Biochemistry 674: Nucleic Acids **Your Name:** _____

Exam II, November 18, 1997 **Prof. Jason Kahn**

You do not need a calculator.

Guess if you don't know.

Give clear and concise answers.

You have 85 minutes for this exam.

1. Methods (20 pts)

(a) (4 pts) Draw ATP, and indicate which phosphorus atoms should be ^{32}P radiolabel for use with (*i*) T4 polynucleotide kinase and (*ii*) the Klenow fragment of DNA polymerase I (which of course would actually use dATP).

ATP

(b) (5 pts) On the substrate below, probes made with Klenow can be made hotter than probes made with kinase, and T4 DNA polymerase, which has a more active $3' \rightarrow 5'$ exonuclease activity, is even better. Sketch the product of the Klenow fill-in reaction with labeled dATP and unlabeled d(C,G,T)TP, and explain the above observation.

5′ HO-GATCG ——————————— C-OH 3′

3′ HO-C ——————————— GCTAG-OH 5′

(c) (4 pts) "PCRanoia", as in "paranoia", is a common affliction in molecular biology labs, especially labs which look at ancient or single-copy DNA. This condition arises from the exquisite sensitivity of PCR. What common problem can arise from the ability to amplify tiny amounts of DNA, and why is it worse when template is available in limited quantities?

(d) (7 pts) Modification-interference is used to identify nucleic acid sites which affect protein binding upon chemical modification, e.g. phosphate ethylation. Explain how **enhancement** as opposed to the usual decrease of protein binding due to modification could arise in this experiment if the binding protein causes DNA deformation, and sketch the resulting appearance of the final denaturing (sequencing) gel, showing 3 lanes: bound, free, and modified but not fractionated.

2. DNA replication (20 pts)

(a) (10 pts) Draw two sketches of the *E.* coli DNA polymerase III replication fork according to the dimeric Pol III model, one sketch for the beginning and one for the end (*i.e.* 1 sec later) of Okazaki fragment synthesis. Include the DNA, pol III cores, and sliding clamps, and label 5′ and 3′ ends. You have room to try twice.

Beginning of Okazaki fragment synthesis

End of fragment synthesis

(b) (6 pts) Sketch a theta structure DNA replication intermediate. If you used a pulse of radiolabel for 1 sec, starting 1 sec after initiation of replication (at 1000 nt/sec, for a 12000-bp plasmid), and then quenched, what would the resulting microscopic autoradiograph look like? What would it look like if replication were unidirectional?

(c) (4 pts) Why have some scientists who engineered DNA polymerases for improved DNA sequencing removed the polymerases' 3′ to 5′ exonuclease activities, or else recommended the use of pyrophosphatase in sequencing reactions?

3. Protein-DNA interaction (20 pts)

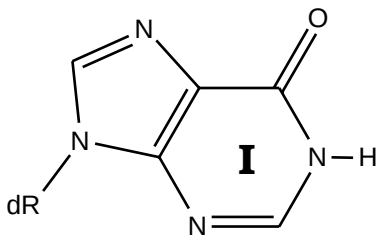
(a) (6 pts) How many base pairs of DNA does a single zinc finger (ZnF) module recognize? Why do all ZnF DNA-binding proteins comprise >1 module? What is the role of the zinc in the zinc finger?

(b) (2 pts) What eating utensil model describes the GCN4-DNA interaction?

(c) (6 pts) Briefly describe two ways whereby proteins recognize sequence-specific DNA sites besides direct protein-nucleobase hydrogen bonding.

(d) (6 pts) TBP normally binds the AT-rich TATA box. Substitution of G-C for A-T base pairs reduces binding, but substitution of Inosine-C base pairs for A-T does not (*i.e.* TBP still binds). Draw in the Cytosine of the I-C pair. How can this observation be used as evidence for minor-groove binding?

Inosine:



4. Transcription (20 pts)

(a) (4 pts) Describe two changes in the ternary RNA•DNA•RNAP complex which occur as *Escherichia coli* RNA polymerase escapes the promoter (converts from initiation to elongation).

(b) (3 pts) Why do RNA polymerases, unlike DNA polymerases, have to be absolutely processive?

(c) (3 pts) Explain how the sigma subunit can confer sequence-specific DNA binding on core RNAP even though isolated sigma does not bind DNA.

Polarity is a phenomenon whereby a nonsense codon in a polycistronic bacterial mRNA leads to decreased expression of downstream genes, which normally would be translated off the same mRNA. A proposed mechanism holds that this has to do with the action of rho (ρ) factor and coupled transcription-translation.

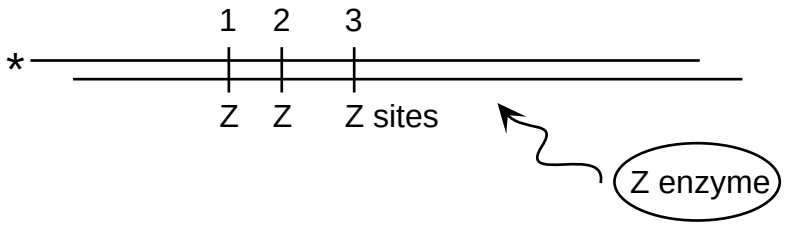
(d) (5 pts) Sketch and describe the general features of ρ-dependent transcription termination. Recall that ρ loads onto long stretches of relatively unstructured naked RNA. If you can't answer this, explain ρ-independent transcription termination instead, but skip part (e).

(e) (5 pts) Sketch and explain how polarity arises.

5. Experimental manifestations (20 pts).

(a) (2 pts) What is the name of the visualization program demonstrated in class and used for web demos?

(b) The following experiment has been used to test for restriction enzyme sliding. The molecule shown is exposed to restriction enzyme Z for a short time, such that digestion is incomplete.



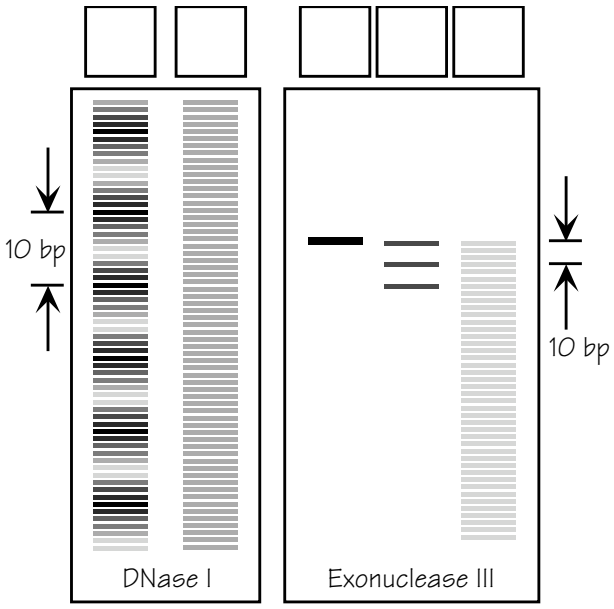
(2 pts) If the enzyme finds its site only by 3-dimensional diffusion, what would the relative rates of cleavage of the three sites be?

(3 pts) If the enzyme slides to find its site, and we assume that it cuts whenever it locates a site and then falls off the DNA, what will the relative cleavage rates be and why (qualitatively—which site will be fastest/slowest/intermediate)?

(2 pts) What would a gel of the diffusion vs. sliding reaction mixes look like (i.e. sketch the two lanes, assuming ~10% cleavage of the total number of sites)? Assume that the extent of reaction is the same for each. In real life, salt concentration could be used to switch search modes.

(c) (6 pts) What is the equation for fractional saturation Θ as a function of total protein concentration $[P]_t$, for a simple non-cooperative DNA binding equilibrium at negligible [DNA]? Sketch the graph of Θ versus $[P]_t$. What famous oxygen-binding protein has a similar binding isotherm for its ligand?

(d) (5 pts) In the boxes above each lane on the gels below, write "T", "R" or "N" according to whether the lane provides evidence for rotational, translational, or no nucleosome positioning.



Question	Score
1	
2	
3	
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