Your Name: _____ **TA:**_____

7.012 Quiz 2 Answers

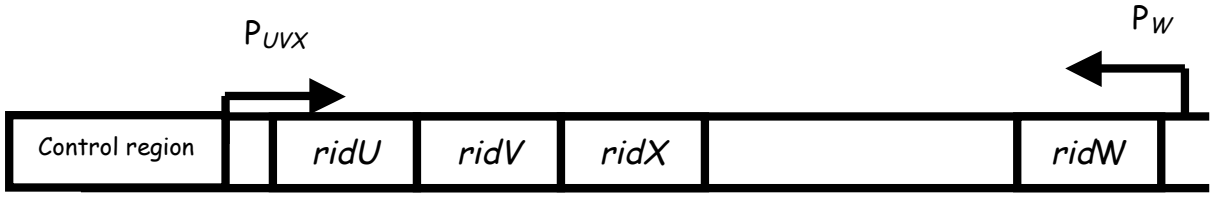
A≥85	**~12% of test takers**
B≥72	**~31.2% of test takers**
C≥60	**~34.1% of test takers**
D≥50	**~16.3% of test takers**
F≥49	**~6.2% of test takers**

Regrade requests (with a note attached indicating the problem and part you want looked at) accepted until Thursday November 4th, 5pm.

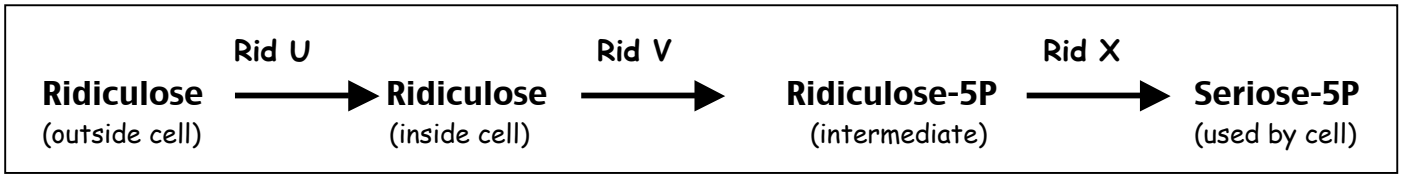
Question	Value	Score
1	17	
2	16	
3	30	
4	17	
5	20	
	100	

Question 1

In the bacterium *Funditus fabricatus,* the metabolism of the sugar ridiculose is dependent on the *rid* operon shown below.



The ridiculose operon encodes the enzymes shown in the following pathway.



The *ridW* gene is constitutively expressed. The expression of *ridU, ridV*, and *ridX* genes is off in the absence of ridiculose and is **activated** by the product of the *ridW* gene in the presence of ridiculose.

There is an artificial inducer of *ridUVX* expression, called GIG-L, and an artificial substrate for Rid X, called STRN that turns **red** in the presence of active Rid X protein.

a) Several specific Rid- mutants of *F. fabricatus* are shown below. Predict their phenotypes when grown in the presence of STRN, with and without the addition of the inducer, GIG-L. **10 pts**

(Fill in the chart with either RED or WHITE)

Strain of *F. Fabricatus*	**+ GIG-L**	**- GIG-L**
WT	RED	WHITE
M1 (deletion of P_{UVX})	White	White
M2 (control region that can't bind RidW protein)	White	White
M3 (RidW protein that can't bind ridiculose)	White	White
M4 (nonsense mutation early in *ridX*)	White	White
M1337 (RidW protein that always binds control region)	Red	Red

Name:_____ TA:_____

b) Predict whether the following *F. fabricatus* strains, that are merodiploid for the ridiculose operon, will grow on minimal media with or without ridiculose as the **only** carbon source.

Fill in the chart with **YES** if the merodiploid will GROW or

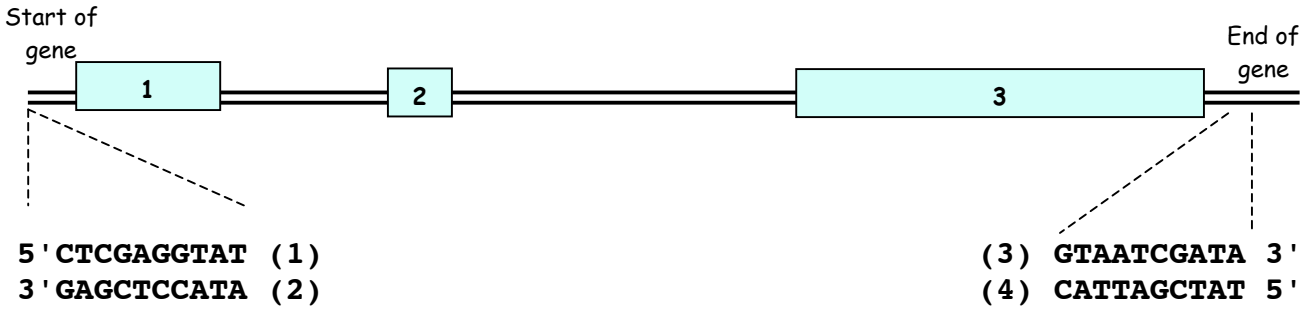
NO if the merodiploid will NOT GROW

7 pts (5 points for the first column, 2 pts for entire last column)

Merodiploid	**+Ridiculose**	**- Ridiculose**
WT / WT	YES	NO
M1 / M2 (deletion of P_{UVX}) (control region that can't bind RidW)	NO	NO
M2/ M3 (control region that can't bind RidW) (RidW protein that can't bind ridiculose)	YES	NO
M3 / M1337 (RidW protein that can't bind ridiculose) (RidW protein that always binds control region)	YES	NO
M4 / M 1 (nonsense mutation early in *ridX*) (deletion of P_{UVX})	NO	NO
M1337 / M4 (RidW protein that always binds control region) (nonsense mutation early in *ridX*)	YES	NO

Question 2

You believe that a disruption in a gene, *sokS*, may contribute to an interesting disease phenotype in cardinals. You wish to PCR amplify and sequence *sokS* from both wild type and diseased cardinals. The *sokS* gene is shown below. Exons are represented by numbered boxes and the terminal sequences are depicted in bold.



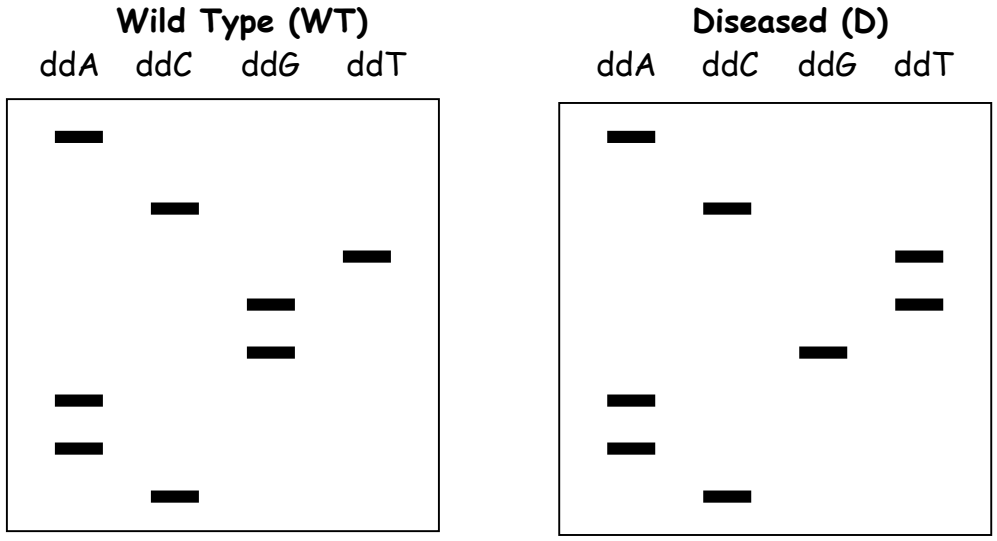
a) For PCR amplification, the primers should be identical to which of the following sequences? (Circle one.) 3 pts

1 and 3 2 and 4 [1 and 4] 2 and 3

b) Which primer(s) should you use in **one** sequencing reaction to obtain sequence that looks most similar to the mRNA (the coding strand)? 2 pts

[1] 2 3 4

You use the dideoxy sequencing method to sequence your PCR products. You see the following pattern in the sequencing gel representing the sequence spanning the end of the first intron and the beginning of exon 2.



c) These gels correspond to which WT and diseased sequences respectively? (WT, D) (Circle i, ii, iii, or iv.) 4 pts

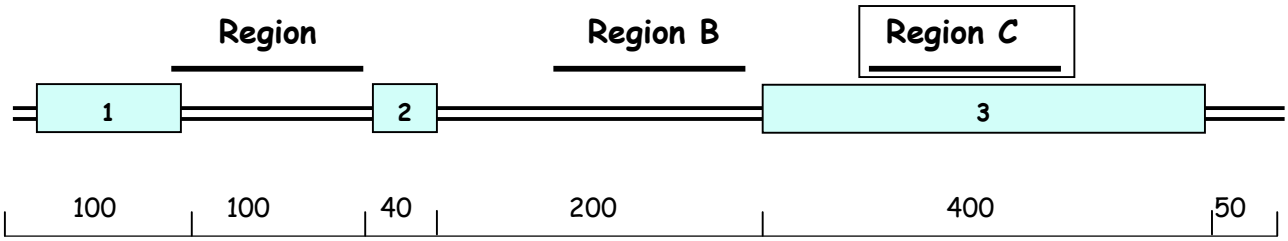
i) 5'-ACTGGAAC-3', 5'-ACTTGAAC-3' ii) 5'-TGACCTTG-3', 5'-TGAACTTG-3'

[iii)] 5'-CAAGGTCA-3', 5'-CAAGTTCA-3' iv) 5'-GTTCCAGT-3', 5'-5'-GTTCAAGT-3'

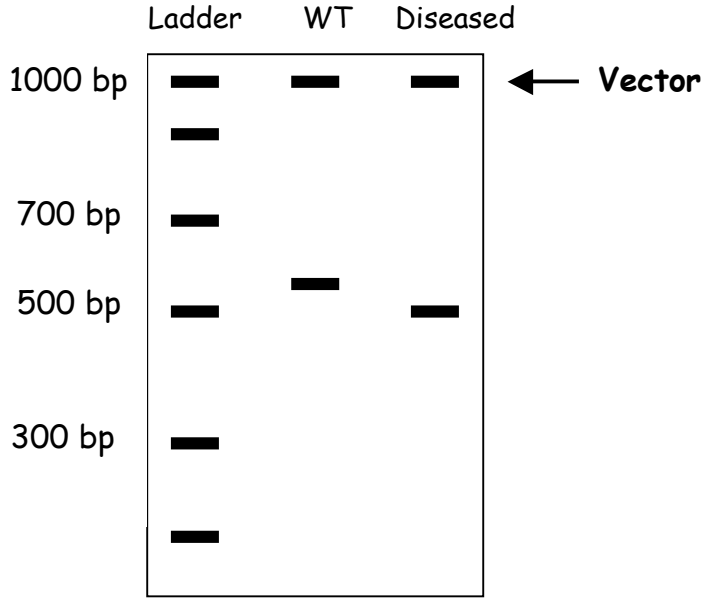
Name:_____ TA:_____

To determine how this mutated sequence might affect the mRNA and the protein product, you obtain two cDNA libraries: one derived from wild type cardinal RNA and one derived from diseased cardinal RNA.

d) You choose to use a radioactively labeled DNA probe to screen these cDNA libraries for clones containing *sokS*. Which region of *sokS* would make the **best** probe for screening the available cDNA libraries? (Circle either Region A, Region B, or Region C.) 4 pts



Your probe hybridizes to cDNA clones in both cDNA libraries. You purify the plasmids from single clones and cut them with the restriction enzyme used for cloning to verify insert size. The gel is shown below.



e) Based on all evidence above, what is the **most likely** explanation for the difference in restriction enzyme digestion patterns of the *sokS* cDNA clones? 3 pts

i) A mutation in the *sokS* cDNA from the diseased cardinal cDNA library gives rise to an additional restriction enzyme site.

ii) The mRNA encoded by the *sokS* gene from the diseased cardinal is incorrectly spliced.

iii) There is likely a problem with the gel and it should be rerun.

iv) The *sokS* gene from the diseased cardinal acquired a spontaneous insertion.

v) The mRNA encoded by the *sokS* gene from the diseased cardinal has no poly A tail.

Question 3

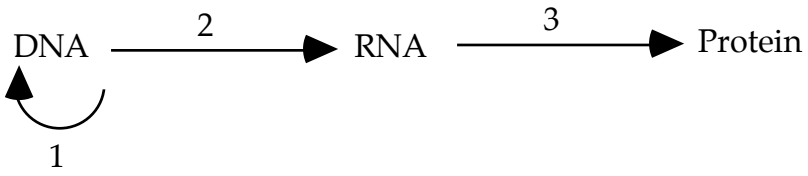
a) Match the following. Choose **only one** answer for each blank below. 10 pts

A. Unwinds DNA
B. Where Okazaki fragments are synthesized
C. Where DNA can be replicated continuously
D. Synthesizes RNA primers on DNA
E. Synthesizes DNA primers on RNA
F. Catalyzes the addition of dNTPs to lipids
G. Relieves tension in DNA caused by unwinding
H. 5′ to 3′ proofreading activity
I. 3′ to 5′ proofreading activity

__B__ lagging strand __I__ DNA polymerase __G__ topoisomerase

__D__ primase __A__ helicase

b) Where in the eukaryotic cell does the following processes of the central dogma occur? One word answer for each, and please write legibly. 6 pts



Process 1____nucleus_____

Process 2_____nucleus_____

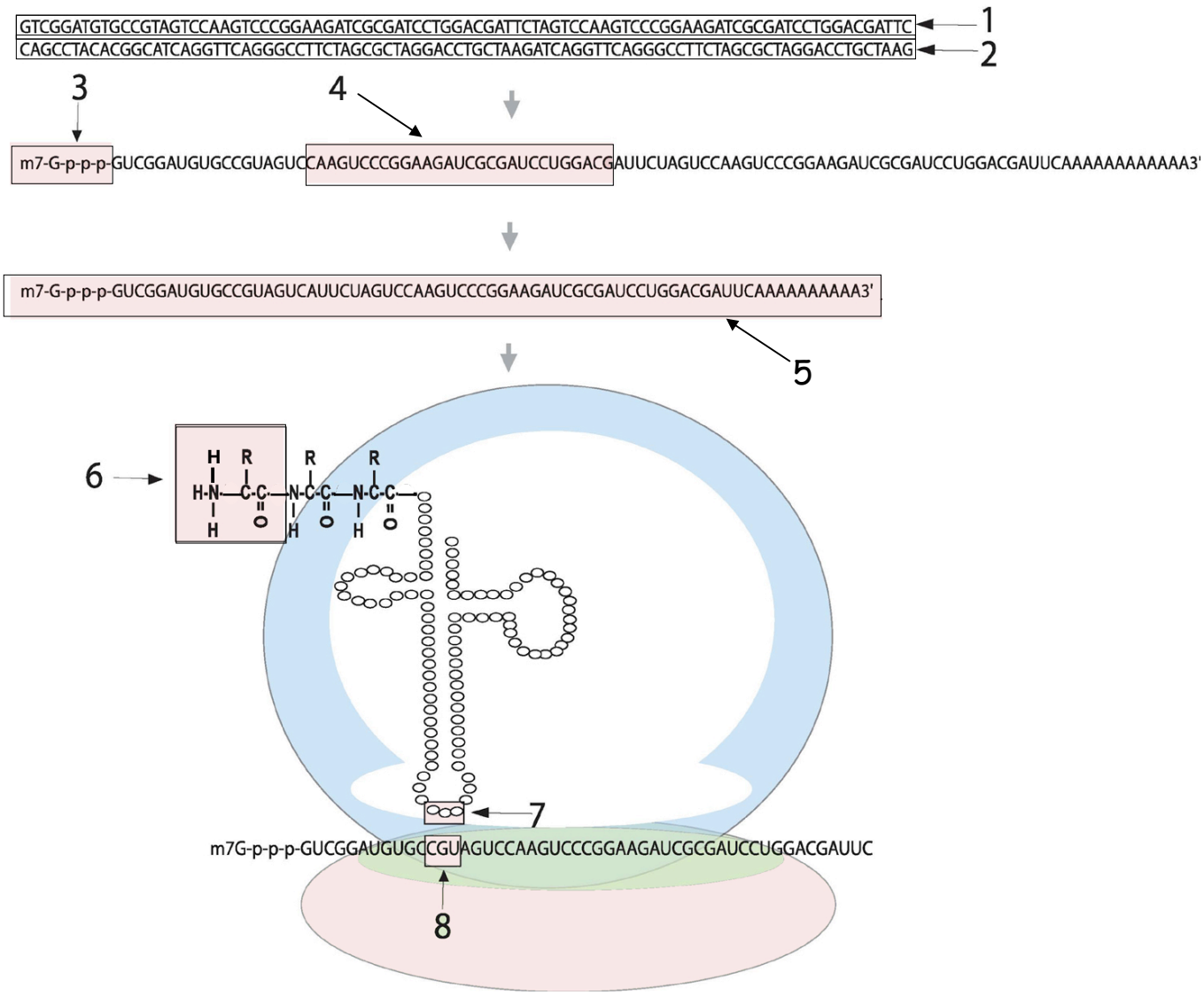
Process 3_____cytoplasm_____

Name:_____ TA:_____

c) Below are schematics of transcription, splicing and translation. Match the numbered boxes with the following terms. Use each number only once. It's okay to leave blanks.

8 pts

__3_ 5' cap	_____3' Cap	__6_ Amino acid	_7__ Anti-codon
__1__ Coding (Non-template) strand		__8_ Codon	____ Exon
__4__Intron		__5_ mature mRNA	____ PolyA tail
____ Pre-mature mRNA		__2__ Template strand	____ tRNA



d) In the box below, write the sequence that would be in box 7 above. Designate the 5' and 3' orientations. 3 pts

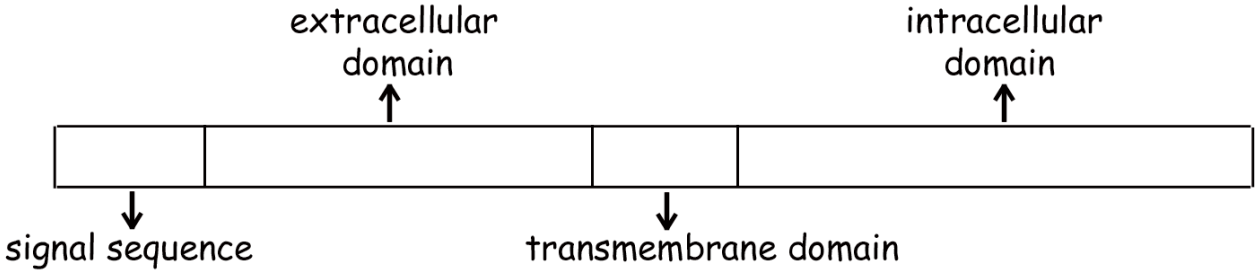
5' – ACG – 3'

e) Circle the **specific** name of the structure that is in box 6 above. 3 pts.

Alanine Arginine Cysteine Cytosine Cyanide Methionine Threonine Uracil

Question 4

Below is a diagram of a transmembrane protein called Soxwin.



Normally Soxwin is found embedded in the plasma membrane. You obtain a mutant in which Soxwin is mislocalized. The mutation resides in the DNA that encodes the transmembrane domain.

coding sequence in WT:
…GTTATATTTCTCGTATGGCTCGGCGTCTTATGG…
val ile phe leu val trp leu gly val leu trp

coding sequence in mutant:
…GTTATATGTTCTCGTATGGCTCGGCGTCTTATGG…
val ile cys ser arg met ala arg arg leu met

a) What type of mutation occurred in the *soxwin* gene? Circle your answer(s). 2pts

deletion [frameshift] [insertion] missense nonsense silent

b) How has this mutation changed the **chemical property** of the transmembrane domain? Fill in each blank with **one** word. 2pts

From __**hydrophobic, non-polar**_ in WT to _**hydrophilic, polar, or charged**__ in mutant

c) Where do you expect the majority of the mutant Soxwin to accumulate? Circle your answer. 3 pts

cytoplasm endoplasmic reticulum golgi apparatus
mitochondria nucleus [outside the cell]
peroxisomes plasma membrane ribosomes

Name:_____ TA:_____

d) There's another *soxwin* mutant in which a missense mutation abolishes the function of the signal sequence. Where would you expect the majority of this mutant Soxwin protein to accumulate? Circle your answer. 3 pts

cytoplasm endoplasmic reticulum golgi apparatus

mitochondria nucleus outside the cell

peroxisomes plasma membrane ribosomes

e) Match the following. (Multiple answers may be chosen for each blank.)

7 pts

Destinations of proteins	Molecular events
____C___ cytoplasm	A. co-translational transport
____B, C___ mitochondria	B. post-translational transport
____B,C___nucleus	C. entire protein synthesized on a free ribosome
____A,D___ extracellular space	D. signal sequence recognized by SRP

Question 5

a) A plasmid is a… (Circle your answer(s).) 2pts

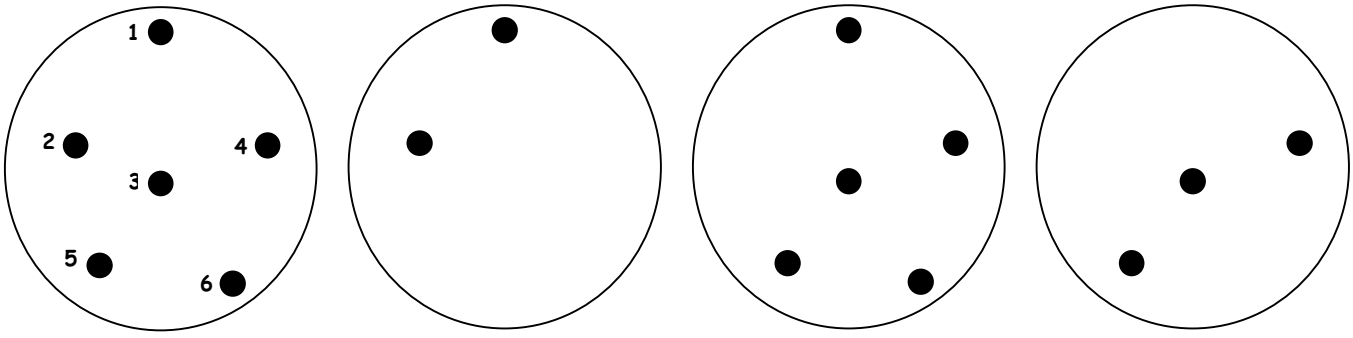
bacterium | circular piece of DNA | cell | multipurpose enzyme | petri plate | vesicle

b) Match each vector feature with its function. Not all answers need be used. 8 pts

____B____Restriction site	A) Required for expression of insert
	B) Allows for insertion of DNA into vector
____E____Origin of replication	C) Encodes an enzyme to cut DNA
	D) Enables selectability for strain that has taken up the vector
____A____Promoter	E) Required for duplication of vector
	F) Required for SRP to bind
____D____Drug resistance	G) Site for ribosome to bind

c) To obtain the gene that rescues a tryptophan biosynthesis *E.coli* mutant strain named, NY-*trp*-Zup, you construct a genomic library from wild-type *E.coli* by cutting the genome with *Bam*HI and inserting the fragments into *p*GoSOX!, a plasmid which has been very successful in the lab. *p*GoSox! contains the genes for tetracycline and kanamycin resistances and has a unique *Bam* HI restriction site that maps to the kanamycin resistance gene. You transform the library into NY-*trp*-Zup and plate the transformants onto rich agar medium. You replica plate the colonies onto different media shown below.

Below are the plates shown in the same orientation after colonies form.



Rich Medium | Minimal medium (lacks tryptophan) | Rich +Tetracycline | Rich +Kanamycin

i) Which colony (ies) contain the **original** *p*GoSOX!? 3 pts 1@

None | **1** | **2** | **3** | **4** | **5** | **6**

ii) Which colony (ies) carry *p*GoSOX! containing an insert? 4 pts 2@

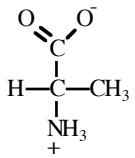
None | **1** | **2** | **3** | **4** | **5** | **6**

iii) Which colony (ies) would you choose to further study the gene encoding the tryptophan biosynthetic enzyme that is deficient in NY-*trp*-Zup? 3 pts 3@

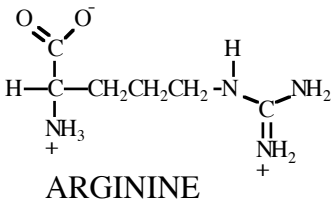
None | **1** | **2** | **3** | **4** | **5** | **6**

Name:_____ TA:_____

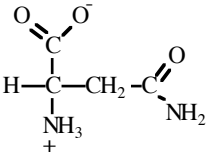
STRUCTURES OF AMINO ACIDS at pH 7.0



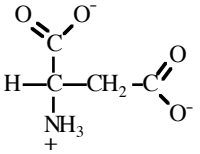
ALANINE
(ala)



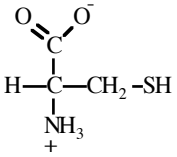
ARGININE
(arg)



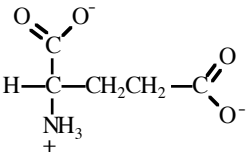
ASPARAGINE
(asn)



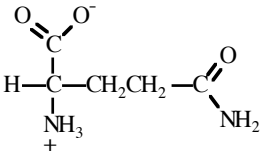
ASPARTIC ACID
(asp)



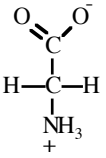
CYSTEINE
(cys)



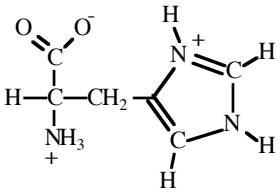
GLUTAMIC ACID
(glu)



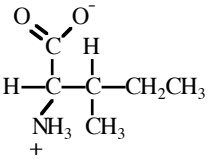
GLUTAMINE
(gln)



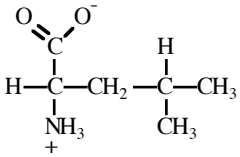
GLYCINE
(gly)



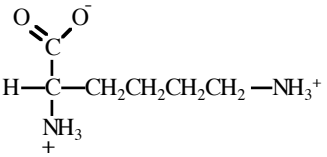
HISTIDINE
(his)



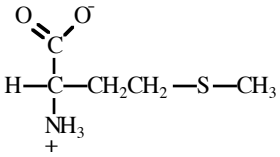
ISOLEUCINE
(ile)



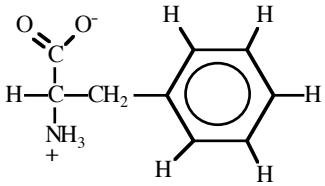
LEUCINE
(leu)



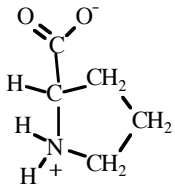
LYSINE
(lys)



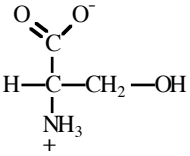
METHIONINE
(met)



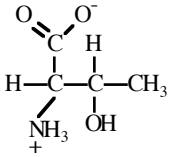
PHENYLALANINE
(phe)



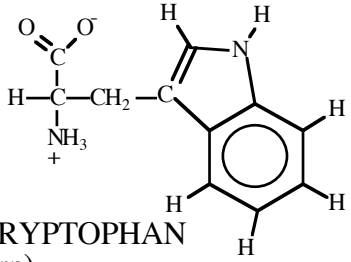
PROLINE
(pro)



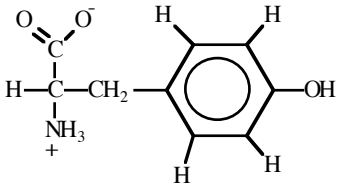
SERINE
(ser)



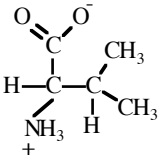
THREONINE
(thr)



TRYPTOPHAN
(trp)



TYROSINE
(tyr)



VALINE
(val)

The Genetic Code

	U	C	A	G	
U	UUU **phe (F)**	UCU **ser (S)**	UAU **tyr (Y)**	UGU **cys (C)**	U
	UUC **phe (F)**	UCC **ser (S)**	UAC **tyr (Y)**	UGC **cys (C)**	C
	UUA **leu (L)**	UCA **ser (S)**	**UAA STOP**	**UGA STOP**	A
	UUG **leu (L)**	UCG **ser (S)**	**UAG STOP**	UGG **trp (W)**	G
C	CUU **leu (L)**	CCU **pro (P)**	CAU **his (H)**	CGU **arg (R)**	U
	CUC **leu (L)**	CCC **pro (P)**	CAC **his (H)**	CGC **arg (R)**	C
	CUA **leu (L)**	CCA **pro (P)**	CAA **gln (Q)**	CGA **arg (R)**	A
	CUG **leu (L)**	CCG **pro (P)**	CAG **gln (Q)**	CGG **arg (R)**	G
A	AUU **ile (I)**	ACU **thr (T)**	AAU **asn (N)**	AGU **ser (S)**	U
	AUC **ile (I)**	ACC **thr (T)**	AAC **asn (N)**	AGC **ser (S)**	C
	AUA **ile (I)**	ACA **thr (T)**	AAA **lys (K)**	AGA **arg (R)**	A
	AUG met (M)	ACG **thr (T)**	AAG **lys (K)**	AGG **arg (R)**	G
G	GUU **val (V)**	GCU **ala (A)**	GAU **asp (D)**	GGU **gly (G)**	U
	GUC **val (V)**	GCC **ala (A)**	GAC **asp (D)**	GGC **gly (G)**	C
	GUA **val (V)**	GCA **ala (A)**	GAA **glu (E)**	GGA **gly (G)**	A
	GUG **val (V)**	GCG **ala (A)**	GAG **glu (E)**	GGG **gly (G)**	G