Name: _____

7.03 Exam II -- 2015

Name: _____
(write your name on every page of this exam)

Exam starts at 11:05 am and ends at 11:55 am.

Please write your name on each page.

Only writing on the FRONT of every page will be graded. (You may use the backs, but only as scratch paper.)

Question 1	32 pts
Question 2	35 pts
Question 3	33 pts

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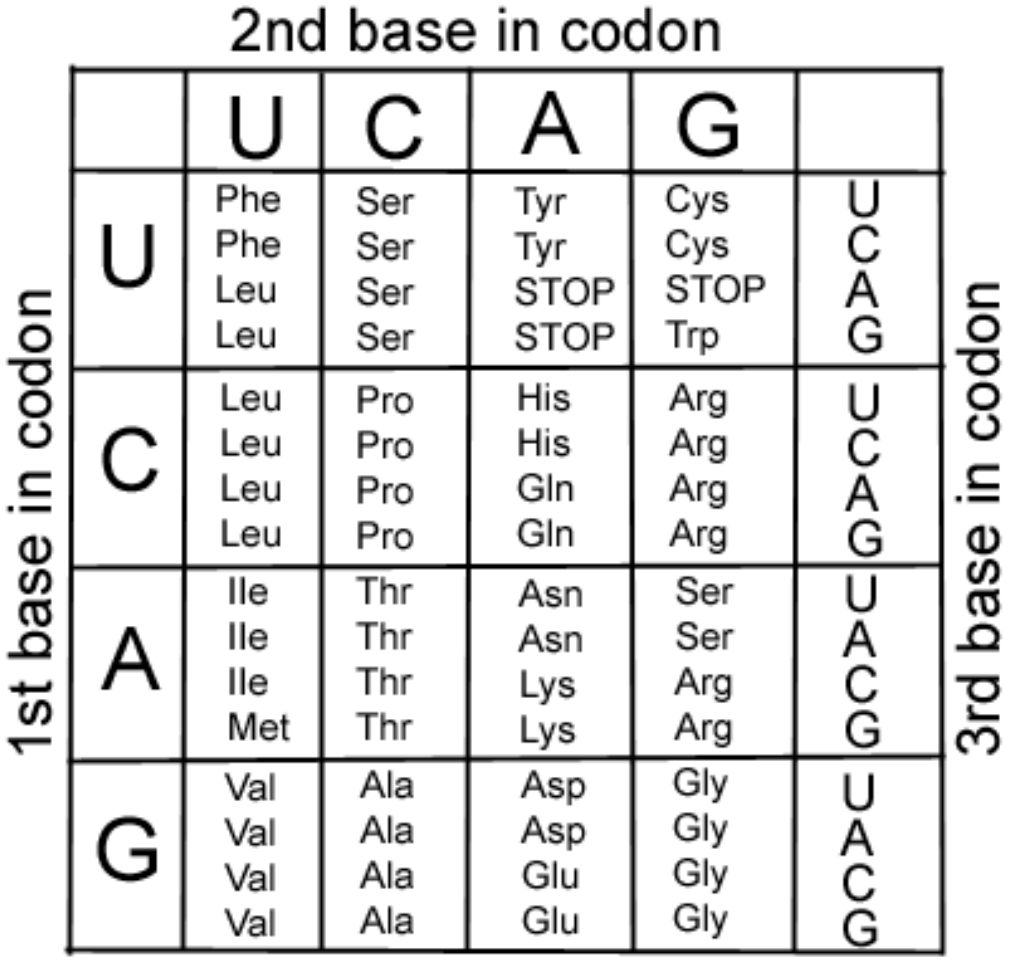
Genetic code



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Question 1 –

(a) (8 pts.**)** Hydroxylamine causes C:G to T:A base pair changes, resulting from **[C to T]** or **[G to A]** mutations. After treatment of lambda phage with hydroxylamin*e* you isolate a mutant that forms tiny plaques on a wild-type *E. coli* host, but large plaques on a host that carries an amb*e*r (UAG) suppressor. List the codons in wild-type lambda phage that could hav*e* been mutated to produce th*e* mutant phage.

Mutated lambda mRNA: 5' UAG 3'

Mutated lambda DNA: 5' TAG 3'

Original lambda: 5' CAG 3' (Gln) [+4]

5' TGG 3' (Trp) [+4]

The G in TAG was not the cause of a mutation, because hydroxylamine can only cause a G to A switch, but not an A to G switch.

-2 points for adding more codons (other than CGG which could be made into TAA w/ hydroxylamine)

(b) (8 pts.**)** Would you expect mutagenesis of the phage mutant described above by treatment of the phage with hydroxylamine to yield revertants that can make large plaques on wild-type *E. coli*? Why or why not?

No [+3]

Hydroxylamine can only cause C to T or G to A changes. Thus 5' TAG 3' can only be changed to TAA. TAA, gives UAA mRNA – a stop codon. So, hydroxylamine cannot cause back mutations. [+5]

No points awarded for stating back mutations are rare. This is true but we could still identify them.

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(c) (16 pts.**)** The following sequence (and encoded amino acids) is contained within the coding sequence of a wild-type *E. coli* gene:

CTC TCT TTC ATG ACT AGG CTG TTT AAG

leu ser phe met thr arg leu phe lys

A mutant is isolated that has an additional A residue giving the sequence:

CTC TCT TTC ATG AC**A**T AGG CTG TTT AAG

Describe a possible suppressor mutation that might revert the defect of the mutation shown above (do not simply describe a back mutation). For your answer:

(i) State whether this is an intragenic or extragenic suppressor

It must be an intragenic suppressor, either a -1 or +2 frameshift, that must occur prior to the inserted G residue following the inserted A (in order to not generate a TAG stop). Note – amber suppressor mutation will not work, as two different stop codons are generated (TAG and TAA) and the amber suppressor is specific for TAG.

[+4 given for any suppressor]

Max of 12/16 points on this question if gave something other than fixing the frameshift as long as consistent between (i) and (ii) and gave correct answer in (iv)

(ii) show the exact sequence change that gives the suppressor mutation

Deletion of any of the underlined bases or insertion of 2 bases before the G of the TAG would work to restore the frame.

CTC TCT TTC ATG ACAT AGG CTG TTT AAG

For example, the "C" deletion would cause the following:

CTC TCT TTC ATG AAT AGG CTG TTT AAG

[+4 for any sequence consistent with answer in (i)]

(iii) give the amino acid sequence of the mutant gene sequence with the suppressor, and:

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If deleted A → ATG CAT AGG…

leu ser phe met his arg leu phe lys

If deleted C → ATG AAT AGG…

leu ser phe met asn arg leu phe lys

If deleted T → ATG ACA AAG…

leu ser phe met thr arg leu phe lys

If deleted A → AGT ACA TGG CTG…

leu ser phe met thr trp leu phe lys

[+ 4 for one of these sequences]

No points awarded for changes that did not restore frameshift

(iv) describe any properties that this part of the protein sequence must have in order for the suppressor to restore function to the mutated gene.

The mutation must not lie in a region of the protein necessary for catalysis or protein folding. [+4]

If deleted the T and so recovered WT at the amino acid level, [+4] given for stating that there are no specific properties required

If did not restore frameshift, [+4] given for stating that it must be at the end of the protein so as to not to interfere with structure/ catalytic activity

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Question 2 –

A segment of the *E. coli* chromosome carries the genes RecA, LeuB, and MotC. Phage P1 is grown on wild type *E. Coli* and the resulting phage are used to infect a recA-, leuB-, and motC- recipient strain that is Amp^R. Rec+ transductants are selected and then tested for the presence of the other markers. The phenotypes of 100 Rec+ transductants are given below.

Phenotype	Number
Leu+ Mot+	48
Leu- Mot+	2
Leu- Mot-	50

(a) (10 pts.) Draw a map giving the relative order and the cotransductional frequencies between the genes RecA, LeuB, and MotC.

RecA ---------- MotC ---------- LeuB [+5]

50% (between RecA and MotC) [+2.5]

48% (between RecA and LeuB) [+2.5]

They don't need to list the LeuB to MotC distance

-2.5 if LeuB placed in middle
-5 if RecA placed in middle

(b) (5 pts.) You have isolated an Hfr derivative of wild type *E. Coli* that transfers the RecA marker early and LeuB and MotC markers late. Draw a diagram of the Hfr showing where F is inserted and its orientation (you don't need to show Amp^R).

RecA ------ ;;;;;; ----- > ------ ;;;;;;; ---------- MotC --------- LeuB

(The greater than arrow is the Ori transfer and the semicolons are insertion sequences)

[+ 2.5 Insertion Location]

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[+2.5 oriT orientation]

[-4 if all genes placed within F plasmid insertion sequences]

(c) (5 pts.) In a cotransduction mapping experiment, P1 phage is grown on the Hfr strain described in **(b)**. These phage are then used to infect a leuB-, motC- recipient strain. Would you expect the cotransduction frequency of Leu+ and Mot+ to be greater than or less than 50%? Why?

Greater than 50%. [+2.5]

We know from A that LeuB and MotC are near each other. We also know that the plasmid did not integrate between LeuB and MotC. [+2.5]

(d) (5 pts.) How would you select for an F' Leu+ and Mot+ strain derived from the Hfr strain described in part b? (Be sure to give the genotypes of any strains that you would use).

Mate the Hfr with an F^- Leu- and Mot- strain that expresses another antibiotic resistance gene (like Kan) Select for strains that are Leu+, Mot+ and Kan^R after a short mating.

Here, the short mating is critical to prevent whole chromosome transfer. The resistance gene of of minor importance.

[+2 mating to F- leu- mot-]
[+2 short mating]
[+1 other antibiotic resistance]

[-4 for using phage transduction]
[-5 for any media plating without crossing to other bacterial strain]
[-4 if Leu+ was placed on donor F']

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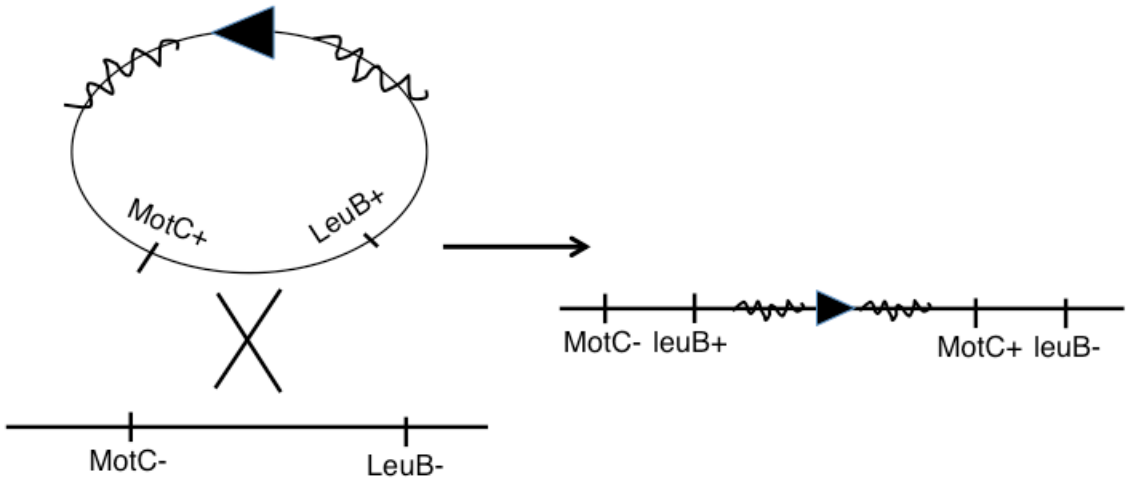
(e) (10 pts.) An F' carrying the LeuB+ and MotC+ markers is isolated from the Hfr strain described in part b. You introduce this F' into a leuB- motC- strain and then isolate an Hfr that can transfer RecA+ efficiently. This Hfr transfers LeuB+ early but transfers Mot+ late. Draw a diagram of the recombination event that produced this Hfr.



[+2 for drawing a recombination event]
[+2 for the final result drawn giving the observed phenotype]
[+2 for the result of the drawn recombination event giving the observed phenotype]
[+3 for drawing the correct, single event from plasmid to genome]
[+1 for proper location of recombination event]

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Question 3

The hexose transporter genes (HXTs) are all regulated by the level of glucose in the media. They are expressed in the presence of glucose but not expressed otherwise. We can graphically depict this as

Glucose -----> HXT

(a **2 points**) Explain in <u>one short sentence</u> the likely advantage of this regulation.

Since glucose is a hexose, the cell saves energy by only expressing HXTs when glucose is present to transport.

You decide to use a genetic strategy to discover some of the pathways regulating this process. You choose one of the HXT genes, HXT1, and use a reporter assay, combining the HXT1 promoter with a lacZ reporter. Using this assay you isolate two mutants: rgt1- and mth1- with the following phenotypes:

	HXK1 reporter expression		Interpretation
	No glucose	With glucose	
WT	white	blue	regulated
rgt1-	blue	blue	constitutive
mth1-	blue	blue	constitutive

(b **3 points**) Fill in the interpretation column in the table above to describe each strain's phenotype. In each case, choose from either 'regulated', 'uninducible', or 'constitutive'.

[1 point for each]

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(c **4 points**) You perform tetrad analysis and determine that neither RGT1 nor MTH1 are linked to HXT1. Given this and the information in the table, draw two regulatory diagrams: one connecting glucose, RTG1 and HXT1, and the other connecting glucose, MTH1 and HXT1.

(Recall that a diagram between galactose, GAL80 and GAL1 looks like: Galactose → GAL80 --l GAL1).

Diagram I: glucose, RTG1 and HXT1

glucose --l RTG1 --l HXT1

Diagram II: glucose, MTH1 and HXT1

glucose --l MTH1 --l HXT1

[+1 for each correct arrow/bar]

(d **6 points**) You would now want to determine how RTG1 and MTH1 are ordered. Assuming a single linear pathway from glucose to HXT1, draw the two possible models connecting glucose, MTH1, RTG1 and HXT1.

Model I:

Glucose --l MTH1 → RTG1 --l HXT1

-1 wrong sign on one arrow

Model II:

Glucose --l RTG1 → MTH1 --l HXT1

-1 wrong sign on one arrow

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(e 2 points) Unfortunately, you quickly realize that you cannot determine with the mutants in hand which of the models is the correct one. Why? Explain in one short sentence.

Can't do epistasis test because single mutants have the same phenotype.

(f 3 points) Fortunately, you next obtain one additional mutant, mth1*, which is very tightly linked (in same gene) to mth1-, and has the following phenotype:

	HXK1 reporter expression (lacZ)		Interpretation
	No glucose	With glucose	
mth1*	white	white	uninducible [1 pt]

Is this mutant constitutive or uninducible? Fill in the interpretation column in the table. Why can you now use this mutant to help determine which of the models in (d) is correct? Explain in one short sentence.

2 points – can do epistasis test because mth1* and rtg1- have different phenotypes

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(g 3 points)

To order the MTH1 and RGT1 in a linear pathway, you now mate the mth1* mutant with the rgt1- mutant, resulting in a diploid mth1* RGT1+ / MTH1+ rgt1- that shows normal regulation of the reporter. You sporulate the diploid and analyze 180 tetrads, obtaining the following results:

A: 31 tetrads		B: 27 tetrads		C: 122 tetrads	
ß-galactosidase activity		ß-galactosidase activity		ß-galactosidase activity	
-glucose	+ glucose	- glucose	+ glucose	- glucose	+ glucose
blue	blue	white	white	blue	blue
blue	blue	white	white	white	white
white	blue	blue	blue	blue	blue
white	blue	blue	blue	white	blue

Assign tetrads A, B, C to the Parental Ditype, Tetratype, and Non-Parental Ditype categories.

A = NPD
B = PD
C = TT

[1 point/ correct category]

(h **4 points**) What is the phenotype of the double mutant mth1*rgt1-? Identify the strain(s) in the tetrad(s) from which you inferred this (e.g., by circling it on the table above).

Double mutant is constitutive [2 points]

Correct one – either 2 in NPD or one in TT [2 points]

-1 if circled whole tetrad
-2 if identified WT spores as double mutant

(i 6 points) Which of the models in (d) is the one consistent with the results in (g)? Write the model here.
Glucose --| MTH1 → RTG1 --| HXT1

[-3 if inconsistent model with part h]