7.03 Exam 2

Name: KEY

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Exam starts at 12:05 and ends at 12:55

Please write your name on each page.

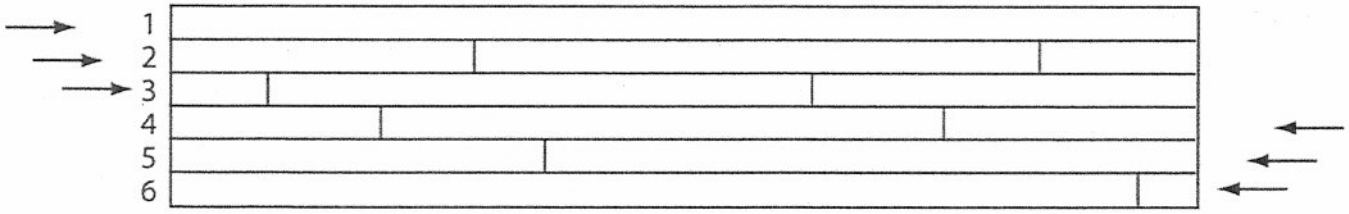
Please...

- Look over the entire exam so you don't spend too much time on hard questions leaving easy questions unanswered.
- Check your answers to make sure that they make sense.
- To help us give partial credit, show your work and state any assumptions that you make.

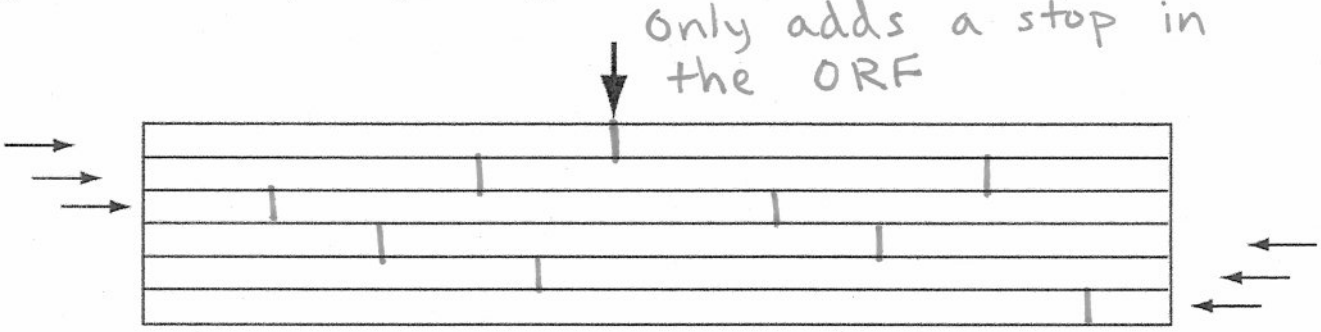
Question 1	36 points
Question 2	32 points
Question 3	32 points

Name: KEY

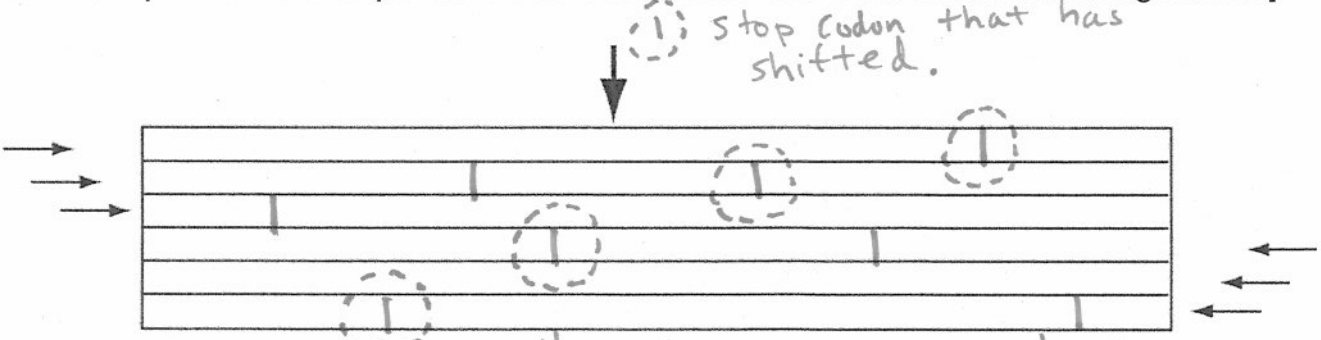
1. The diagram below shows a 300 base pair (i.e. 100 codon) segment in the middle of a bacterial gene displayed in a six-frame translation program similar to StarORF. Rows 1 - 3 represent the three reading frames read from left to right and rows 4 - 6 represent the three reading frames read from right to left. The arrows show the relationships among the three reading frames in each direction. Vertical marks in each row represent the positions of stop codons in that reading frame. Row 1 is the open reading frame for the gene.



(a 8 pts.) Imagine that a nonsense mutation occurred in the bacterial gene at the position of the vertical arrow. In the blank diagram below mark the positions of all of the stop codons that you know must exist, as they would appear in all six frames after the mutation occurred.



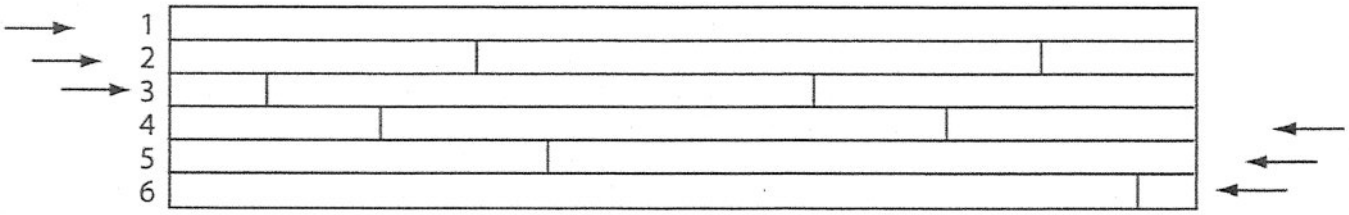
(b 8 pts.) Imagine that the wild type gene was altered by *deletion* of a single base pair at the position of the vertical arrow. In the blank diagram below mark the new positions of the stop codons in all six frames after the mutation occurred. [Be careful, this can be tricky – the question is really asking how the already marked stop codons will be affected by deletion of one base pair from the sequence. Be sure to consider the effect on all six reading frames.]



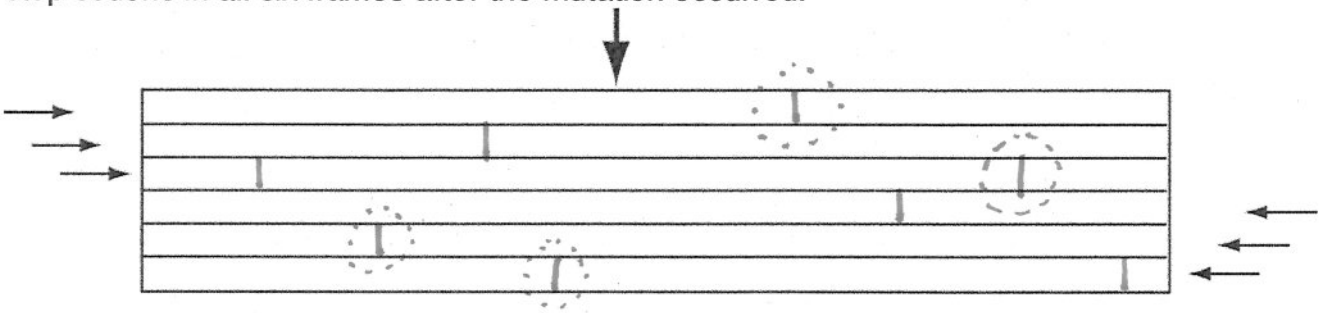
Frameshifts will not create or destroy stop codons. Stops will just move from one frame to another.

Name: KEY

The diagram from the first page is reproduced here for reference.



(c 8 pts.) Imagine that the wild type gene was altered by *insertion* of a single base pair at the position of the vertical arrow. In the blank diagram below mark the new the positions of the stop codons in all six frames after the mutation occurred.



(d 6 pts.) If you wanted to generate an amber mutation in a bacterial gene would you prefer to use a mutagen that causes G•C to A•T transitions or a mutagen that causes A•T to G•C transitions. Explain your reasoning. (The sequences of the three stop codons are: $^{5'}$UAG$^{3'}$ (amber), $^{5'}$UAA$^{3'}$ (ochre), and $^{5'}$UGA$^{3'}$.) G•C → A•T

No codons can be changed into stops by A•T → G•C mutations.

(e 6 pts.) Write out the sequence of the anticodon segment of an amber suppressing tRNA (remember to label 5' and 3' ends and that U substitutes for T in RNA). If you wanted to generate an amber suppressor mutation would you prefer to use a mutagen that causes G•C to A•T transitions or a mutagen that causes A•T to G•C transitions. Explain.

3' (A U C) 5' tRNA anticodon that can suppress amber.

mRNA 5' — U A G — 3'
↑ amber

* no anticodon can be changed to an amber suppressor by a A•T → G•C mutation.

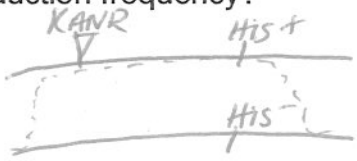
Name: KEY

2. **(a** 6 pts.) You have isolated a His$^-$ *E. coli* strain and you wish to find a linked Tn5 insertion. To do this you grow phage P1 on a large mixed collection of different random Tn5 insertion strains and use the resulting lysate to infect His$^-$ mutant strain and select for kanamycin resistance (Kan^r). Among 1,000 Kan^r transductants you find one that can grow on medium that lacks histidine. What can you definitely say about the distance between the Tn5 insertion site in this strain and the His$^-$ mutation in terms of the size of one P1 phage headfull?

The distance is less than 1 headfull apart as one transductant can grow on medium that lacks His.

POINTS Full or NOT

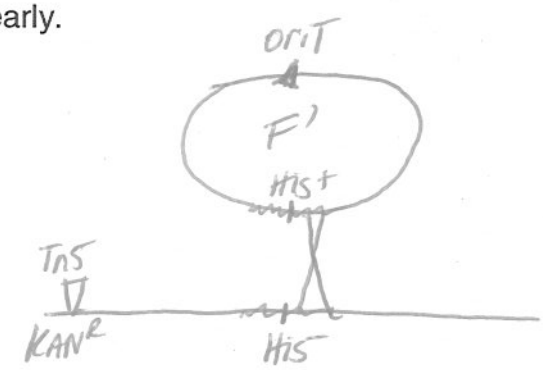
(b 6 pts.) Next you grow P1 phage on the Kan^r His$^+$ strain described above and then use the resulting lysate to infect your His$^-$ mutant strain. From 50 Kan^r transductants you find 35 are His$^-$ and 15 are His$^+$. What is the distance between Tn5 and His$^-$ mutation expressed as a cotransduction frequency?

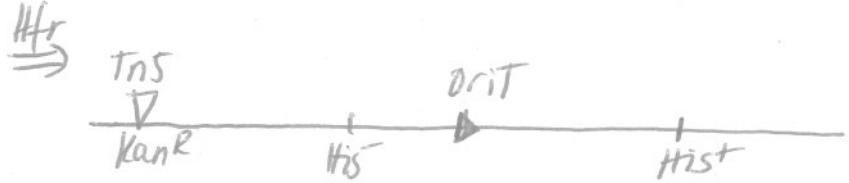


$\frac{15}{50} \times 100 = 30\%$

POINTS Full or NOT

(c 8 pts.) You have available to you a strain with an F' factor that carries a wild type segment of the *E. coli* chromosome including the region corresponding to the location of the His$^-$ mutation (i.e. the F' will complement the His$^-$ mutation). You mate this F' into a Kan^r His$^-$ strain isolated in part **(b)** and then from the resulting F' strain you isolate an Hfr that can transfer Kan^r early but transfers His$^+$ late. Diagram the recombination event between the F' factor and the *E. coli* chromosome and the Hfr that is produced. Your diagram should clearly distinguish sequences derived from the F factor (including the position and orientation of the origin of transfer) and sequences derived from the chromosome. Also the relative positions of the Tn5 insertion, the His$^+$ and His$^-$ alleles and the position of the crossover event should be shown clearly.



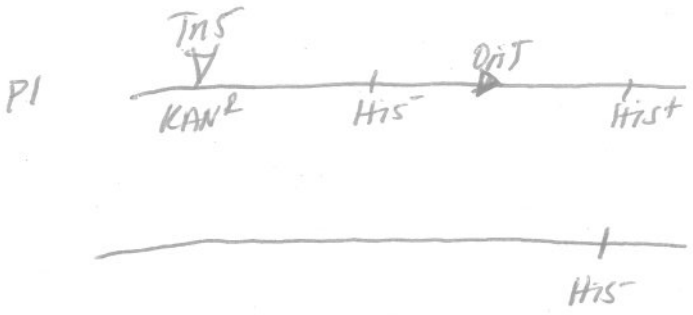


① KANR early
② His+ late

POINTS
4 points: correct Hfr
1 point: F) his+
1 point: KanR: His-
2 points: region of homology

Name: KEY

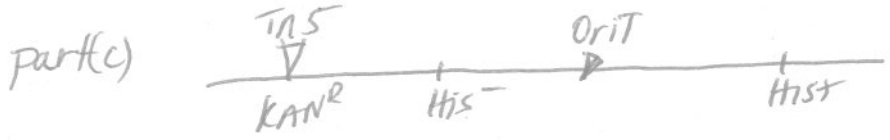
(d 6 pts.) You grow P1 phage on the Hfr strain described in part **(c)**. You use the resulting phage lysate to infect the original His$^-$ mutant strain selecting for Kan^r transductants. What fraction of these transductants would you expect to be His$^+$? Explain your reasoning and remember that the F factor is about the same size as one P1 phage headfull.)



More than one P1 phage headfull, therefore 0 transductants will be His$^+$

POINTS
+3 for 0 transductants
+3 for explanation

(e 8 pts.) As noted above, the Hfr strain described in part **(c)** transfers Kan^r early and His$^+$ late. You set up a mating between this Hfr strain and a His$^-$ mutant as a recipient. You then select for formation of a rare F' derivitave of this strain that can transfer His$^+$ to the recipient after only a brief mating period. Starting with the diagram of the Hfr from part **(c)**, show the position of a recombination event and the F' that would be produced that would allow early transfer of His$^+$.



His$^+$ transfired early

POINTS: 4 points (showing Hfr turn)
2 points (homology)
2 points (product)

Name: KEY

3. You are studying the regulation of sucrose utilization in yeast. Yeast cells use the enzyme invertase to break down sucrose (into glucose and fructose). Invertase is not produced when there is glucose available in the growth medium. Suc1 is the gene for invertase and an assay for Suc1 mRNA shows that the Suc1 gene is not transcribed when glucose is present. You have isolated a Suc1$^-$ mutant, which fails to make invertase under any condition (i.e. the mutant is uninducible). Mating of Suc1$^-$ to a wild type haploid strain produces a diploid that exhibits normal regulation of invertase.

(a 8 pts.) By mutagenizing a wild type strain and screening for mutants that expresses invertase in the presence of glucose you isolate two constitutive mutants designated Inv-1 and Inv-2. Mating an Inv -1 mutant to wild type yields diploids that show normal invertase regulation. You mate an Inv-1 mutant to a Suc1$^-$ mutant (the resulting diploids also show normal regulation of invertase) and sporulation of these diploids produces tetrads of the following types:

Type 1: 2 uninducible; 2 constitutive
Type 2: 2 uninducible; 1 constitutive; 1 regulated
Type 3: 2 uninducible; 2 regulated

Out of 20 tetrads you find 3 of Type 1, 13 of Type 2, and 4 of Type 3. Given all of this information, describe the relevant genetic properties of the Inv-1 mutant and its relationship to Suc1 (explain your reasoning). Deduce the type of regulatory function affected by the Inv-1 mutation. Finally, diagram a model to explain the effects of glucose and the wild type Inv1 gene on Suc1 expression.

Suc1$^-$ = uninducible

$\frac{Suc1^-}{+}$ = wt ∴ recessive ∴LOF

Inv-1 = constitutive (+1)

$\frac{Inv-1}{+}$ = wt ∴ recessive ∴LOF (+1)

$\frac{Inv-1\ \ +}{+\ \ Suc1^-}$ = wt

3 : 13 : 4 approximates 1:4:1.
∴ Suc1$^-$ and Inv-1 are unlinked.
Inv-1 thus functions in trans. (+2)

Inv-1 is recessive, constitutive, trans-acting.
Its normal function is thus negative
∴ repressor. (+2)

Glu → Inv-1 ⊣ Suc1$^-$ (+1)

Sporulate

Type 1	Type 2	Type 3
U	U	U
U	U	U
C	C	wt
C	wt	wt
PD	TT	NPD
3	13	4

(+1)

parentals
Inv-1 + : C
\+ Suc1$^-$: U

recombinants
Inv-1 Suc1$^-$: U (from tetrad data)
\+ + : wt

Name: KEY

(b 8 pts.) Next, you examine the constitutive Inv-2 mutation. Mating an Inv-2 mutant to wild type yields diploids that show constitutive invertase expression. Mating an Inv-2 mutant to a Suc1$^-$ mutant yield diploids that also show constitutive invertase expression. Sporulation of these diploids produces 20 tetrads that are all the same type: 2 uninducible; 2 constitutive. Describe the relevant genetic properties of the Inv-2 mutant and its relationship to Suc1. Deduce the type of regulatory function affected by the Inv-2 mutation.

$\frac{Inv\text{-}2}{+}$ = constitutive ∴ dominant (+1)

Inv-2 = constitutive (+1)
Suc1- = uninducible

(+1) $\frac{Inv\text{-}2 \quad +}{+ \quad Suc1^-}$ = constitutive

↓ sporulate

C
U
C
S

all PD ∴ completely linked. (+2)
in eukaryotes, this means Inv-2 acts in cis. (+1)

Thus, Inv-2 is dominant, constitutive, and cis-acting.
An operator that doesn't allow repressor binding (operator-) fits this description. (+2)

(c 8 pts.) Next you isolate a mutant (named Inv-3) that gives uninducible invertase expression regardless of whether or not glucose is present. An Inv-3 mutant mated to wild type produces diploids that have uninducible invertase expression. A cross of an Inv-3 mutant to an Inv-1 mutant yields 20 tetrads that are all the same type: 2 uninducible; 2 constitutive. Based on these observations propose a mechanism for the Inv-3 mutation, describing the gene or type of gene that it affects and how the mutation alters gene function. Be as precise as you can and explain your reasoning.

Inv-1 = constitutive
Inv-3 = uninducible (+1)
$\frac{Inv\text{-}3}{+}$ = uninducible ∴ dominant (+1)

(+1) $\frac{Inv\text{-}3 \quad +}{+ \quad Inv\text{-}1}$

↓

U
U
C
C

all PD ∴ Completely linked (+2)

So, Inv-3 is dominant, uninducible, and trans-acting.
From (a) we know that its wt allele is a repressor.
Thus, Inv-3 is a repressor that binds to the operator whether glu is bound or not. (+2)

Since Inv-1 and Inv-3 are completely linked, they are likely alleles of the same gene.

Since Inv-1 is unlinked to Suc1-, Inv-3 is also unlinked to Suc1- and works in trans (+1) with respect to Suc1.

Name: KEY

(d 8 pts.) If you mated an Inv-2 mutant to an Inv-3 mutant, what would you expect the phenotype of the resulting diploid to be? After sporulation of these diploids what types of tetrads would you expect to see? If 24 tetrads were dissected how many of each type would you expect?

$\frac{Inv2\ +}{+\ Inv3}$ = (+2) constitutive — based on below diploid diagram. → sporulate

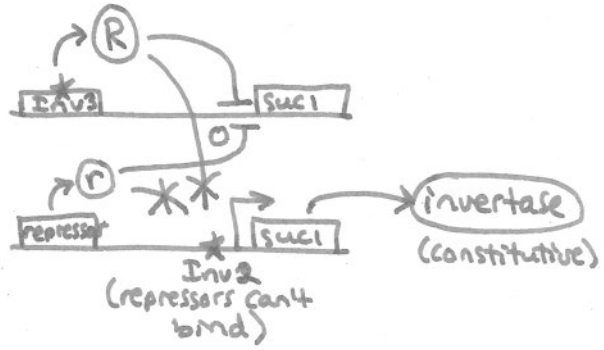
parental
Inv2 + : constitutive
\+ Inv3 : uninducible
recombinant
\+ + : wt (regulated)
Inv2 Inv3 : because Inv2 acts downstream of Inv3, its phenotype wins out in the double mutant (see double mutant diagram lower left)
∴ constitutive (+2)

Inv2 = constitutive
Inv3 = uninducible

R: unregulatable repressor

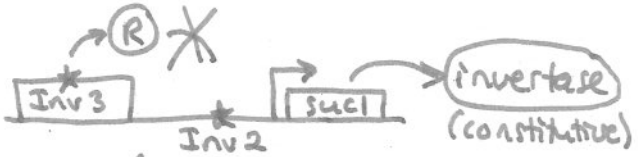
diploid:

r: regulatable repressor

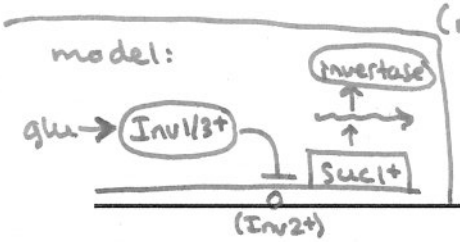


Inv-2 and Inv-3 are unlinked, so there should be a 1:4:1 ratio of PD:TT:NPD. So:

double mutant haploid:



model:



C	C	wt	+3
C	C	wt	
C	C	C	
C	wt	C	
PD	TT	NPD	
4	16	4	+1

Question 1 36 points:_____

Question 2 32 points:_____

Question 3 32 points:_____

Total :_____