Chem 135: Third Midterm

November 16th, 2012

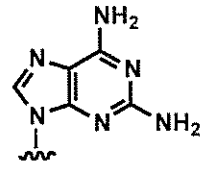
Please provide all answers in the spaces provided. You are allowed to use a calculator for this exam, and you may use (previously disassembled) molecular model kits. Including the title page, there should be **6** total questions spread over **5** pages. There is also a sixth page that should be blank. You can use this last page for scratch paper if you need it, but please remember to copy your answers into the appropriate exam question space.

Name: Key

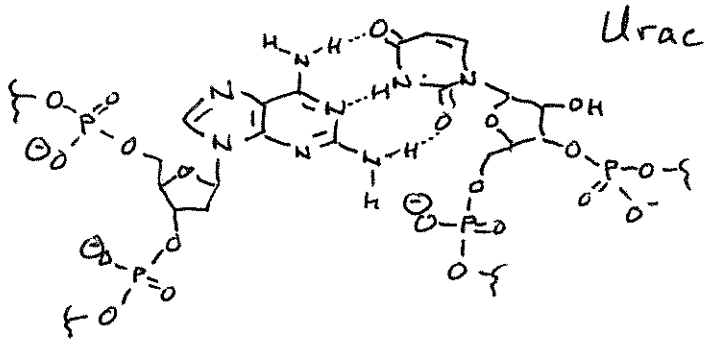
(1)	_____	(14 points)
(2)	_____	(10 points)
(3)	_____	(12 points)
(4)	_____	(24 points)
(5)	_____	(18 points)
(6)	_____	(22 points)
TOTAL	_____	(100 points)

1. 2,6-Diaminopurine (DAP, shown below) is an artificial nucleotide base that can pair with at least one of the "natural" nucleotide bases.

DAP:



a. In the space below, draw DAP as it would appear in an DNA segment, including the sugar and the backbone phosphates. Next, provide the *name and full structure* of the natural **RNA** base it would most likely complement. Also indicate the Watson-Crick-Franklin-type interactions that would occur between the bases (8 points).

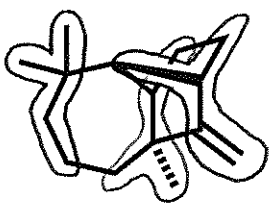


Uracil is the best match.

b. Considering the complementary base that you chose above, suppose you exchanged its "normal" base pairing partner in an RNA sequence with the DAP group. Would the melting temperature of the resulting duplex region increase, decrease, or stay the same? Briefly explain your answer (6 points).

DAP-U pairs have 3 hydrogen bonds, while A-U pairs have only 2. Therefore, T_m will increase.

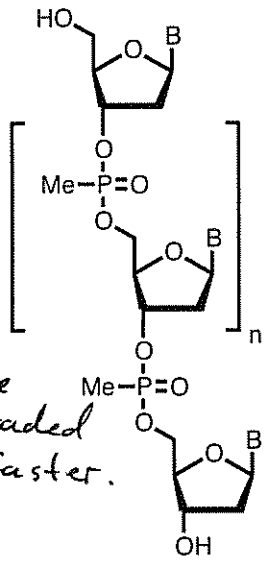
2. Circle the individual terpene units in longifolene. What is the name of the linear precursor that would give rise to this compound (10 points)?



longifolene

it is a sesquiterpene made from farnesyl pyrophosphate.

3. As noted in lecture on Wednesday, there are many therapeutic possibilities of synthetic DNA strands and structures that mimic them. One type of DNA mimic that has been explored has methylphosphonate groups as the backbone, as shown in the structure on the right.



methylphosphonate DNA
(B = nucleotide base)

   a. Compared to an analogous segment of natural DNA, would you expect methylphosphonate DNA to be degraded faster, slower, or at the same rate at pH 10? Briefly explain your answer (6 points).

The phosphonate groups have no negative charge, so they will be more susceptible to nucleophilic attack. It would thus be degraded faster.

   b. When methylphosphonate DNA hybridizes to a complementary sequence of normal DNA, would you expect the resulting duplex to have a higher, lower, or identical melting temperature to that of an analogous double-stranded segment of natural DNA? Briefly explain your answer (6 points).

The lack of backbone charge alleviates interstrand repulsion. Therefore, T_m will increase.

4. Suppose you have discovered a new transcription factor that binds to a specific double stranded DNA (dsDNA) sequence. Your initial experiments have determined that the protein functions as a dimer.

   a. Based on the other examples of DNA binding proteins that were discussed in class, what property would you expect the recognition site of this transcription factor to have? Provide an example of such a dsDNA sequence that is 8 bases in length. Also label the directionality of the strands (8 points).

The site will be palindromic. One example answer:

5'-GTAATTAC-3'
3'-CATTAATG-5'

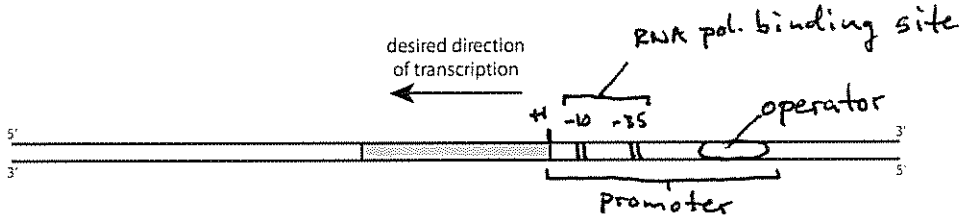
   b. Upon binding, your transcription factor decreases the ability of an adjacent gene to recruit RNA polymerase, thus lowering the amount of mRNA that is produced. This is an example of which type of regulation (4 points)?

Negative regulation

   c. What is the term for a transcription factor that has this function (4 points)?

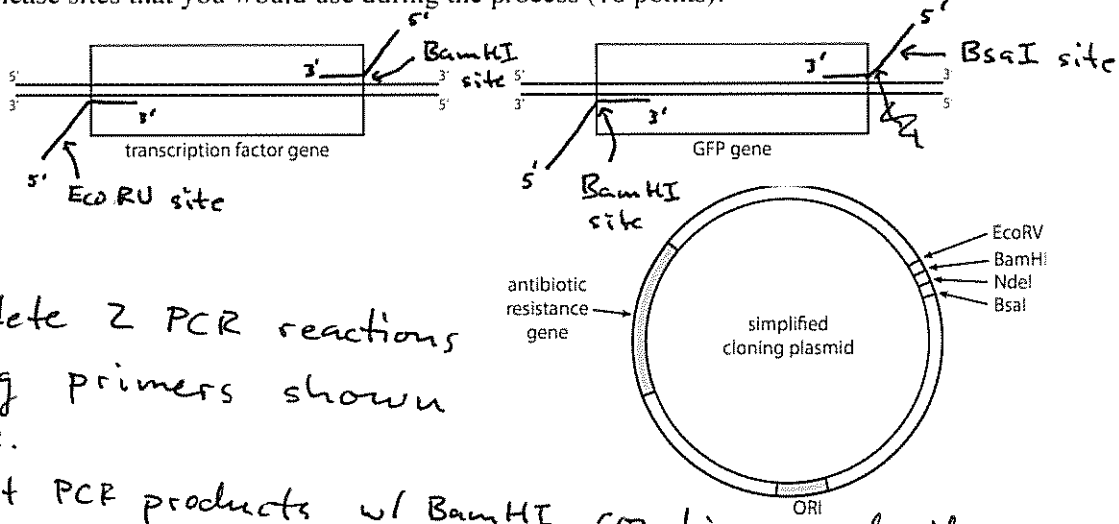
Repressor

d. The shaded region of the diagram below indicates a gene that will be transcribed into mRNA. Add the following features to the drawing and clearly label them: (1) the RNA polymerase binding site(s), (2) the operator, and (3) the promoter. Finally, indicate which of the two DNA strands will be transcribed (8 points).



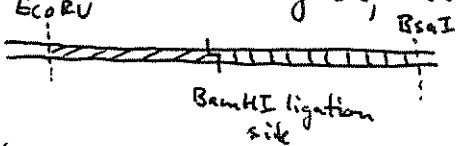
5. One common technique in biology is the generation of fusion proteins, which consist of two different proteins that are joined together into a single polypeptide strand. For example, one might connect the transcription factor discussed above to a green fluorescent protein (GFP) segment. The resulting protein product would bind to a specific location in chromosomal DNA, which could be visualized by tracking the fluorescence of the GFP portion.

Assume that the two proteins to be joined are encoded by the two separate, linear segments of DNA shown below. Outline a strategy that you could use to (1) join the two boxed protein-encoding segments together, and (2) ligate the final gene product into the plasmid shown below. Provide clear drawings as part of your answer, and include the names and specific locations of any restriction endonuclease sites that you would use during the process (18 points).



1) Complete 2 PCR reactions using primers shown above.

2) Digest PCR products w/ BamHI, combine, and then expose to DNA ligase, ATP, & Mg^{2+}. This will yield:

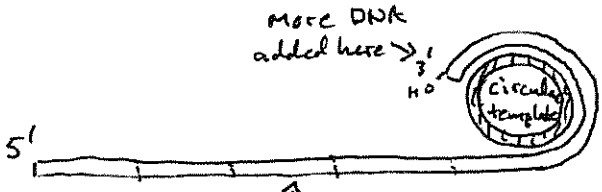


3) Digest plasmid with EcoRU and BsaI. Run a gel to remove excised region. Also digest linear gene (above) with the same endonucleases.

4) Combine digested products and ligate with DNA ligase, ATP, and Mg^{2+}.

6. Small, cyclic oligonucleotides have been shown to be efficient templates for DNA polymerases, in what is called "Rolling Circle DNA Synthesis." In these experiments, rings of single-stranded DNA 50 to 75 nucleotides in size are used. When incubated with DNA polymerase, Mg^{2+}, a DNA primer that is 15-20 bases in length, and deoxynucleotide triphosphates, the usual double-stranded product is formed. However, double-stranded DNA is highly strained when bent into such a small circle, and therefore the strands are forced to separate as the polymerase continues along the circular path.

   a. Based on this consideration, describe the product of a Rolling Circle DNA Synthesis reaction and provide a sketch that shows how this product is formed (8 points).

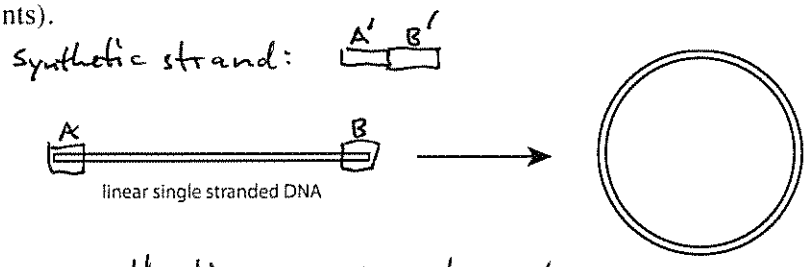


New ssDNA ← a long ssDNA strand will be produced. It will have a series of repeated copies of the circle complement.

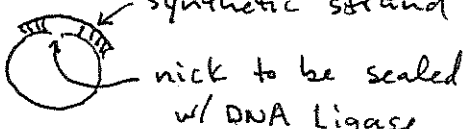
   b. Describe the product of a Rolling Circle DNA Synthesis reaction wherein the 3'-overhanging end sequence of a eukaryotic chromosome serves as the primer for a circular oligonucleotide. What cellular process might this technique mimic (6 points)?

This would extend the 3' end with a long string of repetitive ssDNA. This would mimic the activity of telomerase.

   c. Suppose you would like to synthesize one of the circular, single stranded DNA templates described above from a linear single stranded piece of DNA obtained using chemical synthesis techniques. How would you accomplish this? You may use any reagents or enzymes that you see fit (8 points).



1) Add a synthetic DNA strand that is complementary to both ends. This will bring the circle together.



2) Use DNA Kinase to add phosphate group to 5'-ends.

3) Seal link w/ DNA ligase + ATP + Mg^{2+}

4) Heat to remove synthitic DNA "splint". Gel purify if desired.