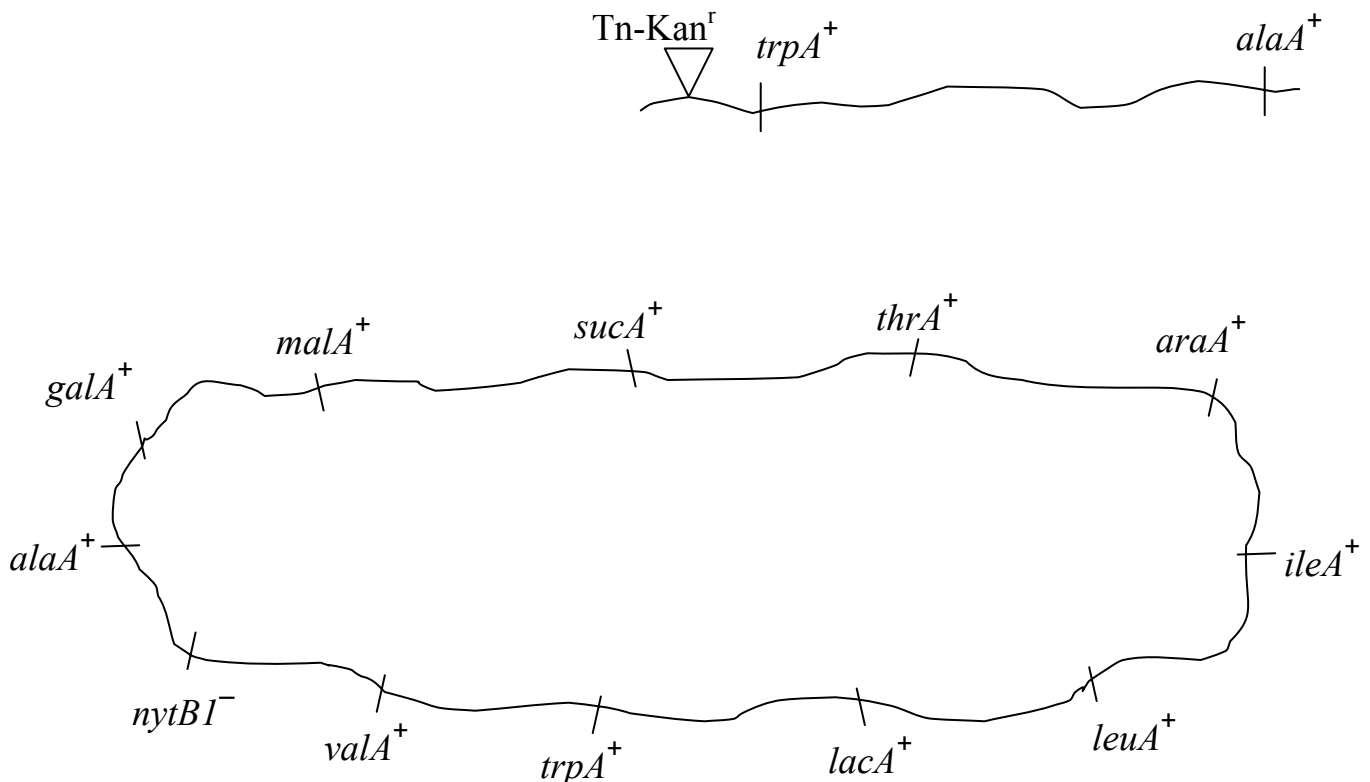
(c, 8 pts) Can you conclude if *nytA1*⁻ acts in cis or in trans with respect to *nytT*? **If so**, state whether *nytA1*⁻ acts in cis or in trans, and state what was the most important piece of information (for example, which strain in the table) you used to reach your conclusion.

(d, 10 pts) Diagram **all possible models** for regulatory pathways for *nytT* that can explain the behavior of the *nytA1*⁻ and *nytB1*⁻ mutations. (Please diagram only linear pathways in which each gene is controlled by no more than one regulator. Please do not include any steps that invoke unknown players.) For each model, include **only** the following: wild-type *nytA*, *nytB*, and *nytT*, and “bright light.”

3. After you perform the experiments from Question #2, you decide to continue studying the regulation of the bacterial gene *nytT*, which is expressed only when the bacterial strain is grown in the dark. You decide to map the two mutations, *nytA1*⁻ and *nytB1*⁻, which you isolated in Question #2. Please refer to the table in the introduction to Question #2 for information about how these mutations affect the regulation of *nytT*.

You find that the *nytA* and *nytB* loci are linked using P1 cotransduction experiments. You isolate a transposon insertion that carries a gene encoding kanamycin resistance. This transposon insertion is near to, but not between, the *nytA* and *nytB* loci.

(a, 6 pts) You grow P1 phage on an otherwise wild-type strain that contains the transposon insertion carrying kanamycin resistance. You use the resulting lysate to infect a *nytB1*⁻ strain, and select for kanamycin resistance. Drawn below are the *E. coli* chromosome and the DNA transduced by P1 during this cotransduction experiment. (Please note that these drawings are not to scale.) **Redraw** the DNA transduced by P1 so that it lines up with the homologous region of the *E. coli* chromosome. Then **draw in** the recombination events necessary to achieve the cotransduction of Tn-Kan^r and the *nytB* locus.



Name: _____

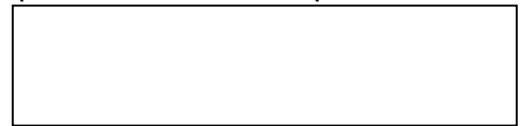
(b, 5 pts) In the transduction experiment described in part **(a)**, out of a total of 50 Kan^r cotransductants, 15 can express the *nytT* gene in the dark and 35 cannot. **Express the distance** between the transposon and the *nytB* locus as a cotransduction frequency.

To map the *nytA* and *nytB* loci, you set up two reciprocal crosses:

In the **first cross**, you grow P1 phage on a Kan^r strain that contains the transposon insertion and the *nytA1*⁻ mutation, and use the resulting phage lysate to infect a *nytB1*⁻ strain. You select for kanamycin resistance (Kan^r), and among 100 Kan^r transductants, you find that only 13 are able to express *nytT*. (All 13 show normal *nytT* regulation.)

In the **second cross**, you grow P1 phage on a Kan^r strain that contains the transposon insertion and the *nytB1*⁻ mutation, and use the resulting phage lysate to infect a *nytA1*⁻ strain. You select for kanamycin resistance (Kan^r), and among 100 Kan^r transductants, you find that only 3 are able to express *nytT*. (All 3 show normal *nytT* regulation.)

(c, 6 pts) Draw a genetic map showing the correct relative positions of the transposon insertion (Tn-Kan^r) and the *nytA* and *nytB* loci in this box:



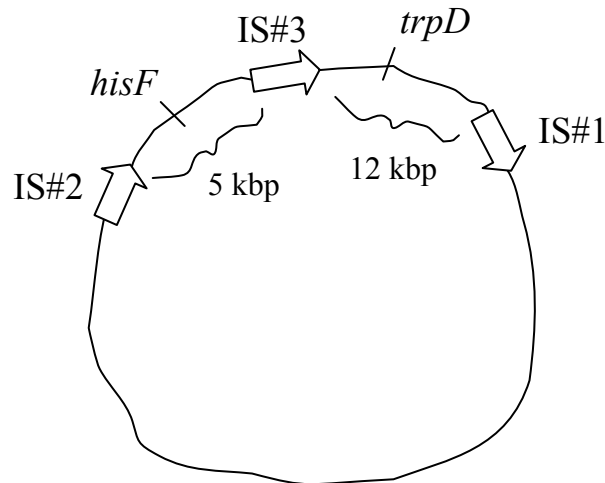
(d, 6 pts) Based on the gene order **that you drew in part (c)**, state the chromosomal genotype of a transductant that must have resulted from a quadruple crossover event between the transduced DNA and the bacterial chromosome of the recipient **in the first cross**. (Be sure to indicate the chromosomal genotype at both the *nytA* and *nytB* loci.)

Name: _____

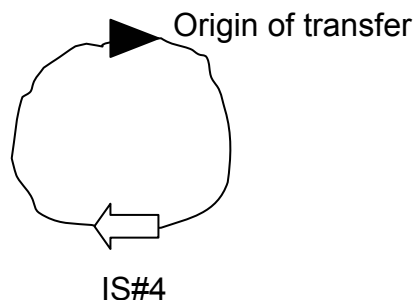
(e, 6 pts) Based on the gene order **that you drew in part (c)**, which of the following is the most reasonable distance between Tn-Kan^r and the *nytA* locus, as expressed as a cotransduction frequency? (**Your choices are:** 20%, 30%, OR 40%.)

4. You are studying a strain of *E. coli* whose total genome size is **4,639 kilobase pairs** (kbp). The chromosome of this *E. coli* strain is diagrammed below, and its three insertion sequences are indicated. Note that this drawing is **not to scale**.

NOTE: Assume that the size of each insertion sequence is 1 kbp.



You are utilizing a form of the F factor that is **95 kbp** in length. This F factor has a single IS sequence and a single origin of transfer, as indicated:



Fill in the chart on the next page, which considers cells containing the above chromosome and F factor.

Name: _____

(22 pts) Fill in the chart below. Two boxes have been done for you.

NOTE: Assume each cell described in Column 1 contains only what is listed -- NO OTHER recombination events have occurred in each cell besides those listed.

Type of cell	What is the size of the circular <i>E. coli</i> chromosome in the cell?	What is the size of the extrachromosomal circle of DNA in the cell?	Can <i>hisF</i> be transferred efficiently, inefficiently, OR never?	Can <i>trpD</i> be transferred efficiently, inefficiently, OR never?
An F ⁻ bacterial cell	4,639 kbp	0 kbp (there isn't one)		
An F ⁺ bacterial cell				
An Hfr cell (named " Hfr A ") resulting from recombination between IS#4 and IS#3				
A cell resulting from recombination between IS#2 and IS#1 in "Hfr A"				