ANSWERS TO Exam Questions from Exam 2 – Mutations, Bacterial Genetics, and Bacterial Gene Regulation

1. Drawn below is part of a wild-type gene.

(a)

...GCTAAGTATTGCTCAAGATTAGGATGATAAATAACTGG-3′
...CGATTCATAACGAGTTCTAATCCTACTATTTATTGACC-5′

change TA basepair to CG (for example)

(b)

...GCTAAGTATTGCTCAAGATTAGGATGATAAATAACTGG-3′
...CGATTCATAACGAGTTCTAATCCTACTATTTATTGACC-5′

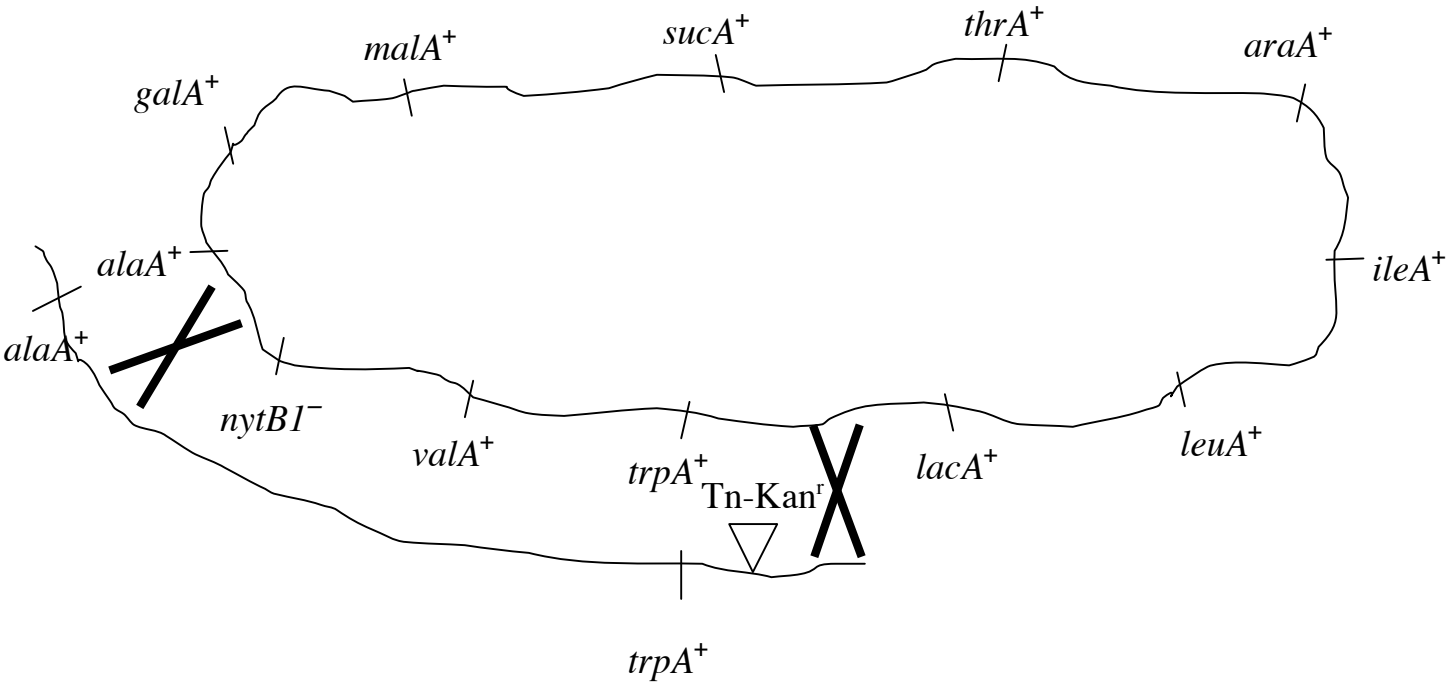
add in a TA basepair (for example)

(c) gln-tRNA, glu-tRNA, lys-tRNA, ser-tRNA, leu-tRNA, tyr-tRNA

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.
(a) yes, uninducible, strain 2
(b) yes, recessive, strain 4
(c) yes, trans, the P1 transduction experiment
(d) light --] A → B → T
light --] B → A → T
light --] A/B → T

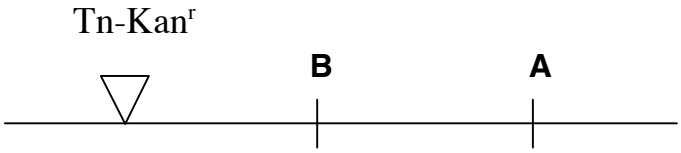
3. After you perform the experiments from Question #2, you decide to continue studying the regulation of the bacterial gene *nytT*, which is expressed only when the bacterial strain is grown in the dark.

(a)



(b) 30%

(c)



(d) Tn B− A−

(e) 20%

4. You are studying a strain of *E. coli* whose total genome size is **4,639 kilobase pairs** (kbp).

Type of cell	What is the size of the circular *E. coli* chromosome in the cell?	What is the size of the extrachromosomal circle of DNA in the cell?	Can *hisF* be transferred efficiently, inefficiently, OR never?	Can *trpD* be transferred efficiently, inefficiently, OR never?
An F^- bacterial cell	4,639 kbp	0 kbp (there isn't one)	Never	Never
An F^+ bacterial cell	4639	95	Never	Never
An Hfr cell (**named "<u>Hfr A</u>"**) resulting from recombination between IS#4 and IS#3	4734	0	efficiently	Inefficiently
A cell resulting from recombination between IS#2 and IS#1 in "Hfr A"	4620	114	Efficiently	Efficiently

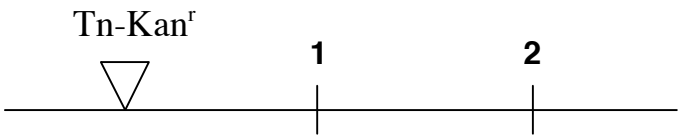
5. You have isolated a mutation in the Lac I gene; this mutation causes constitutive LacZYA gene expression.
Propose **two different** explanations for why the amber-suppressing mutant allele of the gene encoding $tRNA^{trp}$ fails to suppress this particular amber mutation.

Answer: Either the efficiency of the tRNA suppressor only creates low levels of the wild-type form of the protein, and such low levels are not sufficient for the protein to function properly **OR** Inserting a tryptophan residue at that position in the protein creates a non-functional protein.

6. You have isolated an *E. coli* mutant which you call Lac1^{-}. This mutant cannot grow on the sugar lactose as the only carbon source.

(a) 80%

(b)



(c) Z^{-}

(d) P^{-} or I^{S}

7. You have identified a new strain of *E. coli* that can grow on starch.

(a) operator, because constitutive cis dominant

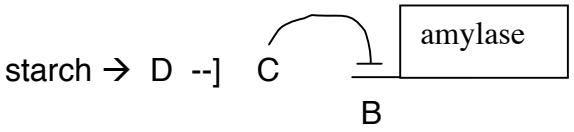
(b) repressor, because constitutive trans recessive

(c) activator, because trans uninducible, recessive

(d) D is earlier than B

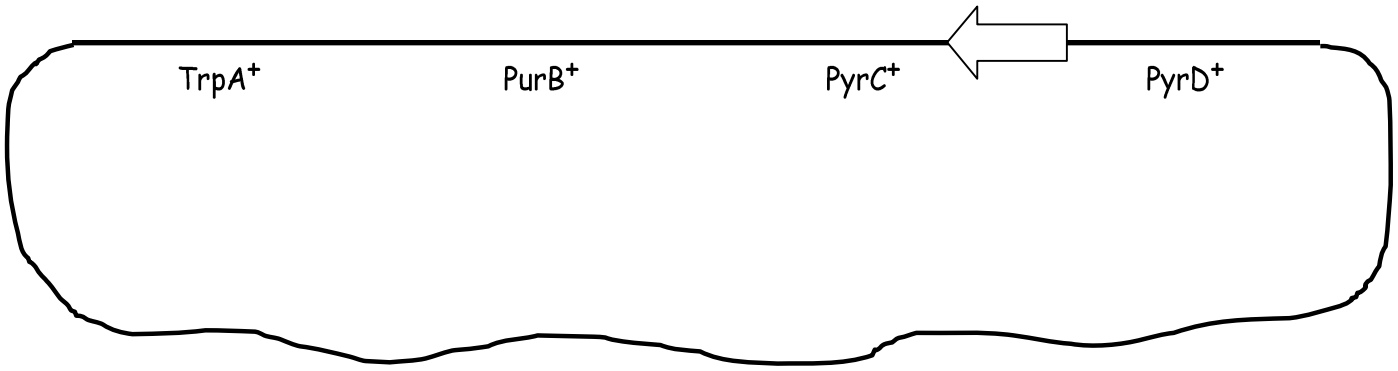
(e) D is earlier than C

(f)

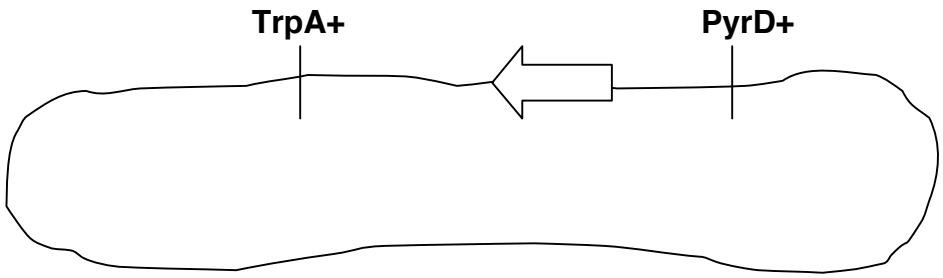


8. Below is a diagram of the F factor showing the direction of the origin of transfer (ori T) and an **IS2 element** carried on this F plasmid.

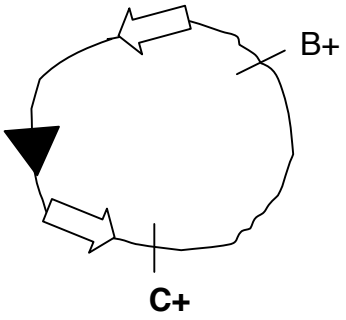
(a)



(b)



(c) The F' plasmid:



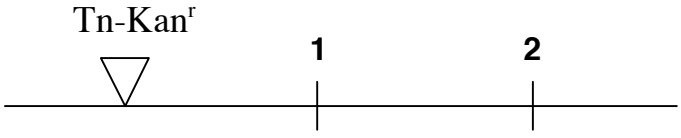
(d) the mutation in PurB gives a dominant phenotype

9. The Mot genes of *E. coli* are required for motility (swimming) of these bacteria.

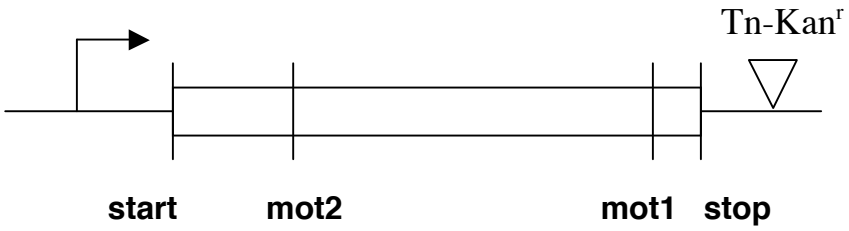
(a) 70%

(b) no – the two loci could be unlinked or could be tightly linked and you'd get the same result

(c)



(d)



(e) an amber nonsense mutation

(f) 5'-CTA-3'
3'-GAT-5'
the bottom strand is used as a template for transcription

10. Raffinose is a sugar that requires the lactose permease (the LacY gene product) to enter an *E. coli* cell.

(a) I^- or O^C or I^{-d}

(b) I^-

(c) O^C or I^{-d}

(d) on the chromosome

11. You are studying the regulation of ubiquinone synthesis in bacteria.

(a) repressor, because constitutive recessive trans

(b) ubiquinone → A → B --] Ubi1

ubiquinone → B → A --] Ubi1

(c) because the double mutant phenotype would be the same for the two models, since the two mutations cause the same phenotype

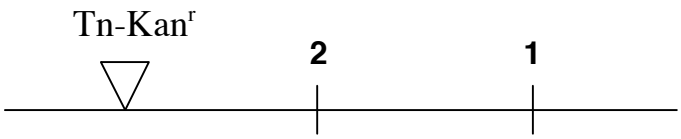
(d) ubiquinone → B → A --] Ubi1

(e) B^S activates the activity/expression of A regardless of the presence of ubiquinone

12. You have isolated a **Tn5** insertion in an otherwise wild-type *E. coli* strain that you think may be linked to the **Lac** operon.

(a) 70%

(b)



(c) the second cross

(d) KanR 2^- 1^- would give constitutive expression

13. The codon for tryptophan is $^{5'}$UGG$^{3'}$.

(a) 5'-CCA-3'

(b) 5'-TGG-3'
3'-ACC-5'

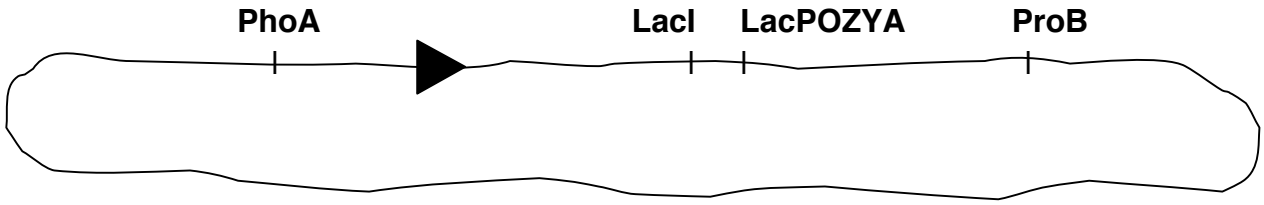
(c) 5'-CUA-3'

(d) yes

(e) amber

14. The region of the *E. coli* chromosome near the **Lac** operon is diagrammed below:

(a)

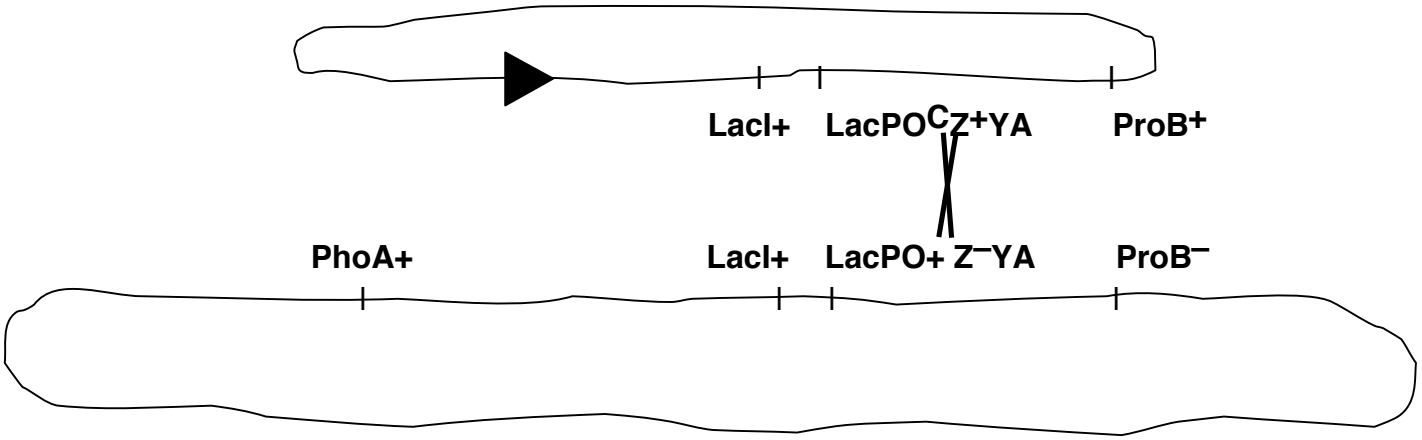


(b) no

(c) look for constitutive expression of LacZ in strains grown without lactose

(d) six

(e)



(f) an Hfr

15. For each of the two following subparts (one for the lac operon and one for the mal operon), predict the number of units of enzyme activity that will be displayed by a strain of the given genotype, grown under the given conditions.

(a)

	ß-galactosidase activity –IPTG	+IPTG
Lac O^{+} Z^{+} / F' Lac O^{c} Z^{-}	1	100
Lac I^{+} O^{+} Z^{+} Y^{-} / F' Lac I^{-} O^{+} Z^{+} Y^{+}	2	200
Lac I^{+} O^{c} Z^{+} / F' Lac I^{-} O^{+} Z^{+}	101	200
Lac I^{-d} O^{c} Z^{+} / F' Lac I^{s} P^{-} O^{+} Z^{+}	100	100

(b)

	maltase activity –maltose	maltase activity +maltose
$MalT^{-}\ Q^{+}$ / F' $MalT^{+}\ Q^{-}$	1	100
$MalT^{C}\ Q^{+}$ / F' $MalT^{+}\ Q^{-}$	100	100
$MalT^{C}\ Q^{-}$ / F' $MalT^{-}\ Q^{+}$	100	100

16. You are studying the regulation of methanol utilization in bacteria.

(a) repressor, because trans, recessive, constitutive

(b) because the B2 mutation gives a dominant phenotype

(c) repressor

(d) B1 causes a loss of function of the B gene. B2 is a superrepressor allele that encodes a form of B that represses even when methanol is present

(e) methanol --] B → A --] Mox
methanol --] A → B --] Mox

(f) because B2 causes a phenotype that is distinguishable from the phenotype caused by the A mutation

(g) methanol --] A → B --] Mox

17. Phage T4 expresses an enzyme lysozyme, which enables the phage to lyse infected bacterial cells.

(a) 1 m.u.

(b) 120,000 base pairs in length

(c) one possibility:
CGA (arginine)
5'-CGA-3'
3'-GCT-5'
the bottom strand is used as a template in transcription

the other possibility:
UGG (tryptophan)
5'-TGG-3'
3'-ACC-5'
the bottom strand is used as a template in transcription

18. You have isolated a **Tn5** insertion in an otherwise wild-type *E. coli* strain; this transposon is near to but not within the group of lac genes on the *E. coli* chromosome.

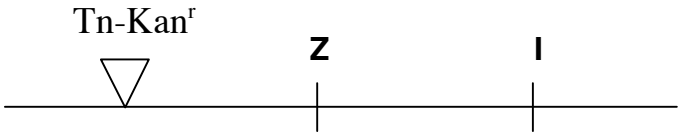
(a) 60%

(b)

Number of transuctants	ß-galactosidase	permease	Genotype
578	uninducible	regulated	Z^- Y^+ I^+
400	constitutive	constitutive	Z^+ Y^+ I^-
20	uninducible	constitutive	Z^- Y^+ I^-
2	regulated	regulated	Z^+ Y^+ I^+

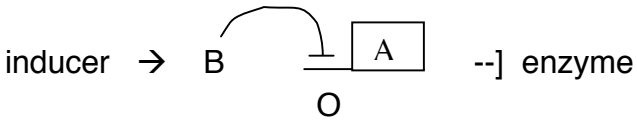
(c) 58%

(d)



19. An enzyme that you are interested in from *E. coli* is regulated by the following scheme:

(a)



(b)

	– inducer	+ inducer
B^*	**yes**	**yes**
O^-_A	**no**	**no**
$B^* O^-_A$	**no**	**no**

(c)

	– inducer	+ inducer
A^*	**yes**	**yes**
$A^*/ F' A^+$	**yes**	**yes**
$O^+_A \ A^*/ F' O^-_A \ A^+$	**yes**	**no**

20. In order to study regulation of starch degradation in *E. coli*, you isolate a **Tn5::LacZ** insertion in the gene for the starch-degrading enzyme amylase. Construct a model to explain amylase regulation that is consistent with all of this information.

ANSWER: starch → 1 --] 2 --] amylase

21. The following sequence (and some of the encoded amino acids) lies within the coding sequence of a wild-type *E. coli* gene, "gene X":

(a) take away the T that immediately follows the A that was added in the original mutation

`...CTC TCT TTC ATG ACA AGG GGG GGG TAA GCT AA...`

(b) `...leu ser phe met thr arg gly gly.`

(c) no stipulations – the protein returns to being wild-type if this intragenic suppressor mutation occurs

(d) a mutation in a tRNA gene (the possibilities are: tyr, leu, ser, trp, gln, or glu) such that the tRNA now recognizes the nonsense codon TAG and inserts an amino acid when the stop codon is read. For example, if you choose leu:

The DNA change in the anticodon portion of the tRNA gene would be from wild-type:

5'-CAA-3'

3'-GTT-5'

To mutant: 5'-CTA-3'

3'-GAT-5'

(e) `...leu ser phe met thr (leu) gly gly val ser.`

(f) the changing of the last amino acids of this protein from "`arg gly gly`" to "`(leu) gly gly val ser`" must not affect the function of the protein