

Name: \_\_\_\_\_KEY\_\_\_\_\_

## 7.012 Exam Two KEY -- 2006

Exam starts at 10:05 am and ends at 10:55 am.

There are 9 pages including this cover page & the genetic code.

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**            **24 pts**\_\_\_\_\_

**Question 2**            **11 pts**\_\_\_\_\_

**Question 3**            **24 pts**\_\_\_\_\_

**Question 4**            **25 pts**\_\_\_\_\_

**Question 5**            **16 pts**\_\_\_\_\_

**TOTAL**            **out of 100**\_\_\_\_\_

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**1. (24 pts)** You are working with a piece of DNA of the sequence:

5' -TATTGAGCTCCCGGAT-3'  
3' -ATAACTCGAGGGCCTA-5'

**(a, 5 pts)** You cut the above piece of DNA with a restriction enzyme that recognizes the sequence 5'-GAGCTC-3' and cuts on the 3' side of the A within this sequence. Draw all products that result from this digestion. Make sure to draw the nucleotide sequence of both strands, and label all 5' and 3' ends.

5' -TATTGA-3'                      5' -GCTCCCGGAT-3'  
3' -ATAACTCG-5'                      3' -AGGGCCTA-5'

**(b, 5 pts)** Now you ligate the DNA you produced in part (a) to the sequence below, which you have also cut with the same restriction enzyme. Draw the shortest DNA product that could form from ligating a piece of DNA from part (a) to a piece of DNA from part (b). Make sure to draw the nucleotide sequence of both strands, and label all 5' and 3' ends.

5' -TAGAGCTCCGCAATG-3'  
3' -ATCTCGAGGCGTTAC-5'

**This sequence becomes the following when cut:**

5' -TAGA-3'                      5' -GCTCCGCAATG-3'  
3' -ATCTCG-5'                      3' -AGGCGTTAC-5'

**The shortest product of ligation would be if you ligated the piece on the left from part (b) to the piece on the left from part (a). To do this, you have to flip the piece from part (a) on the left around so that its sticky ends line up with those of the piece from part (b).**

5' -TAGA-3'                      5' -GCTCAATA-3'  
3' -ATCTCG-5'                      3' -AGTTAT-5'

**giving the final answer**

5' -TAGAGCTCAATA-3'  
3' -ATCTCGAGTTAT-5'

**(c, 6 pts)** Restriction enzymes have DNA binding domains. Name three other different types of proteins (that are not restriction enzymes) that have DNA binding domains.

**Acceptable answers are any proteins whose job it is to act upon DNA. These include: activators and/or repressors (which are both transcription factors), DNA polymerase, RNA polymerase, helicase, primase, ligase, topoisomerase, telomerase, histones (part of the nucleosome), nuclease, DNA repair enzymes, and methylase.**

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Which reaction is catalyzed by each of the enzymes listed below? Answer by stating which specific type of bond is affected (e.g. "covalent" is not specific enough), and whether each enzyme catalyzes the formation or the breaking of that type of bond.

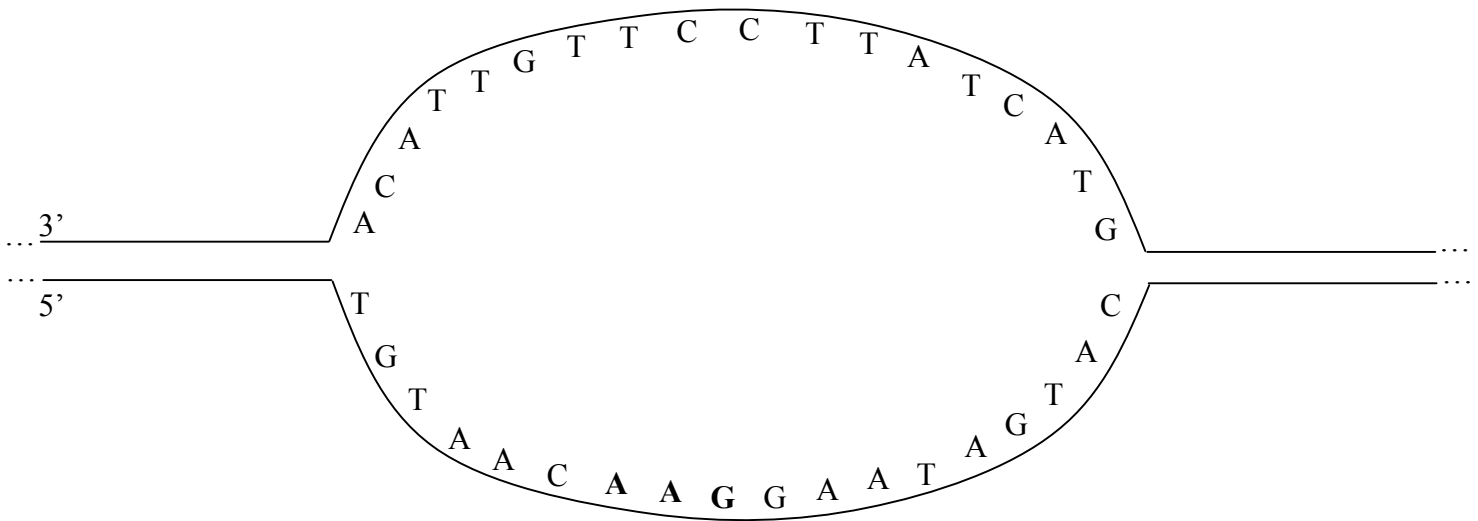
(d, 4 pts) the *T. aquaticus* "Taq" polymerase used in PCR

Formation of phosphodiester bonds. (PCR is just many rounds of DNA replication.)

(e, 4 pts) a restriction enzyme

Breaking of phosphodiester bonds.

2. (11 pts) Below is shown a picture of a replication bubble.



(a, 3 pts) The primer 5'-CUU-3' is being used to replicate this piece of DNA. Would that primer anneal to the upper strand of DNA in the picture or the lower strand?

The lower strand. The sequence 5'-CUU-3' would basepair to its antiparallel complement, 3'-GAA-5', which only exists at the site bolded above.

(b, 5 pts) Write what the product of DNA replication would be if the first five nucleotides had been added onto this primer by DNA polymerase. Label the 5' and 3' ends. Make sure to include the primer.

5'-CUUGTTAC-3'. Replication proceeds 5'--> 3', such that nucleotides are always added on to the 3' end of the primer. This is DNA replication, so the nucleotides added on to the RNA primer are deoxyribonucleotides.

(c, 3 pts) Would that strand that you have drawn in part (b) be a leading strand or a lagging strand?

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A leading strand. DNA replication occurs in a “leading fashion” when the direction of fork opening by helicase is the same direction as replication is occurring. This allows replication to keep on going continuously, as helicase unwinds more and more DNA. Helicase would be unwinding in these two directions:

← and →

to expand this replication bubble, and our strand is replicating this way:

←

so it must be a leading strand.

**3. (24 pts)** You are studying a very short protein-encoding gene whose sequence is shown below. The region of sequence shown is from the transcriptional start site to the transcriptional stop site. The gene’s one small intron is shown for you in bold.

5’ –CTACGTACTAGCTATTCC**GATCTATA**CTCGATCTAGTCGCATTCCGATTTCGATCGTAC–3’  
3’ –GATGCATGATCGATAAGG**CTAGATAT**GAGCTAGATCAGCGTAAGGCTAAGCTAGCATG–5’

**(a, 4 pts)** Which strand is used as a template in transcription, the upper strand or the lower strand?

**The upper strand. The only strand that has a start codon (5’-ATG-3’, bolded and underlined above) is the bottom strand, so the bottom strand must look like the mRNA. This means that the upper strand is the one that is used as a template.**

**(b, 4 pts)** How many nucleotides long would the final processed mRNA made from this gene be (not including the 5’ cap and the 3’ polyA tail)?

**50 nucleotides. The sequence shown goes from the transcriptional start site to the transcriptional stop site. Thus the whole 58 nucleotides are transcribed, but then the 8 nucleotides of the intron are spliced out, leading to a final mRNA of 50 nucleotides, which looks like this:**

**5’- GUACGAUCGAAUCGGAAAUGCGACUAGAUCGAGGGAAUAGCUAGUACGUAG-3’**

**(c, 4 pts)** How many amino acids long would the protein product of this gene be?

**8 amino acids. The mRNA looks like this after splicing the intron out:**

**5’-GUACGAUCGAAUCGGAAAUGCGACUAGAUCGAGGGAAUAGCUAGUACGUAG-3’**

**The AUG encodes methionine, the first amino acid. You then have seven other codons that code for amino acids until the stop codon.**

**1<sup>st</sup> codon: AUG**

**2<sup>nd</sup> codon: CGA**

**3<sup>rd</sup> codon: CUA**

**4<sup>th</sup> codon: GAU**

**5<sup>th</sup> codon: CGA**

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6<sup>th</sup> codon: GGG  
7<sup>th</sup> codon: AAU  
8<sup>th</sup> codon: AGC  
stop codon: UAG

**(d, 4 pts)** What is the sequence of the anticodon of the tRNA that would bind to the third codon of this mRNA as it was being translated? Make sure to label the 5' and 3' ends of the anticodon sequence.

**5-UAG-3'.**

**The third codon is 5'-CUA-3'. Thus the tRNA that basepairs with this section of the mRNA would be 3'-GAU-5'.**

**(e, 4 pts)** What is the amino acid that would be on the C-terminus end of the protein produced from this gene?

**Serine (ser). The last codon of this gene is the 8<sup>th</sup> codon: AGC. If you look up AGC on the genetic code, it codes for serine.**

**(f, 4 pts)** Say that the C:G basepair that is underlined in the sequence above were changed to an A:T basepair to make a mutant form of this gene. Which kind of mutation would this be? Your choices are: a frameshift mutation, a nonsense mutation, a silent mutation, or a missense mutation.

**A missense mutation. This changes the 4<sup>th</sup> codon from a GAU to a UAU. GAU encodes asp (aspartic acid) and UAU encodes tyr (tyrosine), so this would be a missense mutation.**

**4. (25 pts)** You are interested in how a specific protein-encoding mouse gene named Hrt1 is regulated. Full activity of the Hrt1 gene product is present in heart cells, but no activity of this gene product is present in liver cells. You hypothesize that the Hrt1 gene product is regulated in one of the following ways (which are listed in no particular order):

- 1) whether the mRNA is translated or not
- 2) whether the protein product is stable or immediately completely degraded
- 3) whether the gene is transcribed or not
- 4) whether the protein product is phosphorylated or not
- 5) whether the pre-mRNA is spliced to include all 5 exons or only 4 of those exons

You prepare four samples from cells: all of the mature mRNAs in heart cells, all of the mature mRNAs in liver cells, all of the proteins in heart cells, all of the proteins in liver cells. You load each sample into a separate lane in a gel, and separate the components by size via gel electrophoresis. You then detect presence/absence of any band corresponding to Hrt1 RNA or protein.

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(a, 5 pts) Towards which pole of the gel will the Hrt1 RNA run? Your choices are: positive, negative, neither, both, or inconclusive. Explain your answer.

**The positive pole. RNA is negatively charged so it will be attracted to the positive pole. RNA is negative because it is made of nucleotides, and part of every nucleotide is a phosphate group, and phosphate groups are very negative.**

(b, 5 pts) Towards which pole of the gel will the Hrt1 protein run? Your choices are: positive, negative, neither, both, or inconclusive. Explain your answer.

**Inconclusive. Proteins can be of any total charge. Each protein's charge depends upon the amino acid composition of that protein. If the protein has more negatively charged amino acids (glutamic acid and aspartic acid) than positively charged amino acids (histidine, lysine, arginine), then the protein will be overall negative and will run towards the positive pole. If the protein has more positively charged amino acids, it will run towards the negative pole. If it has the same number of positive and negative amino acids, it will not run either way because it will be neutral. Given that you have no information about the makeup of Hrt1 protein, you do not know which way it will run.**

You perform the type of experiment described above, and detect any Hrt1 mRNA that is present in heart cells or in liver cells. At which level (of the five levels listed above) do you think the Hrt1 gene product is regulated? List all possible levels (i.e. #1 through #5) given that result.

|            |                       |   |
|------------|-----------------------|---|
| (c, 5 pts) | RNA from heart cells: | a band is present   |
|            | RNA from liver cells: | a band with the same intensity is present that ran the same distance in the gel |

**1, 2, 4**

**#1 is possible because it may be that, although the same RNA is present in both heart cells and liver cells, the way that Hrt1 is active only in heart cells is that the mRNA is translated only in heart cells (and not in liver cells).**

**#2 is possible because all that you know is that mRNA is made in both cell populations. Therefore the regulation of the gene must occur post-transcriptionally. Degradation of a protein occurs post-translationally and is thus still an option for regulation.**

**#3 is NOT possible because there is mRNA produced from Hrt1 in both heart and liver cells, so this Hrt1 gene is not regulated at the level of transcription.**

**#4 is possible because all that you know is that mRNA is made in both cell populations. Therefore the regulation of the gene must occur post-transcriptionally. Phosphorylation occurs post-translationally and is thus still an option for regulation.**

**#5 is NOT possible because, if the pre-mRNA from Hrt1 were regulated by splicing, the mature mRNA would be a different length in heart cells than in liver cells, and this result tells you that the mRNA is the same length in both cell populations.**

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Now say that, INSTEAD, you perform the type of experiment described above, but detect any Hrt1 **protein** that is present in heart cells or in liver cells. At which level (of the five levels listed above) do you think the Hrt1 gene product is regulated? For each potential result, list **all** possible levels (i.e. #1 through #5) given that one result.

**(d, 5 pts)** Protein from heart cells: a band is present  
Protein from liver cells: a band with the same intensity is present that ran a different distance in the gel

**4, 5**

**#1 is NOT possible because Hrt1 protein has clearly been translated in both cell populations, because you can see protein in both lanes. Thus this gene is not regulated at the level of translation.**

**#2 is NOT possible because, if the gene product were regulated by immediate and complete protein degradation, then one of these two lanes would have no protein in it.**

**#3 is NOT possible because there is Hrt1 protein in both lanes, so Hrt1 mRNA must have been produced from the Hrt1 gene in both heart and liver cells, so this Hrt1 gene is not regulated at the level of transcription.**

**#4 is possible because, if the gene product was regulated by phosphorylation, then the protein would be present in both cell types, but it would be a slightly different size and charge in one cell type.**

**#5 is possible because, if the pre-mRNA from Hrt1 were regulated by splicing, then protein would be present in both lanes, but the protein would be of a different size in one of the two lanes.**

**(e, 5 pts)** Protein from heart cells: a band is present  
Protein from liver cells: no band is present

**1, 2, 3**

**#1 is possible because it may be that, although the same RNA is present in both heart cells and liver cells, the way that Hrt1 is active only in heart cells is that the mRNA is translated only in heart cells (and not in liver cells).**

**#2 is possible because seeing no protein in liver cells could mean that the protein is never made in liver cells, or it could mean that the protein is made but then immediately completely degraded.**

**#3 is possible because it could be that there is no protein in liver cells because the Hrt1 gene is simply never transcribed into mRNA in liver cells.**

**#4 is NOT possible because, if the gene product was regulated by phosphorylation, then the protein would be present in both cell types, but it would be a slightly different size and charge in one cell type.**

**#5 is NOT possible because, if the pre-mRNA from Hrt1 were regulated by splicing, then protein would be present in both lanes, but the protein would be of a different size.**

**5. (16 pts)** You are studying a **mouse** gene named MtoA. You know that the protein product of this gene localizes to mitochondria in mouse cells. Your final goal is that you want to know whether mouse MtoA protein is capable of localizing to mitochondria if produced in **yeast** cells. You decide to make a plasmid that contains a gene that is a fusion of the mouse MtoA gene to the **jellyfish** GFP gene. You make the plasmid correctly in a test tube, transform it into ***E. coli***, select for ampicillin, and allow the *E. coli* to grow up into colonies so as to make many copies of the plasmid. You then extract the plasmid from *E. coli*, and then transform it into yeast. You allow the transformed yeast to grow up into colonies, and you then look at these yeast containing the plasmid under a fluorescence microscope, and see green fluorescence in the mitochondria.

**(a, 4 pts)** Answer YES or NO for each of the following organisms. Must your plasmid contain an origin of replication that works...

...in mice? **NO.** The plasmid will never be put into mouse cells, so it will never have to replicate in mouse cells, so it does not need a mouse origin of replication. (Remember, an origin is a binding site for DNA polymerase and thus a start site for DNA replication.)

...in yeast? **YES.** In the last step of your experiment, you put the plasmid into a yeast cell and then grow that yeast cell up into a colony of yeast cells ( $10^7$  cells). Each one of those  $10^7$  cells must get a plasmid, so the plasmid must be able to replicate in yeast, so it must have a yeast origin of replication.

...in jellyfish? **NO.** The plasmid will never be put into jellyfish cells, so it will never have to replicate in jellyfish cells, so it does not need a jellyfish origin of replication.

...in *E. coli*? **YES.** In the middle of your experiment, you put the plasmid into an *E. coli* cell and then grow that bacterial cell up into a colony of cells ( $10^7$  cells) to make many copies of the plasmid. Each one of those  $10^7$  cells must get a plasmid, so the plasmid must be able to replicate in bacteria, so it must have a yeast origin of replication.

**(b, 3 pts)** Must your plasmid contain a promoter that works in mice? If yes, state which specific gene on the plasmid will be controlled by that promoter.

**NO.** This plasmid will never be put into mouse cells, so no genes on the plasmid will ever need to be transcribed by mouse RNA polymerase. (Remember, a promoter is a binding site for RNA polymerase and therefore a start signal for transcription.)

**(c, 3 pts)** Must your plasmid contain a promoter that works in yeast? If yes, state which specific gene on the plasmid will be controlled by that promoter.

**YES,** the gene that encodes the MtoA-GFP fusion protein. You want the hybrid protein MtoA-GFP to be expressed in yeast cells, so the coding region that encodes that fusion protein would have to be under control of a yeast promoter.

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**(d, 3 pts)** Must your plasmid contain a promoter that works in jellyfish? If yes, state which specific gene on the plasmid will be controlled by that promoter.

**NO. This plasmid will never be put into jellyfish cells, so no genes on the plasmid will ever need to be transcribed by jellyfish RNA polymerase. (Remember, a promoter is a binding site for RNA polymerase and therefore a start signal for transcription.)**

**(e, 3 pts)** Must your plasmid contain a promoter that works in *E. coli*? If yes, state which specific gene on the plasmid will be controlled by that promoter.

**YES, the gene that encodes for resistance to the antibiotic compound ampicillin. When you transform the *E. coli* cells with the plasmid, you select for ampicillin, which means that the plasmid must confer resistance to ampicillin. This means that the coding region of the ampicillin resistance gene would have to be under control of a bacterial promoter. Note that ampicillin is a small molecule, not a gene and not a protein. The gene on the plasmid is a gene that encodes a protein that confers resistance to ampicillin.**