Biochemistry 674 **Your Name:**_____

Prof. Jason D. Kahn **Exam #2: November 16, 1999**

Nucleic Acids **University of Maryland, College Park**

You have 80 minutes for this exam.

Exams written in pencil or erasable ink will not be re-graded under any circumstances.

Explanations should be concise.

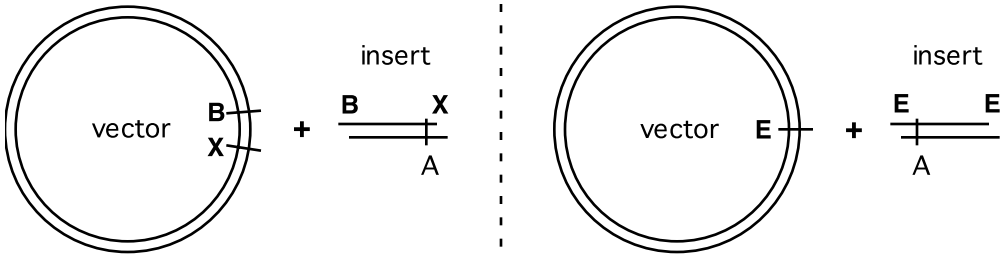
You will not need a calculator for this exam, and no other study aids or materials are permitted.

Useful equation:

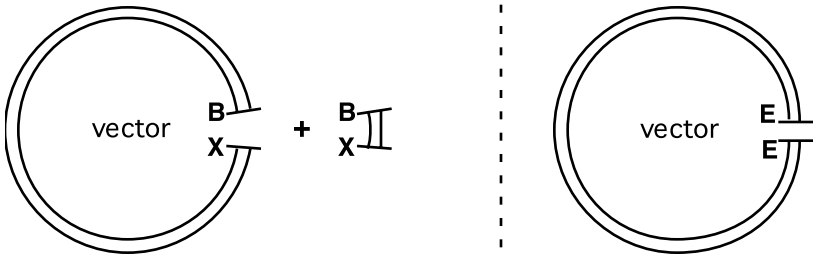
$\Theta = P / (P + K)$

1. (18 pts) Enzymatic manipulations and cloning

Directional cloning (using different sticky ends at each end of an insert to be cloned) is a good thing. To illustrate why, consider cloning the fragment at the right in each panel below into the plasmid at the left, after cleavage with the indicated enzymes. The "A" sites are orientation markers: we will not cut with A but please include the site in drawings you make below.



(0 pts) Draw the initial restriction products after vector cleavage, before addition of insert.



(a; 10 pts, ~1 each) Draw possible circular ligation products of vector + insert , assuming vector is not purified and no phosphatase is used. Omit all vector multimers and species with >3 inserts.

(b; 8 pts) Draw the possible ligation products if phosphatase treatment is used appropriately (and tell me when that is). Assuming that inverted repeats don't clone, indicate the clonable products.

2. (20 pts) Protein-nucleic acid interaction methods

(a; 5 pts) Sketch the graph of fractional saturation vs. protein concentration for a simple protein-DNA interaction. Label axes and K_D (this is independent of parts b and c).

Protection against restriction enzyme digestion can be used to study protein-DNA interaction For example, BamH I restriction enzyme digestion can be used to measure the binding affinity of the generic DNA Binding Domain (DBD) below for its specific site as indicated.

(b; 8 pts) Sketch a native gel showing an experiment using BamH I digestion to determine a binding constant for the DBD, including the appropriate control lanes. Label the gel bands and the lanes. Hint: this is not a gel shift experiment, results would be the same after SDS treatment.

(c; 7 pts) What additional information could Xba I digestion provide in this experiment? Sketch the experimental manifestation of your answer.

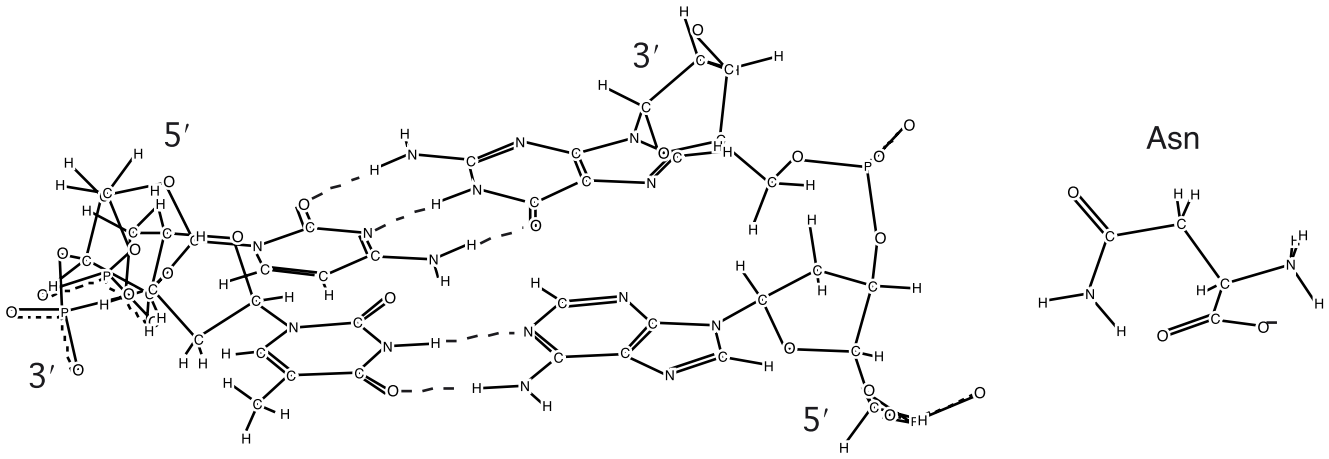
3. (22 pts) Specificity and structure

(a; 2 pts) What is the H-bond donor-acceptor matrix in the major groove for the base pairs below:

5′ C–G T–A 3′	

One common mode of DNA recognition is the use of hydrogen bond networks, such that one amino acid can recognize two base pairs simultaneously by forming multiple hydrogen bonds.

(b; 3 pts) On the sketch below (the same base pairs as in a), add a plausible bridging asparagine side chain between the two base pairs.



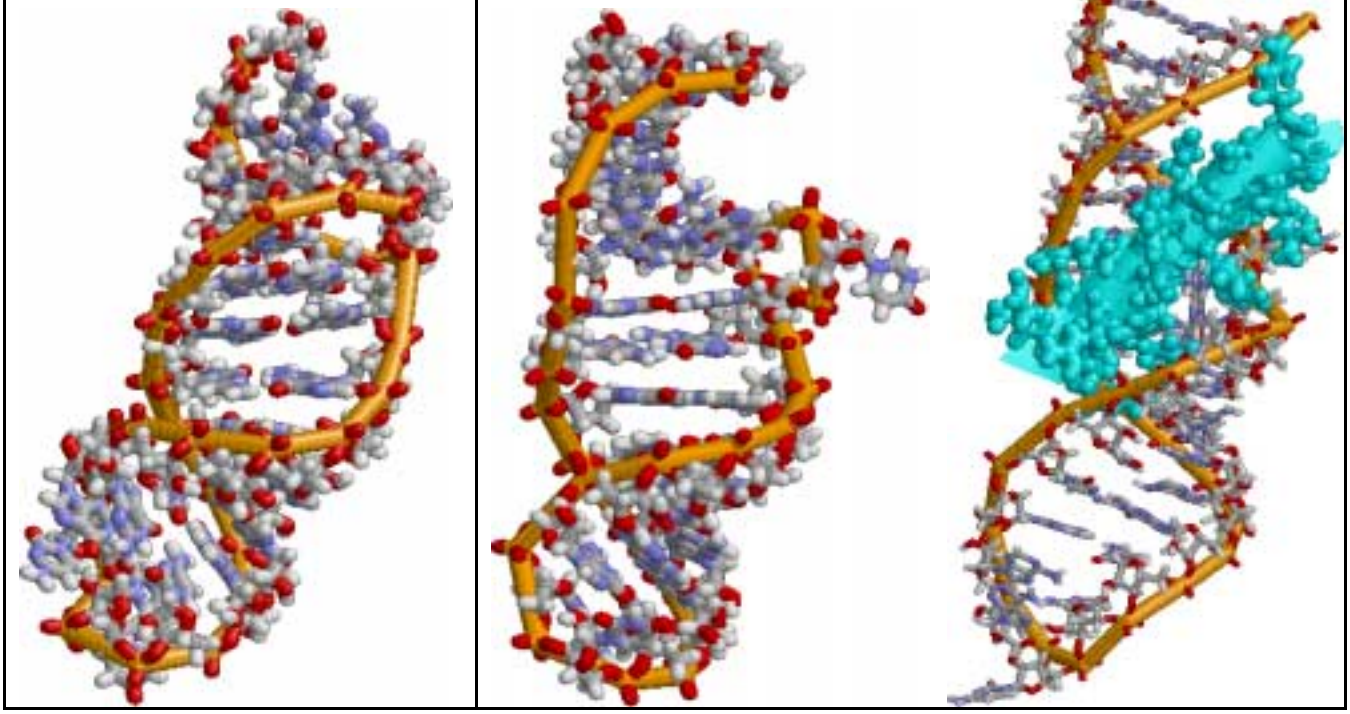
(c; 7 pts) Of the 16 dinucleotides, choose three which could be excluded (i.e. to which binding would be disfavored) given the asparagine positioned as you have drawn it. Explain your reasoning briefly, once. Why are there 16 possible dinucleotides to be recognized rather than just 10?

(d; 2 pts) What kind of DNA recognition (direct readout etc.) is typified by the TATA box binding protein (TBP)?

The pictures below show the Rev Response Element (RRE) RNA before (left) and after (middle) Rev protein binding, and the protein•RNA complex (right, from a different lab)

FREE RNA:

BOUND RNA:



(e; 8 pts) Identify two related general features of the protein-RNA recognition event shown which are typical of RNA recognition but unusual for dsDNA recognition. On the other hand, what aspect of the bound structure is reminiscent of protein-DNA recognition?

4. (20 pts) DNA replication

(a; 4 pts) Pyrophosphorolysis is the microscopic reversal of nucleotide incorporation. Write out the chemical reactions for nucleotide addition to a primer and for pyrophosphorolysis.

(b; 6 pts) Pyrophosphorolysis appears to have potential for proofreading: a DNA polymerase could adapt a 3′→ 5′ exonuclease active site to simply "reverse off" a misincorporated base at no energy cost. What's wrong with this picture? What important kinetic partitioning step does the real 3′ → 5′ exonuclease make possible as part of increasing fidelity?

(c; 6 pts) List two ways in which chromosomal DNA replication burns ATP energy in ways that are not related directly to the synthetic reaction above. What is the biological function of each energy-dissipating reaction?

(d; 4 pts) Briefly describe the Meselson-Stahl experiment and its significance.

5. (20 pts) Miscellaneous: Chromatin, cell cycle, telomeres

(a; 6 pts) Discuss why a translationally positioned nucleosome is also a more stable nucleosome than one formed on random DNA. (Hint: any sequence can be packaged.)What is the experimental signature of translational positioning?

(b; 4 pt) Sketch the essential idea of the licensing model for eukaryotic DNA replication.

(c; 10 pts) Give two reasons why organisms with linear chromosomes (like us) need telomeres. What does the telomerase enzyme use as a template? Why is telomerase under study as an anti-aging target? What are possible side-effects? If anti-aging worked perfectly, would it be good or bad?

Do Not Write Below This Line

Score:

Question 1: _____ out of 18: Enzymatic manipulations and cloning methods

Question 2: _____ out of 20: Protein-nucleic acid interaction methods

Question 3: _____ out of 22: Specificity and structure

Question 4: _____ out of 20: DNA replication

Question 5: _____ out of 20: Miscellaneous: Chromatin, cell cycle, telomeres

Total: _____ out of 100