Biochemistry 661 **Your Name:** _____

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

Final Exam (200 points total) **October 14, 2010**

You have 90 minutes for this exam.

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. Methods (40 pts):

(a; 24 pts) Explain why the electrophoretic mobility shift assay is better than footprinting for looking for very weak nucleic acid binding by proteins. Explain why footprinting requires much hotter DNA or RNA than EMSA (think about what the gels look like). On the other hand, list one of the several major advantages of footprinting over EMSA in terms of the methods' results or applicability.

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(b; 16 pts) Briefly describe the essential steps of the Roche 454 method of next-gen sequencing.

2. Nucleosome Structure and Stability (48 pts):

(a; 16 pts) Draw two sketches of the structure of the Nucleosome, one showing DNA gyres on a tuna can and one showing the disposition of the histones from above. Draw the $(H3/H4)_2$ tetramer as one large oval blob and each H2A/H2B dimer as a smaller circular blob.

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(b; 16 pts) Explain the origin of the observed periodicity of AT-rich vs. GC-rich sequences in sequences that form rotationally positioned nucleosomes. The periodicity in and of itself provides evidence for overtwisting in the nucleosome, because the average separation between e.g. AA dinucleotides is smaller than in free DNA. Physically, why does it make sense that nucleosome-bound DNA can have a smaller helical repeat? How does overtwisting resolve at least most of the "nucleosome paradox?"

(c; 16 pts) Originally it was thought that nucleosome acetylation might have its effect via dramatic conformational changes in the nucleosome. This turned out not to be the case: what is the actual role (in general) of histone modification? What pair of enzymatic activities is generally associated with activation and repression of transcription? What method is used to track changes in histone modification state across a gene?

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3. DNA Polymerases and DNA Replication (56 pts):

(a; 20 pts) Sketch the two metal ion mechanism for RNA polymerization. Don't worry about the exact arrangement of protein side chains. State the function of each metal ion. One reason this mechanism has been so attractive, besides its universality, is that it may offer some supporting evidence for an RNA world. How so, for example in contrast to an enzymatic reaction mechanism with a covalent E-I intermediate such as the serine protease mechanism?

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(b; 18 pts) We discussed the idea that slow extension of a mismatched primer terminus is fundamental to DNA polymerase fidelity. What rationale did we offer for the relative lack of specificity of the 3′→5′ exonuclease (hint: it's a separate active site)? We did not discuss the experimental evidence for slow extension of a mismatch, but it is actually reasonably straightforward to study if you have an oligonucleotide synthesizer (or IDT's web site) in hand. Describe how you would do such an experiment. It is also helpful to have an exonuclease-deficient mutant available – how does the use of an exo$^-$ mutant polymerase simplify the results?

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(c; 18 pts) The coordination of leading and lagging strand DNA synthesis at the replication fork is a subject of enduring interest. Describe the fundamental puzzle about the timing and list two of the several possible solutions that have been proposed.

4. DNA Repair (36 pts):

(a; 8 pts) What common type of disaster is thought to be the reason for the low viability of RecBCD mutants? What is the general source of information used to fix lesions that affect both strands, like crosslinks or double-strand breaks?

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(b; 28 pts) Mismatch repair (MMR) is essential for mopping up most of the few mistakes made by DNA replicative polymerases.

Name the protein in *E. coli* responsible for mismatch recognition:

How does the MMR machinery know which strand to fix (what marks the daughter strand)?

There have been many mechanisms proposed for the needed information transfer between the marker and the mismatch. Give two simple reasons that this information transfer is a challenge.

Why doesn't simple through-space random looping make sense as a mechanism for information transfer?

What would the consequences be of making the wrong "decision"?

How does the MMR system illustrate the idea that cost is nearly no object when it comes to the integrity of the genome?

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5. Protein-DNA Recognition (20 pts):

(a; 20 pts) Some proteins can recognize specific nucleic acid sequences without hydrogen bonding to the DNA/RNA bases. Provide the name of a protein that recognizes DNA in this manner. Briefly describe where this protein interacts with the DNA, the type of primary interactions that are made, and a key features of the DNA sequence that facilitates binding.

Page	Score
1	
2	
3	
4	
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