

Name KEY

Your ID Number       

**BIOCHEMISTRY 353, SPRING 2002 FIRST HOUR EXAM, Feb. 8, 2002**

Before you start, PRINT your name and record your ID number. Be sure to print your name at the top of each page. Notes of any kind are NOT permitted.

Confine your answers to the space provided. Only answers in the space provided will be graded. If you cross out an entire answer, you may write your new answer in the same amount of space on the back of the page. Be sure to clearly state in a box on the front of the page that the answer is on the back of the page, or your answer will not be seen by the grader.

**THIS EXAM IS 8 PAGES LONG. BEFORE STARTING CHECK THAT YOU HAVE ALL OF THE PAGES**

**DO NOT TURN THIS PAGE OVER UNTIL INSTRUCTED TO DO SO BY A PROCTOR**

Part I        /30

Part II        /20

Part III        /15

Part IV        /16

Part V        /19

**TOTAL        /100**

Name KEY

**Part I. TRUE-FALSE (3 points each)** Circle T for statements which are True and F for statements which are False. (GRADED RIGHT MINUS WRONG; This means that if you got 8 right and 1 wrong and left 1 blank you would get  $8 - 1 = 7 \times 3$  or 21 points.)

- T (F) 1. In the processing of mRNA precursors, the smallest intron is almost always removed first and the largest intron is almost always removed last.  
*5' intron usually removed first*
- (T) F 2. In analyzing the base composition of most double-stranded DNAs,  $T+C=A+G$ ,  $C=G$  and  $T=A$ . *A:T + G:C Base pairs. Chargaff's Rules Helped in producing double helical structure for DNA*
- (T) F 3. If you synthesize the 5'-capped form of an mRNA that is normally capped, divide the mRNA into equal portions, chemically decap half of it and inject the capped mRNA into one set of living eukaryotic cells and the decapped mRNA into a second similar set of cells, one day after injection the level of the decapped mRNA will probably be lower than the level of the capped mRNA.  
*decapped mRNA is more rapidly degraded leading to a lower level*
- T (F) 4. A mutation that inactivated the 3'→5' exonuclease activity of *E. coli* DNA polymerase III would probably have no effect on the rate of mutations in newly replicated *E. coli* DNA. *This is the proofreading exonuclease. Inactivating this activity will therefore increase the rate of mutations*
- T (F) 5. Since there are 61 trinucleotide codons that specify amino acids, and 20 amino acids, three codons specify each amino acid. *The number of codons that specify amino acids is highly variable from 1 to 25.*
- (T) F 6. A graduate student isolated a DNA polymerase from an organism living in an Antarctic glacier. This enzyme is very stable at low temperatures and is very unstable at high temperatures (assume this is true). When this enzyme was used as the DNA polymerase in a standard PCR reaction, the target DNA hardly increases in amount. *In standard PCR there is a cycle of heating at each cycle to denature the DNA. This enzyme will be inactivated. Thermostable polymerases are used.*
- T (F) 7. A key step in demonstrating that DNA is the hereditary material was the demonstration that heating a pathogenic *Pneumococcus* bacterium had no effect on the bacterium's ability to infect and kill mice. *Heating blocked the pathogenic effect. The heat-treated bacteria could transfer a factor (ie. DNA) to live bacteria + make them pathogenic*
- (T) F 8. EDTA chelates magnesium ions (assume this is true). If high levels of EDTA are added to a solution containing an unspliced type II self-splicing intron, the rate of self-splicing will be reduced. *Requires bound  $Mg^{2+}$ . If all  $Mg^{2+}$  is chelated by EDTA splicing (type II) will be reduced*
- (T) F 9. If you very rapidly label the newly synthesized DNA in an *E. coli* containing only 10% of the normal level of *E. coli* DNA ligase, the fragments of newly synthesized DNA (hint: Okazaki fragments) isolated from the bacteria containing the reduced DNA ligase activity will be shorter than those in the bacteria containing normal DNA ligase levels. *ligation slower so short transient Okazaki fragments last longer, + the fragments will be*
- T (F) 10. The most common intranucleotide linkage in nucleic acids is a 2'-3' phosphodiester bond. *3'-5'*

**Part II. Multiple Choice Questions (20 points 4 points each) Circle the letters corresponding to ALL of the correct answers for each question.** (If 4 answers are correct and you circle one, that is not the correct answer). **Grading these questions: If you circle all of the correct answers you get all 4 points. If you make one error you lose 2 points (half credit). If you make two errors in a question you lose all 4 points (no credit).** (For example: If a question has three correct answers and you circle two of the three correct answers and no incorrect answers, you get two points [half credit]. If the question has three correct answers, and you circle two correct and one incorrect answer, you have made two errors, and get no points.)

1. At the same time that *E. coli* are infected with bacteriophage T2, the radioisotopes  $^{32}\text{P}$  and  $^{35}\text{S}$  are added. Which of the following events then occur.

- A. A new set of radioactively labeled proteins appears.
- B. The radioactive isotopes are not incorporated into RNA or protein
- C. A new class of labeled RNA is synthesized
- D. Newly synthesized radioactively labeled RNA becomes associated with ribosomes.
- E. Newly synthesized RNA becomes associated only with newly synthesized ribosomes.

*This experiment showed that T2 proteins were synthesized on pre-existing ribosomes.*

2. Chemically synthesized oligonucleotides are commonly used:

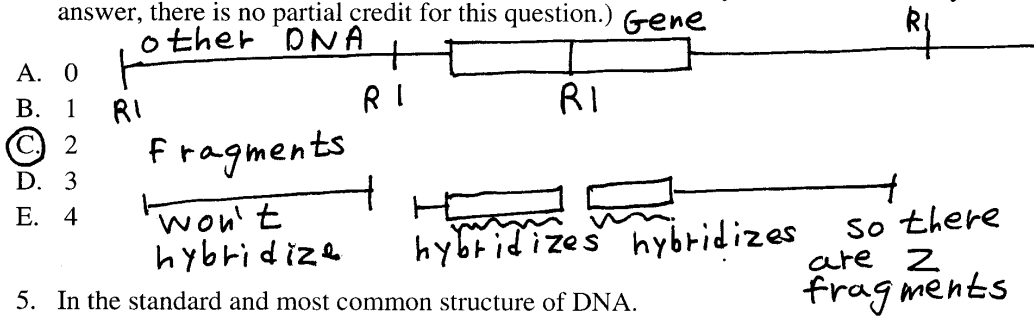
- A. As primers in PCR reactions.
- B. As probes for Western blots (protein blots).
- C. As primers for sequencing DNA.
- D. To construct linkers containing restriction sites.
- E. To introduce mutations into cloned DNA molecules.

3. Which of these DNA sequences, if they were contained within a much longer double stranded DNA molecule, would be likely to be the recognition sequence of a restriction enzyme?

- A. 5'-TAGCTA-3'  
3'-ATCGAT-5'
- B. 5'-GACCAG-3'  
3'-CTGGCC-5'
- C. 5'-AGTC-3'  
3'-TCAG-5'
- D. 5'-GAATTC-3'  
3'-CTTAAG-5'
- E. 5'-ACGT-3'  
3'-TGCA-5'

*palindromes*

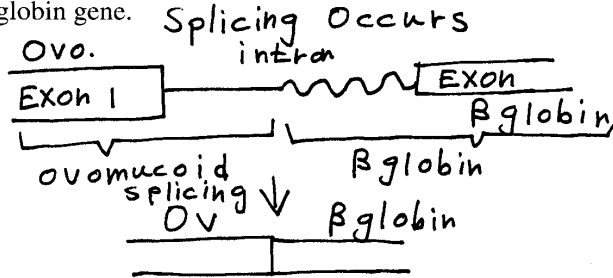
4. Assume that you have cloned the chromosomal copy of a gene that is present in a single copy in the genome of a turtle. The cloned gene contains one EcoRI site near its center. If you label the entire length of the cloned gene by nick translation, denature the labeled DNA to make it single stranded and probe a Southern blot of turtle DNA digested to completion with EcoRI, how many bands are you most likely to see on the autoradiogram? You should assume that the technical aspects of the experiment were carried out correctly. (Since there is only one correct answer, there is no partial credit for this question.)



5. In the standard and most common structure of DNA.
- A. The phosphate backbone of the two strands is linked in the center of the helix by bridging magnesium ions.
- B. The DNA is in 2 helical chains coiled around a common axis running in opposite directions.
- C. The nucleotides are on the inside of the helix and the sugar phosphate backbone is on the outside.
- D. The planes of the nucleotide bases are perpendicular to the axis of the helix.
- E. The helix rotates one complete turn approximately every 10-10.4 nucleotides.

**Part III. (15 points, 5 points each)** Based on your knowledge of splicing pathways, predict whether an RNA transcript made from each of the following hybrid genes will be spliced if incubated under conditions appropriate for splicing of a natural RNA precursor. If you think it will be spliced, briefly describe the mechanism. If you think it will not be spliced, briefly explain the reason for your conclusion. **Simple drawings should be included for full credit.**

1. The first exon and 5' half of the first intron of a gene encoding chicken ovomucoid joined by recombinant DNA techniques to the 3'-half of the second intron and 3'-exon of the mouse  $\beta$ -globin gene.



All of the signals required for splicing are present

5' splice junction (from ovomucoid)

branch site and pyrimidine-rich sequence + 3' splice junction supplied by  $\beta$  globin

The sequence within the intron does not matter as long as the signals recognized by the spliceosome are present. A hybrid intron like this one will work in splicing.

(5' + 3' splice junction branch site + pyr. rich sequence)

Name KEY

2. The 3'-half of a hammerhead ribozyme joined to the 5'-one third of the Tetrahymena ribosomal RNA precursor intron. No splicing or low level incorrect splicing.
- Ⓐ Tetrahymena 5' 1/3 contains the guide sequence but not the conserved core + the entire 3' recognition sequence + 3' junction. This cannot be replaced by the 3' half of the hammerhead because the cleavage site + base pairing element are in the 5' region of the hammerhead. Ⓑ Also there are different cleavage mechanisms used by the two types of introns (either a or b is ok) G based + Mg based

3. An intron in which the region containing the naturally occurring 5'-splice junction has been replaced by the sequence 5'-AGGUAAGUA-3'

Splicing will occur  
This is ~~an~~ excellent 5' splice junction and it will base pair with high efficiency with the complementary sequence in U1 SnRNP.

Part IV. Fill in (16 points, 2 points each, there is no penalty for wrong answers. Only the first written answer will be graded. There may be more than one correct answer)

1. In the genetic code the number of nucleotides that specifies incorporation of an amino acid is

3

2. Exposure of DNA to ultraviolet (UV) light commonly causes formation of Thymine, Thymidine also accepted dimers.

Name KEY

- In converting an mRNA into a double stranded by PCR, the mRNA is first copied into single stranded DNA using the enzyme Reverse Transcriptase, RNA dependent DNA polymerase, RT
- These two nucleotides A:T Adenine and Thymidine form a base pair that normally contains two hydrogen bonds.
- The enzyme DNA polymerase requires a primer to copy DNA.
- In dideoxy DNA sequencing which carbon on the sugar contains a hydrogen (H) instead of its normal OH group. 3 (Normal 2' deoxy sugar 3'-5' phosphodiester dideoxy 2' 3' deoxy)
- DNA sequences are normally written 5' → 3' on the antisense or coding strand.
- The enzyme T4 DNA ligase, bacteriophage T4 DNA ligase (-1 for DNA ligase) is widely used in recombinant DNA research to covalently join blunt ended double stranded DNA fragments in the presence of ATP.

**V. Short Answer (19 points, 3 points each unless otherwise noted)** provide a brief biochemical explanation for each of the following observations or statements. Your answer should reflect the contents of this course. Only answers in the space provided will be graded, so think before you write. If you cross out an entire answer make a box indicating clearly that the answer is on the back, and use an equivalent amount of space on the back of the page. You do not have to fill in the entire space. If you know the answer, one or two short sentences or a simple diagram may be sufficient. If you keep writing, and write something that is incorrect, some credit will be deducted.

- Bacteriophage T2 was used in the experiments performed by Hershey and Chase to help determine the nature of the genetic material. Bacteriophage M13 infects *E. coli* using a strategy that is different strategy than the one employed by T2. The M13 protein coat is removed in the inner membrane of the bacterial cell, where it is sequestered and subsequently used to help envelop the progeny phage DNA. Why would M13 have been much less well suited than T2 was for the experiments carried out by Hershey and Chase? The Hershey Chase experiment depends on the spatial separation of the protein and nucleic acid components of the virus to determine which contains the genetic material. In T2 infection the DNA enters the cell while the protein coat remains outside where it can be physically separated from the cells by simple mechanical force (a blender). ~~If the protein~~ Using centrifugation the cells containing the DNA can be physically separated from the protein (cells + DNA in the pellet, T2 coat protein in the supernatant). If M13 were used and the labeled protein and nucleic acid were both within the cell, both would be found in the cell pellet + this experiment would not provide any useful information on the nature of T2's genetic material. 6

Name KEY

2. (9 points, 3 points each) A solution containing active DNA polymerase I and the  $Mg^{+2}$  salts of dATP, dGTP, dCTP and dTTP is present. The molecules listed below are added to aliquots of this solution. State whether or not you would expect to observe DNA synthesis and provide a brief (1-2 sentence) explanation of your reasoning. (Assume that incubation temperature and buffers were appropriate for testing for DNA synthesis.)

A. A double-stranded closed circular negatively supercoiled DNA 3,000 nucleotides in length.

No DNA synthesis

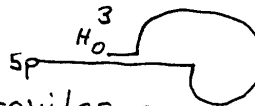
DNA polymerase needs a free 3'-OH to use as a primer. A closed circular molecule has no ends for the nuclease activity of DNA pol I to use. Also, there is nothing to unwind the DNA.

B. A linear single-stranded DNA 3,000 nucleotides in length base paired near the middle of the DNA with a 50 nucleotide linear single-stranded RNA containing at its ends a 5'-hydroxyl group and a 3'-phosphate

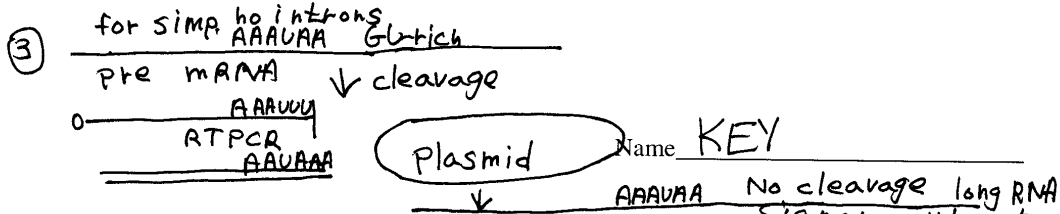
DNA synthesis can use an RNA primer  
No DNA synthesis But DNA synthesis requires a free 3'-OH and here we have a 3'-phosphate so there will be no DNA synthesis

C. A single-stranded DNA 1,000 nucleotides in length that contains 5'-phosphate and 3'-hydroxyl groups at its ends that contains a sequence of 30 nucleotides that can perfectly base pair with a sequence near the center of the DNA.

Yes DNA synthesis will occur



the base paired sequence provides a primer with the correct 3'OH at its end & the single stranded DNA provides a template



3. (4 points) A student used an oligo dT primer to start an RT-PCR reaction to copy an mRNA into double-stranded DNA. This double-stranded DNA sequence was then cloned into a plasmid downstream of a functional transcription initiation site. The DNA was grown up and inserted into the same type of cells that contained the original mRNA. The cytoplasmic mRNA transcript of the gene transcribed (copied) from the gene in the plasmid was much longer than the mRNA copied in the RT-PCR reaction. Explain this observation.

Eukaryotic RNA polymerase makes primary pre-mRNA transcripts that extend far beyond the 3'-end found in the final mRNA in the cytoplasm. An AAUAAA sequence upstream of the cleavage site and a GU-rich sequence downstream of the cleavage site are both important. Since the GU-rich sequence is NOT present in the mRNA copied by RT-PCR, the cleavage and polyadenylation system will not recognize the correct cleavage site & will cleave at the next <sup>downstream</sup> correct recognition site or ~~at~~ at a low rate at many downstream sites leading to longer MRNAs

4. A student isolated a mutant in DNA polymerase III that reduces the frequency of spontaneous mutations in *E. coli*. Provide a plausible explanation for how this mutant might work.

The mutation increased the activity of the 3'→5' exonuclease activity in the polymerase.

This makes it more likely that an incorrect base pair will be removed by the proofreading activity and therefore lowered the frequency of mutations (This would also slow DNA synthesis)

Also acceptable but a bit far-fetched

The mutation changed the geometry of the substrate binding site so that covalent bond formation is less likely with an incorrectly base paired nucleotide