Biochemistry 674 **Your Name:** _____

Nucleic Acids **Prof. Jason Kahn**

Exam II (100 points total) **November 17, 2005**

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 and clear. I have given you more space than you should need, and there's extra space on the last page.

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.

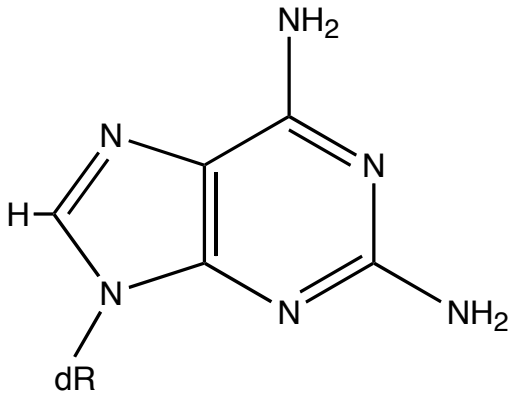
Honor Pledge: At the end of the exam time, please write out the following sentence below and sign it, or talk to me about it:

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

1. Protein-Nucleic Acid Recognition (35 pts):

The modified nucleobase at the right is 2,6-diaminopurine (DAP). It is a useful probe for protein-nucleic acid interaction.

(a; 4 pts) What feature of the DAP-T base pair increases the persistence length and thermal stability of DNA that contains DAP?



Score for the page_____

(b; 11 pts) Today, your favorite protein (YFP) is a transcription factor that binds dsDNA. Its recognition site is:

5′...ACAGGTATAACAGGT...3′
3′...TGCCCATATTGTCCA...5′

We would like to know which groove the protein recognizes. How could you use oligonucleotides with DAP substituted for A or G to probe this question? What are the expected results for a protein that binds in the minor vs. the major groove?

(c; 8 pts) Now, suppose a crystal structure emerges that demonstrates that the experiment you did in (b) gave misleading answers. For example, suppose a position that you had identified as important is not contacted by YFP. How can you explain this result? What is the new most likely physical explanation of the origin of DAP's effect? [In science, there's nothing wrong with being wrong. It's only stupid or criminal behavior that is discouraged.] There's more space on the next page if you need it.

Score for the page_____

(d; 6 pts) Do you think DAP substitution would lead to more stable/better rotationally positioned nucleosomes or not? Explain briefly.

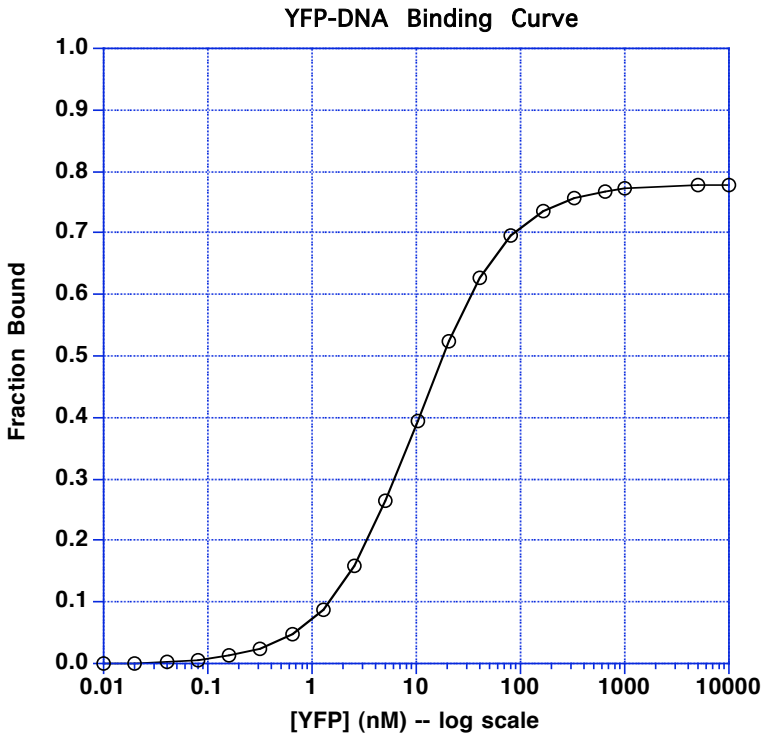
(e; 6 pts) Note that the recognition site for YFP is a direct repeat. What general kind of transcription factor have we mentioned that binds direct repeats? Why are transcription factors typically dimeric?

Score for the page_____

2. Methods and miscellaneous (38 pts):

The curve below shows a protein-DNA binding curve derived from EMSA data. Note the *y*-axis limits.

(a; 4 pts) What is the equation for the fraction of DNA bound as a function of protein concentration for a simple protein-DNA binding curve?



(b; 6 pts) On the graph, sketch the curve you would expect for YFP-DNA binding with a K_d of 20 nM. Indicate how you would determine K_d from the graph.

(c; 12 pts) Give a possible physical explanation for the difference between expectation and reality. How can you modify the equation from (a) to account for the phenomenon? What do you think the true K_d is for the YFP-DNA interaction?

Score for the page_____

(d; 9 pts) ChIP-on-a-chip reports on all of the binding sites for YFP. There are many sequences that match the consensus above but are not observed to have bound YFP in vivo. On the other hand, there are also many genes where the observed fold change in transcriptional activity +/- YFP is much larger than the observed change in occupancy. Briefly discuss a common explanation for these observations.

(e; 7 pts) What is the chromatin modification commonly associated with transcriptional activation? How might you assess its role at the genomic level?

Score for the page_____

3. DNA Replication (27 pts):

(a; 12 pts) Sketch the "trombone model" for the Pol III replication fork at the instant that the lagging strand polymerase is starting to extend an RNA primer. Your diagram should include the core polymerases, the τ dimer, the clamp loader, SSB, β sliding clamps, primase, and helicase.

(b; 6 pts) What is the advantage to the cell in having primer-template DNA but not nicked DNA stabilize the β-core interaction? How does this play out during the synthesis of an Okazaki fragment?

Score for the page_____

(c; 9 pts) How does kinetic partitioning allow the 3′→5′ exonuclease activity of a DNA polymerase to make a large contribution to improved fidelity even though the exo activity itself is may not be particularly selective for mismatched DNA? Give a possible reason for the lethality of an active mutant of the proofreading ε subunit of Pol III.

Page	Score
1	
2	
3	
4	
5	
6	
7	
Total	

Score for the page_____