

DO NOT OPEN UNTIL TOLD TO START
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Genetics 466
November 4, 2008 - Second Exam

Name (print neatly) _____ **ANSWER KEY** _____

10 Digit Student # _____

Signature _____

Page 2	Page 3	Page 4	Page 5	
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Page 6	Page 7	Page 8	Page 9	
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Total

INSTRUCTIONS:

1. There are 10 pages (9 questions) on the exam. *Make sure you have all the pages.*
2. Be sure to provide your student information above.
3. Make your answer clear. Show your work.
4. The last page contains the genetic code, you may tear it off if that is convenient in helping you solve certain problems.
5. If you don't understand what is being asked, raise your hand and ask for help.
6. You have two hours.

1. (9 pts)

The following questions concern the investigations that led to our knowledge of DNA as the hereditary material, and its replication.

a) (2 pts) Name and briefly describe two clues that were crucial to Watson and Crick's correct model for the structure of DNA?

1. X-Ray Diffraction studies indicated helical structure
2. Chargaff's "rules" that in DNA
 content of $[A] = [T]$
 of $[C] = [G]$

b) (2 pts) If the cytosine content of a species DNA is 22%, then what is the thymine

content? 28 %

$$C = 22 \quad A + T = 100 - 44 = 56$$

$$G = 22 \quad A = T \quad T = 28$$

c) (5 pts) Fill in the blanks:

DNA is held together by hydrogen bonds which are broken by the enzyme called

helicase in the process of replication. During DNA replication, the

lagging strand is said to undergo discontinuous replication in which short

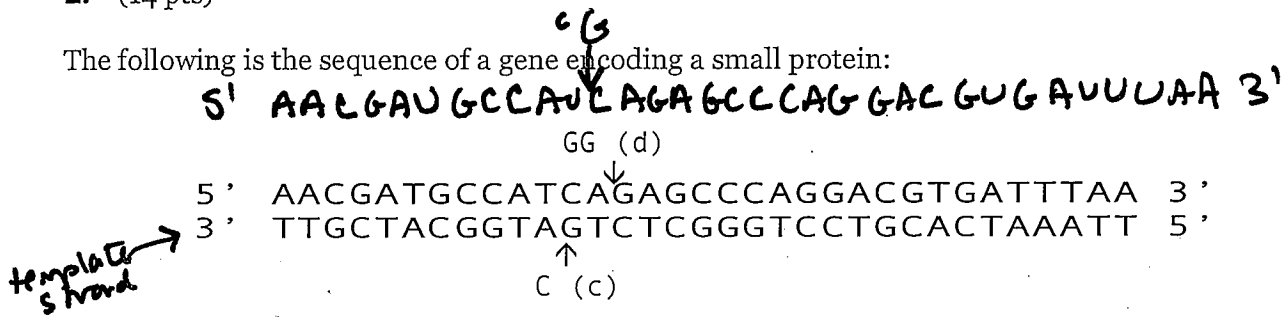
fragments must be synthesized and then ligated to complete the strand.

In eukaryotes, the replication of the ends of chromosomes is maintained by the enzyme

telomerase.

2. (14 pts)

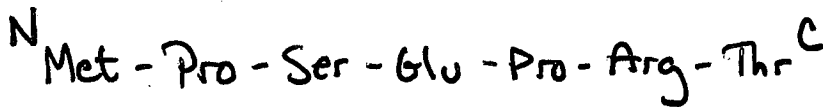
The following is the sequence of a gene encoding a small protein:



a) (3 pts) If transcription proceeds from left to right and begins at the first base shown, what is the sequence of the mRNA from this gene? Label the 5' and 3' ends.

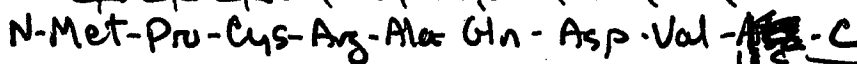
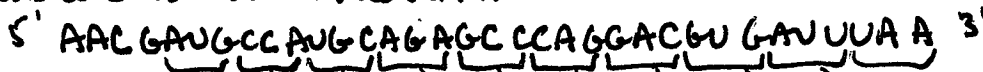


b) (3 pts) Assume translation requires an initiation codon and begins at the first one encountered, what is the protein sequence encoded by the above mRNA? Label the C and N termini.

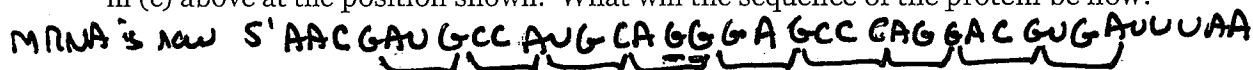


c) (4 pts) Suppose that a C becomes permanently inserted into the position shown due to a mistake during replication. What will the sequence of the protein be now?

means a G is inserted into mRNA



d) (4 pts) Suppose that an additional two G residues become inserted into the mutant gene in (c) above at the position shown. What will the sequence of the protein be now?



3. (11 pts)

Fill in the following table with the appropriate DNA, mRNA, or tRNA bases and the correct amino acids. Assume that transcription and translation are from left to right. Do not concern yourself with "wobble" rules.

GG means original frame

A	T	G	T	T	T	T	G	G	T	A	G	DNA double helix
T	A	C	A	A	A	A	C	C	A	T	C	
A	U	G	U	U	U	U	G	G	U	A	G	mRNA transcribed
U	A	C	A	A	A	A	C	C	A	U	C	tRNA anticodon
M	e	t	P	H	E	T	R	P	-	-	-	amino acids of protein

4. (10 pts)

The following questions concern experiments involved in cracking the genetic code and properties of the translation process.

- a) (2pts) One approach to deciphering the code was to mix bases in different ratios and to observe the ratio of amino acids incorporated into proteins. For example, when U was mixed with G in a 3:1 ratio, the ratio of phenylalanine: leucine was 3:1. What does this reveal about possible sequences encoding leucine?

Since UUU is Phenylalanine

and the base ratio of 3:1 (U:G) means that

$3/4 \times 3/4 \times 3/4 = 27/64$ of protein is UUU = Phe, then
 leu must be $9/64$ or $3/4 \cdot 3/4 \cdot 1/4$ and some permutation of

GUU
 UUG or
 UGU

- b) (2pts) What technical innovation was required so that the specific meaning of each codon could be deciphered?

The ability to synthesize trinucleotides of specific sequences.

- c) (6pts) Fill in the blanks to complete these statements about translation (1/2 pt each)

In bacteria, the first tRNA brought to the ribosome to initiate translation is for the amino acid

f-Met

. This tRNA enters the P site; its positioning is facilitated by the binding of 16S rRNA in the small subunit of the ribosome to the Shine-Delgarno sequence or 5' end of

the mRNA. The second tRNA enters the ribosome via the A site. The first

peptide bond is formed between the carboxyl group of the first amino acid in

the P site and the amino group of the second amino acid. After peptide bond

formation, the tRNAs must move in order for translation of the next codon to proceed, a process

called translocation. There are fewer tRNAs than there are codons because of a principle

called wobble, where certain bases such as the modified base inosine can

pair with more than one codon. Once a tRNA has transferred the growing peptide chain to the

next tRNA, it leaves the ribosome via the exit site, and can then be recycled for use

again.

5. (12 pts)

In cells of the following genotypes, indicate whether *lac* mRNA and β -galactosidase are made. *lac* operon genes are indicated by the symbols IPOZY. Enter a "+" or a "-" to indicate whether the mRNA or enzyme are produced in the absence or presence of lactose as the sole carbon source in the medium.

Genotype	<i>lac</i> mRNA		β -galactosidase enzyme	
	no lactose	lactose	no lactose	lactose
Haploid strains				
I-P ⁺ O ⁺ Z ⁺ Y ⁺	+	+	+	+
I ^S P ⁺ O ^c Z ⁻ Y ⁺	+	+	-	-
I ⁺ P ⁻ O ⁺ Z ⁺ Y ⁺	-	-	-	-
Diploid Strains				
<u>I-P-O^cZ⁻Y⁺</u> I ⁺ P ⁺ O ⁺ Z ⁻ Y ⁺	-	+	-	-
<u>I^SP⁺O⁺Z⁺Y⁺</u> I ^S P ⁺ O ^c Z ⁻ Y ⁺	+	+	-	-
Triploid Strain				
<u>I-P⁺O⁺Z⁻Y⁺</u> <u>I^SP⁺O^cZ⁺Y⁺</u> I ⁺ P ⁻ O ⁺ Z ⁺ Y ⁺	+	+	+	+

6. (18 pts)

Some short answer questions about genetic switches.

- a) (2 pts) A bacteriophage λ (lambda) has a temperature-sensitive mutation in its *cro* gene such that the *cro* protein is active at 30°C but inactive at 42°C. Which pathway will the bacteriophage follow in a nutrient rich medium at 30°C lysis? At 42°C lysogeny? (lysogeny or lysis?)

- b) (2 pts) A bacteriophage λ (lambda) infecting a host cell in a rich medium expresses the *cI* gene even though it has a wild-type *cro* gene. Offer one short explanation for how this situation can occur.

Cro can't repress *cI* because of a mutation in the *cro* binding site in O_R3 . If *cro* can't bind, it can't repress *cI*, *cI* represses *cro* and establishes lysogeny.

- c) (2 pts) A strain of *Bacillus subtilis* is isolated that is unable to activate a group of genes in the forespore. What gene might be defective and why?

The sigma subunit σ^F used in forespore formation. If σ^F is mutant, site of forespore genes are not turned on.

- d) (2 pts) What do the *cI*, *cro*, *lacI*, *Hox*, and *Mat α 2* proteins have in common? Be specific and succinct.

They are each DNA-binding proteins with the helix-turn-helix structural motif.

e) (4 pts) Predict and briefly explain the effect on *GAL1* transcription (increase or decrease), in the presence of galactose alone, of the following mutations:

i. Deletion of the *Gal4* DNA-binding domain.

Decrease, *GAL4* can't bind to UAS if DNA-binding domain is gone

ii. Deletion of the *GAL3* gene.

Decrease, because *GAL3* is required to release inhibition of *Gal4* by *Gal80*.

iii. Addition of six *Gal4*-binding sites to the *Gali* UAS element.

Increase, more *Gal4* binding sites will increase ~~amount of~~ *Gal4* occupancy of UAS and further stimulate transcription

iv. Mutations causing the loss of the SWI-SNF histone acetylase activity.

Decrease, the histone acetylase activity is recruited to the UAS by *Gal4* and increases transcription

f) (2 pts) An *a* yeast cell is found that cannot switch mating-type. Name two genes that could be mutated in this cell.

HO endonuclease

the *HM1Δ* cassette

The *SWI* genes

g) (2 pts) Will the level of *trp* operon transcription increase or decrease in a mutant in which the *trp* codons of the leader peptide are deleted (in the presence of tryptophan)?

Increase. The leader region is required for attenuation mechanism in presence of tryptophan - removal eliminates attenuation

h) (2 pts) In homozygous embryos from a *bicoid* mutant female fly, name two classes of gene expression that will be abnormal.

gap genes

pair-rule genes

segment polarity genes

homeotic genes

7. (11 pts)

The following paragraph concerns the roles of regulatory genes in animal development. Fill in the blank with most correct term, phrase, or description. (1 pt each)

The first set of gene products that regulate embryonic development in the fruit fly are so-called maternal effect proteins, produced by the mother, an example of which is the bicoid, nanos protein, which is expressed in a concentration gradient. The identities of individual or groups of body segments are controlled by the Hox (homeotic) genes; each of these eight genes contains a homeobox, a 180 bp segment of DNA that encodes a protein domain that binds to DNA (does what?). Vertebrates have 38 of these genes which are organized in four (how many?) clusters and expressed in domains along the anteroposterior axis of vertebrate embryos. Most members of the genetic toolkit that governs animal development encode transcription factors or members of signaling pathways (what classes of proteins) and therefore directly or indirectly control the expression of other genes. Many of these genes have diverse roles in the development of individual species, such as the (Sonic) hedgehog gene (give an example) and mutations in the human gene cause defects such as

polydactyly (give an example).
Cancer
holoprosencephaly

8. (8 pts)

You are part of a team conducting a screen for genes involved in fly embryonic development. You are handed males and females of a test strain from a round of mutagenesis where the test chromosome is balanced with a balancer chromosome, i.e. each animal is M*/Balancer where M* represents a possible mutation on the chromosome.

a) (2 pts) You perform the following cross:

♂ M*/Balancer × ♀ M*/Balancer

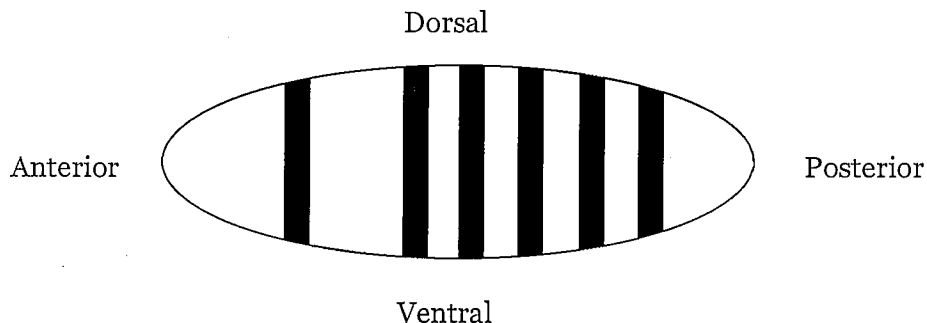
and all of your adult progeny possess the dominant marker of the Balancer, what can you conclude about the nature of the M* mutation?

It is a zygotic lethal mutation

- b) (2 pts) You inspect the embryos from this same cross and find that some are missing just one segment. What percentage of embryos should have this defect?

25%

- c) (4 pts) Using a panel of antibodies to early developmental proteins, you find that all maternal gradients and gap protein patterns are normal, but that the *even-skipped* protein pattern looks like this:



- i) What is the nature of this mutation?

The loss of one stripe indicates it is a regulatory mutant in *eve* stripe 2 enhancer.

- ii) How could you prove your answer in (i) above?

Isolate the enhancer DNA from mutant, inspect integrity and place upstream of reporter gene and put back into flies.

9. (7 pts)

The following questions concern properties of gene organization, expression, and regulation in bacteria and eukaryotes.

- T F In eukaryotes, the ground state of gene expression is "on".
- T F In bacteria, the sigma subunit of RNA polymerase binds to gene promoters.
- T F Eukaryotes possess three different RNA polymerases.
- T F The 5' cap of bacterial mRNAs helps position the transcript for translation in the ribosome.
- T F In eukaryotes, acetylation of histones is associated with increased gene expression.
- T F Eukaryotic mRNAs are typically spliced from larger precursors and poly-adenylated.
- T F Some eukaryotic genes have many enhancers.

		Second letter				
		U	C	A	G	
First letter	U	UUU } Phe UUC } UUA } Leu UUG }	UCU } UCC } Ser UCA } UCG }	UAU } Tyr UAC } UAA Stop UAG Stop	UGU } Cys UGC } UGA Stop UGG Trp	U C A G
	C	CUU } CUC } Leu CUA } CUG }	CCU } CCC } Pro CCA } CCG }	CAU } His CAC } CAA } Gln CAG }	CGU } CGC } Arg CGA } CGG }	U C A G
	A	AUU } AUC } Ile AUA } AUG Met	ACU } ACC } Thr ACA } ACG }	AAU } Asn AAC } AAA } Lys AAG }	AGU } Ser AGC } AGA } Arg AGG }	U C A G
	G	GUU } GUC } Val GUA } GUG }	GCU } GCC } Ala GCA } GCG }	GAU } Asp GAC } GAA } Glu GAG }	GGU } GGC } Gly GGA } GGG }	U C A G
						Third letter

Figure 9-6
Introduction to Genetic Analysis, Ninth Edition
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