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

Prof. Jason Kahn **Exam II, November 16, 2000**

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.
You may need a calculator for this exam. No other study aids or materials are permitted.
Generous partial credit will be given, *i.e.*, if you don't know, guess.

1. Chromatin (20 pts):

(a; 6 pts) We discussed the notion of "marks" left on histone tails by modifying enzymes. Give two examples of such marks. How are marks (in general) believed to be important in gene regulation?

(b; 9 pts) Briefly describe how the chromatin immunoprecipitation assay works, using antibodies to YFTF (your favorite transcription factor). Why does it provide better evidence for the in vivo involvement of YFTF at a particular site than previous methods of correlating in vitro and in vivo footprints?

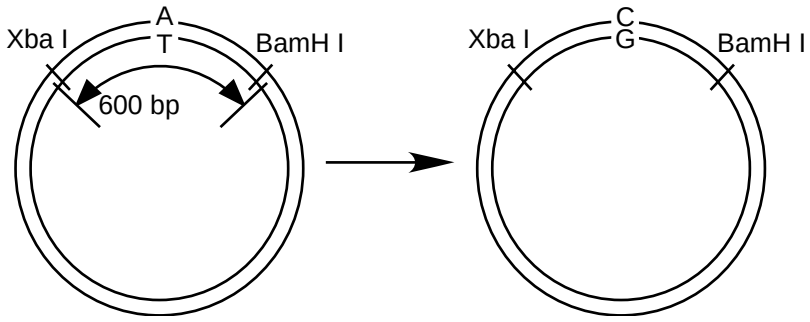
(c; 5 pts) Is the supercoiling induced by nucleosomes restrained or unrestrained? How could you detect the existence of unrestrained supercoiling in vivo?

2. Methods (20 pts).

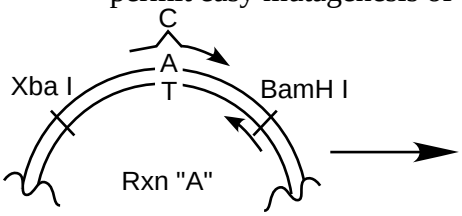
PCR has many uses, among them site-directed mutagenesis. It is straightforward to produce a PCR product which is not identical to the template by including a mismatch in the primer, but this is just one part of a practical method.

In this problem, assume that Xba I and BamH I are the only restriction enzymes available.

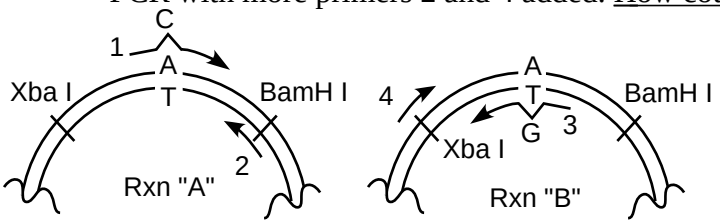
Desired conversion:



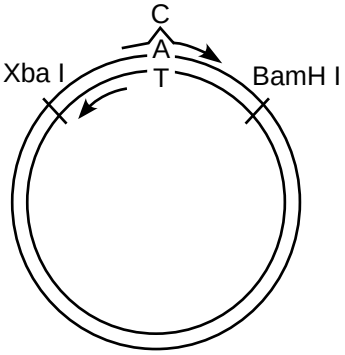
(a; 4 pts) In the example shown below, simply using a mismatched primer in PCR does not permit easy mutagenesis of the plasmid. What is the product and why doesn't it help much?



(b; 8 pts) Using an additional reaction can make the idea work. Sketch the product you would obtain if you mixed the products of PCR reactions A and B below, and did a few cycles of PCR with more primers 2 and 4 added. How could you use it to make the desired mutant?



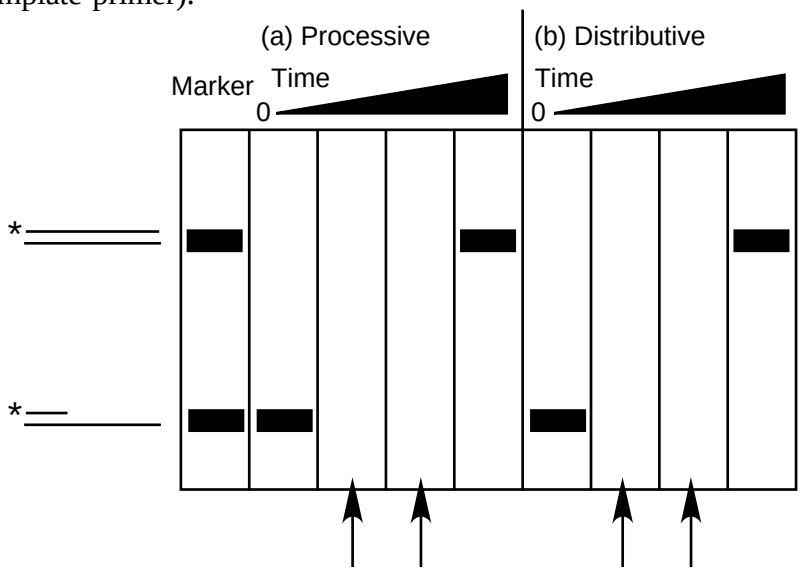
(c; 8 pts) The method can be further simplified. How could you use PCR with the two primers below to make the desired mutant—what enzymes and steps would be needed to get a plasmid with which to transform ?



3. Replication (20 pts).

(a; 6 pts) On the lanes indicated with arrows on the autoradiogram below, draw the product distributions for extension of the indicated template-primer. as a function of time, expected for (a) a processive DNA polymerase and (b) a distributive polymerase. The reactions are done at limiting enzyme (i.e. excess template-primer).

The marker lane includes samples of the starting primer-template and the final product of replication, as indicated by the pictures on the left.



(b; 3 pts) Why is it essential that the replicative DNA polymerase (Pol III in *E. coli*) be highly processive?

(c; 5 pts) What protein is the processivity factor for the Pol III core? What does it look like? What other machinery is needed to handle the processivity factor? How is rapid recycling of the lagging strand Pol III achieved? (Very short answers expected.)

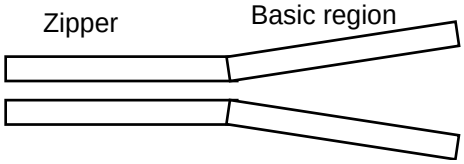
(d; 6 pts) What is the end-replication problem, and how does telomerase provide a solution? How was it demonstrated that a section of the internal RNA of telomerase serves as a template for the enzyme's reverse transcriptase activity?

4. Protein-DNA interaction (20 pts)

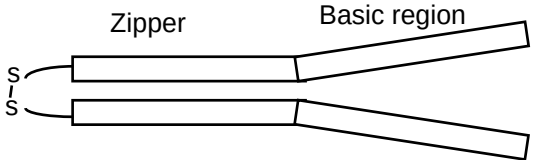
(a; 6 pts) Protein folding or refolding upon DNA binding is a common and fascinating phenomenon. Why might evolution have selected for this in the case of (a) leucine zipper transcription factors and (b) restriction enzymes (separate answers for the two cases)?

Since leucine zippers are monomeric at concentrations up to 10 μM, much higher than the concentrations needed to bind DNA effectively, we know that ΔG (not ΔG^0) is positive for dimerization under binding conditions. It is, however, possible to make bZIP protein models which are linked by disulfides and thereby remain dimeric at essentially zero concentration:

Natural:



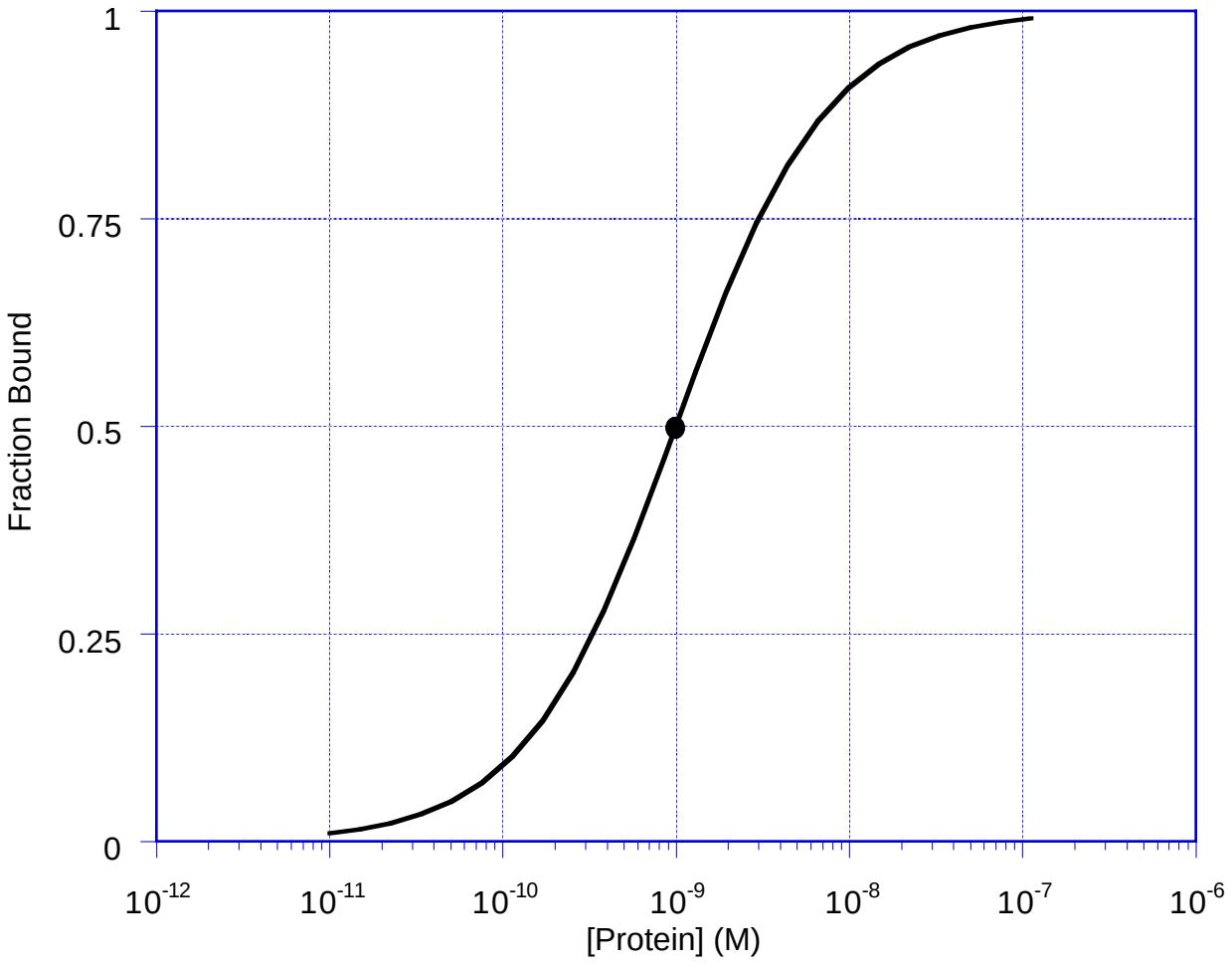
Disulfide-linked:



(b; 6 pts) Will the linked (oxidized) bZIP protein bind with higher or lower affinity than the unlinked (reduced) bZIP? Why?

(c; 4 pts) The graph below shows a binding curve for a garden variety monomeric DNA binding protein. Sketch the curve expected for a reduced bZIP protein that binds 50% of the input DNA at a protein dimer concentration of 1 nM.

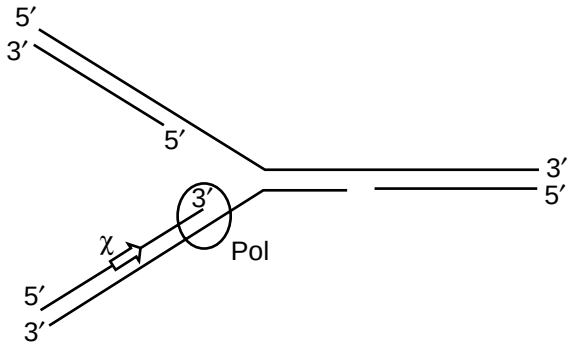
(d; 4 pts) Sketch the curve expected for the oxidized protein. I'm looking for a shift direction and a shape, don't worry about the numerical value of K_{diss}.



5. Recombination (20 pts)

The replication engine shown is headed for trouble due to the nick on the leading strand template. This situation is probably why RecBCD is important for cell viability.

Note: parts a-c below are drawn directly from lecture.



(a; 3 pts) Draw the nucleic acids immediately after the leading strand polymerase copies the last base before the nick. You need not draw proteins.

(b; 7 pts) Sketch the steps in RecBCD + RecA-mediated use of the double-strand break product for strand invasion into the lagging strand duplex. Assume that the lagging strand has been filled in and re-ligated, i.e. it's a clean duplex.

(c; 4 pts) Indicate on your last diagram in (b) how the product of this reaction is equivalent to a Holliday junction plus a replication fork, or draw the junction plus fork below.

(d; 6 pts) Does it matter which way the Holliday junction is resolved, i.e. is there a genetic consequence? Why or why not? Sketch the theta structure resulting from a crossover event (i.e. resolution so as to switch partners), and suggest an experimental (albeit difficult-to-test) prediction from the mechanism you have drawn.

Question	Score
1	/20
2	/20
3	/20
4	/20
5	/20
Total	/100