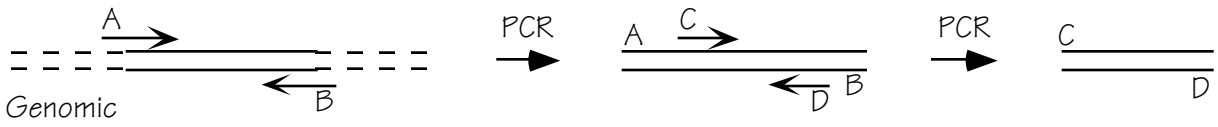
Biochemistry 674, Fall, 1995: Nucleic Acids **Prof. Jason Kahn**

Exam II: November 16, 1995 **Your Name Here:** _____

This exam has five questions worth 20 points each. Answer all five. You do not need a calculator. I have provided more writing space on this exam than on exam I, but your answers should still be concise. There is extra space on the last page if you need it.

1. Methods

a. "Nested PCR" is commonly used to identify the presence of rare sequences in genomic or other complex DNA mixtures. In this method, a PCR amplification is performed and then the products of the reaction are subjected to a second round of PCR using primers that hybridize internally to the expected product of the first round (see the diagram below; letters are just to indicate positions).



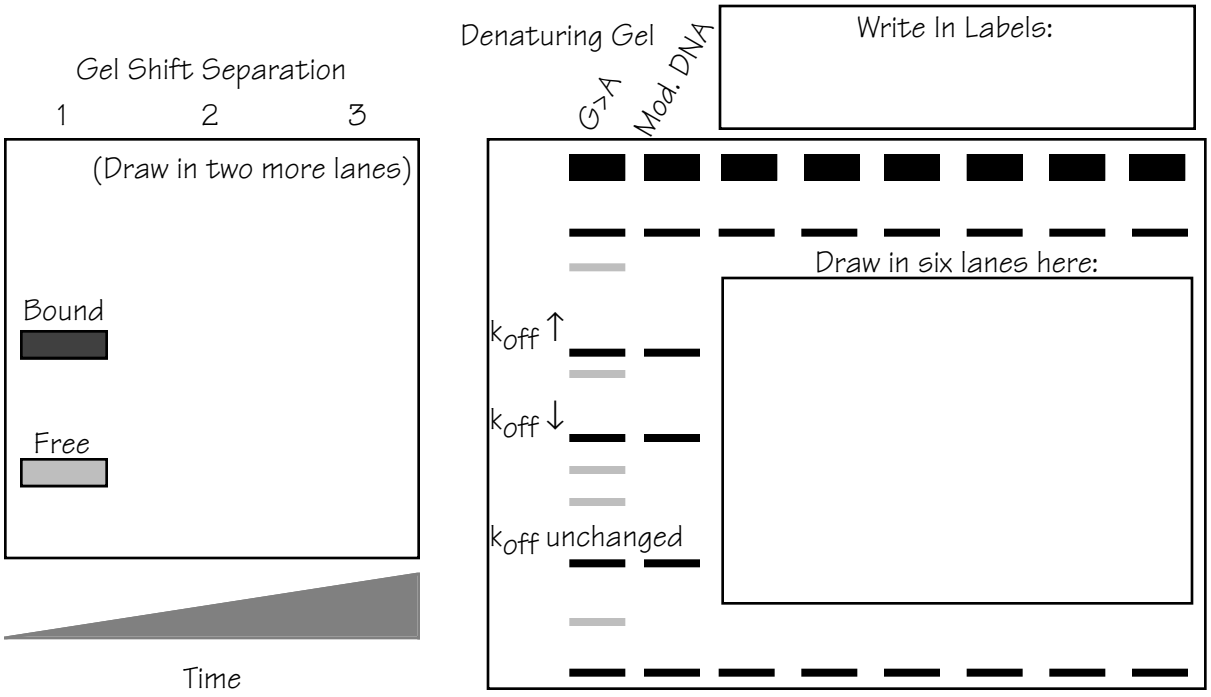
(4 pts) How does the second amplification improve specificity?

(4 pts) Typically the products of the first PCR are diluted before the second reaction. If this is not done, the procedure is likely not to work. Why is this?

b. We have discussed the use of the gel shift assay and modification interference in analyzing the thermodynamics of protein-nucleic acid interaction. In principle, this method could also be applied to kinetics. We want to design a modification-interference experiment that would allow us to identify DNA sites at which modification speeds up or slows down protein-DNA complex dissociation.

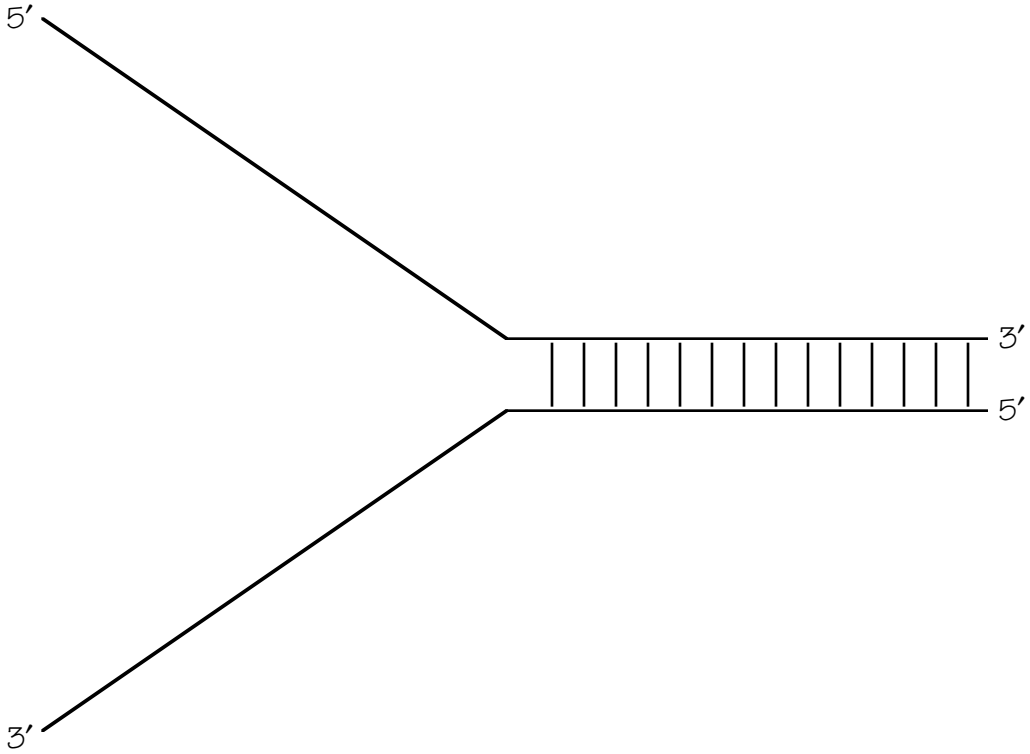
(6 pts) Describe briefly how you would run each step and the purpose of each. Assume that we have lightly DMS-treated DNA available.

(6 pts) Draw the expected results (band position and intensity) in the gel pictures below. Assume that equal amounts of radioactivity are loaded into each of the lanes of the denaturing gel. As indicated, draw the patterns on the denaturing gel that would indicate a an increase, decrease, or no effect on off-rate due to a modification and label which is which. [Note: This was not a successful question.]



2. DNA replication

a. The diagram below shows a prokaryotic DNA replication fork.



(3 pts) Draw in the leading and lagging strands and label them, assuming that we are just beginning a new Okazaki fragment. Distinguish RNA and DNA.

(3 pts) Add polymerase III holoenzymes, sliding clamps, SSB, and the DNA helicase to your diagram. For simplicity, do not draw the holoenzyme dimerization.

(4 pts) The remaining activities are the primase, DNA polymerase I or RNAse H, DNA ligase, and the clamp loader. Briefly describe the function of each but don't draw them in.

(3 pts) Why has evolution chosen to link two polymerase II holoenzymes in a dimeric complex?

b. DNA polymerases typically have associated 3′→5′ exonuclease activities.
(2 pts) What is the function of the 3′→5′ exo in DNA replication?

(5 pts) T4 phage variants have been identified whose mutation rates are increased ("mutator") or decreased ("anti-mutator"), and the 3′→5′ exo has been identified as the activity causing the phenotype. Would the "anti-mutator" have *increased* or *decreased* exo activity? Give two possible reasons that the "anti-mutator" appears to be less fit in terms of evolution than the wild type phage.

3. Sequence-specific recognition of nucleic acids

a. Seeman et al. (1975) proposed that recognition of DNA base pairs will proceed through hydrogen bonding to donors and acceptors in the DNA major and minor grooves.

(3 pts) Draw a plausible structure for recognition of adenine by Asn ($R = -CH_2C(O)NH_2$):

(5 pts) What was the early 80's idea of a "recognition code" for protein-nucleic acid interaction? Briefly describe two structural aspects or recognition modes of protein-DNA complexes that have shown that no such simple code exists.

(4 pts) Besides the difficulty of RNA major groove recognition, how are the RNA-protein complexes described in class fundamentally different from the simple DNA-protein model typified by helix-turn-helix-DNA complexes?

b. Nucleosomes can be rotationally and translationally positioned, and this can be an important element of their function in control of gene expression and other DNA transactions.

(4 pts) Define each type of nucleosome positioning and give one way to experimentally distinguish them.

(4 pts) Which type of nucleosome positioning is easier to explain and control, and why?

4. Facilitated diffusion and action at a distance.

(4 pts) Define facilitated diffusion. Why is it used in biological systems?

(4 pts) Qualitatively, given a protein-DNA association reaction which can be accelerated by sliding, how does the association rate constant depend on salt-concentration, and why?

(4 pts each) You have identified a protein that binds at site A and activates transcription starting from site B. You have been provided with the sets of DNA substrates below. How can they be used to determine whether the interaction is mediated via looping, tracking, or long-range transmission of topological change? For simplicity, make comparisons among results expected for each mechanism only within each set, not between sets. An example of the desired type of answer is given.

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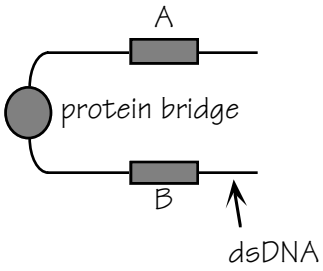
<td> 
</td>
<td>If it's looping, it will still work. If it's sliding or topological transmission, it won't.</td>
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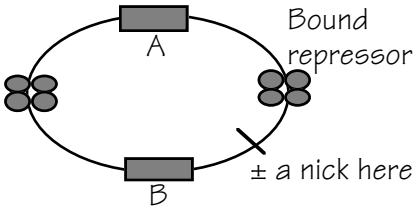
<td> 
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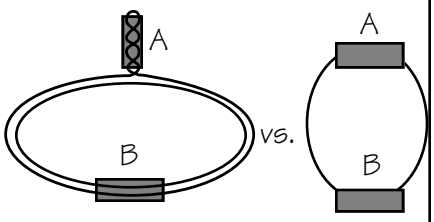
<td> 
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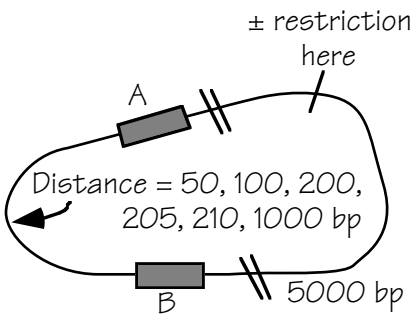
<td> 
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5. Transcription, transcription factors, and structural motifs

a. (5 pts) Draw lines to specify a one-to-one correspondence among the DNA-binding proteins or motifs on the left and the properties on the right.

helix-turn-helix	β-sheet recognition motif
bZIP proteins	non-specific but strong binding, largely electrostatic
histone octamer	requires an ATPase for assembly onto DNA
PCNA	induces DNA strand separation
zinc finger	protein folds only upon binding to DNA
TATA-binding protein	at least 2 and usually more required to bind
RNA polymerase	found in phage repressors and homeodomains

b. The transcription cycle refers to the entire process of generating an RNA.
(5 pts) Give a one-sentence description of each of the four steps of the transcription cycle.

(5 pts) Qualitatively describe the abortive initiation process and its use in a polymerase assay. What important step of initiation is not observed with this assay?

(5 pts) In general, what is the biochemical role of pausing in transcription? Give one illustrative example.

Question	Score
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