Biochemistry 661 **Your Name:** _____

Nucleic Acids, Module I **Prof. Jason Kahn**

Exam I (100 points total) **September 23, 2010**

You have 60 minutes for this exam.

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

Explanations should be concise and clear. I have given you more space than you should need. There is a extra space on the last page if you need it.

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

Generous partial credit will be given, *i.e.*, if you don't know, guess.

Honor Pledge: At the end of the examination time , please write out the following sentence and sign it, or talk to me about it:

"I pledge on my honor that I have not given or received any unauthorized assistance on this examination."

1. DNA Structure and Flexibility (28 pts):

(a; 10 pts) Draw a Watson-Crick A:U pair in RNA. Indicate the pseudodyad axis and the approximate location for the intersection of the A-form helix axis with the base plane. Draw the sugars and include the numbering on one sugar and both bases.

Score for the page_____

(b; 3 pts) We have emphasized over and over what it is that makes the Watson-Crick base pairs special. What is it?

(c; 2 pts) The _____ phosphate on ATP is incorporated into nucleic acid by polymerases. The _____phosphate is added to the end of a nucleic acid by T4 polynucleotide kinase.

(d; 5 pts) Sketch a DNA helix to explain why the cleavage patterns generated by a groove-bound hydroxyl radical generating reagents in the minor groove would be "staggered" to the 3′ side.

(e; 8 pts) Sketch a picture illustrating the idea of persistence length: show five random 300-bp DNA molecules aligned at one end, and indicate the displacement that we talked about in defining the persistence length. Similarly, sketch a set of molecules for a DNA that has an intrinsic 90° bend in the center.

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2. Hybridization (18 pts):

(a; 9 pts) Even though hydrogen bonds are quite strong, to a first approximation they do not contribute very much to the stability of double-stranded nucleic acids. Why not? To a second approximation, they do actually contribute some stabilization. Why? The current nearest-neighbor thermodynamics model includes an H-bond term that is parameterized according to the number of terminal A-T base pairs (as opposed to G-C). Considering the nearest neighbors in the following two sequences, explain why: ATCGA vs. GATCG.

(b; 9 pts) Briefly describe how quantitative (real-time) PCR can be used to count small numbers of RNA transcripts. You don't need to describe the cycling in detail.

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3. RNA Structure (12 pts):

(a; 3 pts) Why don't sequence-specific RNA binding proteins recognize the major groove of fully double stranded RNA?

(b; 3 pts) Why does tertiary folding of RNA usually require divalent metal ions?

(c; 6 pts) Sketch an RNA secondary structure including at least one stem-loop, a bulge, and a three-arm junction.

4. DNA Topology (24 pts):

(a; 9 pts) Many antibiotics (e.g. Ciprofloxacin) are bacterial gyrase (Type II topoisomerase) inhibitors. Why are they specific to bacteria? Why are several topoisomerase poisons used as chemotherapy agents? What is the ideal step in the topoisomerase reaction cycle with which to interfere?

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(b; 15 pts) Sketch two closed circular DNAs, one with $\Delta Lk = +3$ and one with $\Delta Lk = -3$, showing toroidal superhelices, one with writhe = +3 and one with writhe = -3 (i.e. assuming no ΔTw relative to relaxed DNA). Then draw two molecules with the same ΔLk's but with each having $\Delta Tw = -3$ relative to relaxed DNA. It is not easy to do this conversion for positively supercoiled DNA. Why do you think proteins that create or stabilize positive writhe are found in some thermophiles (heat loving organisms)?

5. Sequencing and Methods (18 pts):

(a; 9 pts) Define a scaffold in genome sequencing. What are the "mate pairs" used in shotgun sequencing and how are they used to order contigs?

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(b; 9 pts) Outline how to clone and express a eukaryotic gene in *E. coli* using a plasmid expression vector. Assume you know the genome sequence and have access to a cDNA library. Include a sketch of the plasmid with its key features identified.

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