Biochemistry 465 **Your Printed Name:**_____

Section I

March 4, 1999 **Your SS#:**_____

Exam #1

Prof. Jason D. Kahn **Your Signature:**_____

You have 80 minutes for this exam.

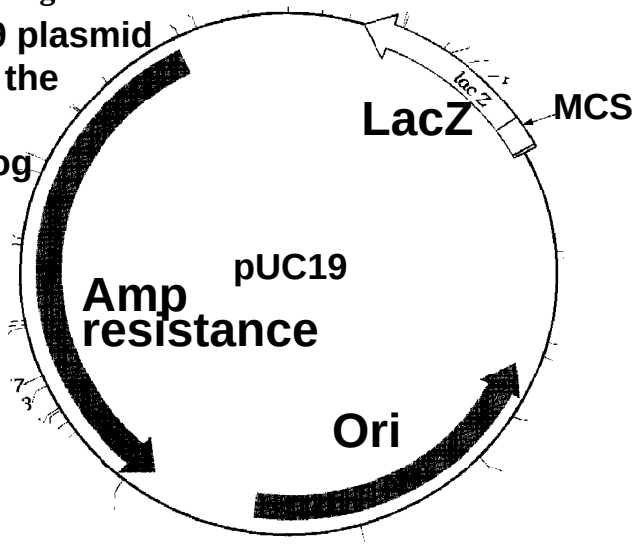
Exams written in pencil or erasable ink will not be re-graded under any circumstances.

Explanations should be concise.

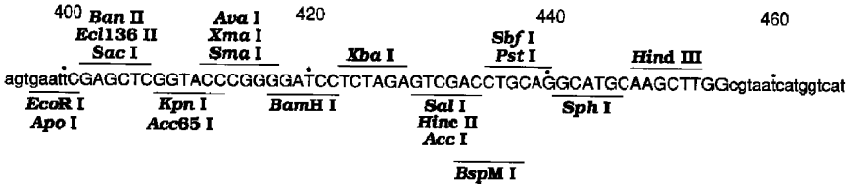
You will not need a calculator for this exam, and no other study aids or materials are permitted.

1. (20 pts) Plasmid-based cloning of DNA.

Map of pUC19 plasmid adapted from the New England Biolabs catalog



MCS:



(a; 6 pts) You are given a DNA restriction fragment with Xba I and Pst I ends. How would you go about cloning it into the pUC19 vector shown above—what steps would you take and what enzymes would each step require?

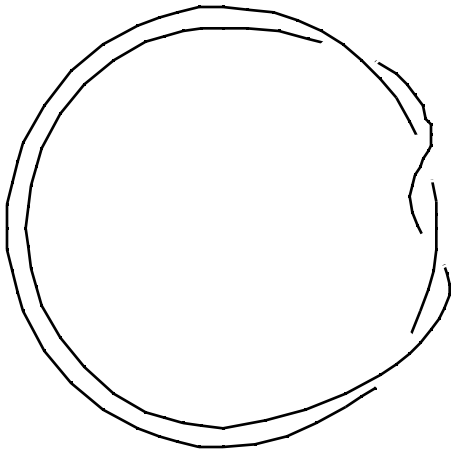
(b, 8 pts) Briefly discuss how the Amp^r gene, the Ori region, and the LacZ gene of pUC19 are essential to the cloning of DNA restriction fragments using blue/white colony screening. What are IPTG and X-gal used for?

(c; 5 pts) Cloning is significantly trickier if you need to clone a fragment whose ends were generated by only one restriction enzyme (e.g. both ends might be Xba I ends). In this case it is much more important to use phosphatase at one step from (a). Which step, and why is phosphatase needed?

(d; 1 pt) What does YFD stand for?

2. DNA Topology and Topoisomerases

The drawing at the right shows a closed circular DNA with altered twist. As usual, we draw only the twist increment (i.e. the parallel strands are assumed to be normal helical B-DNA; the relaxed circular DNA would be represented as non-intersecting circles). Remember that the topological convention is that the two DNA strands run in the same direction.



(a; 4 pts) Assign signs to all of the nodes in the figure and calculate ΔLk relative to the relaxed circle.

(b; 4 pts) Draw the DNA shape which would result if the ΔTw in the figure were converted into plectonemic superhelical writhe (i.e. draw a figure with the same Lk as above but with ΔTw = 0 relative to the relaxed circle). You can draw the double-stranded DNA as one line. Label the nodes.

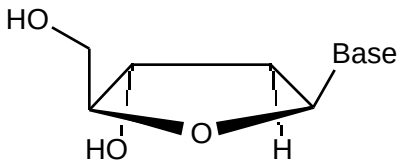
(c; 4 pts) Draw the same DNA with the same Lk but as a toroidal superhelix. Label the nodes.

(d; 4 pts) Ethidium bromide binds to the circle and changes the twist by $\Delta Tw = -2$ relative to the diagram I provided. What will ΔLk, ΔTw, and ΔWr be relative to the relaxed circle?

(e; 4 pts) What is the linking number change induced by a Type I topoisomerase? What general type of mechanism was proposed for the enzyme (a two-word answer would suffice)? Answer the same two questions for Type II topoisomerases.

3. (20 pts) Base pairing and helical structures

(a; 6 pts) Imagine that the sugar drawn at the right is used as the backbone sugar for DNA on the planet Rorrim, with the same phosphate and bases as used here. What helical shape would the DNA adopt, and why? (Hint: draw the ribose that we use on Earth and compare.) Would DNA isolated from bacteria on Rorrim tend to be positively or negatively supercoiled?

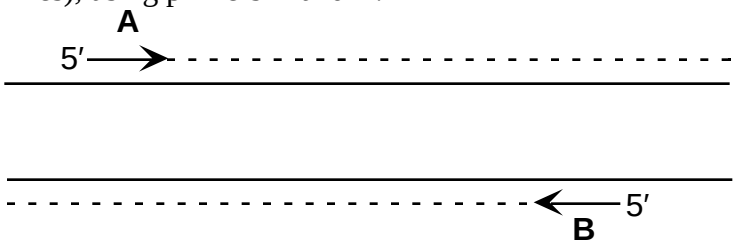


(b; 10 pts) In the space below, draw the a T•A-T triple base pair with the third-strand T binding to the Hoogsteen face of the Watson-Crick A. Is the third strand parallel or antiparallel to the Watson-Crick T strand? Why? You need not draw the sugars.

(c; 4 pts) Draw the pseudodyad axis of the W-C base pair on the diagram you drew above. Why is it called a “pseudo” dyad and what is important about it in terms if the uniqueness of the W-C base pairs vs. other possible H-bonding arrangements?

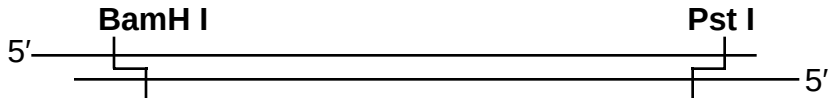
4. PCR, enzymatic methods.

The diagram below shows the products after the first round of PCR amplification of a region of template DNA (solid lines), using primers A and B.



(a; 10 pts) Draw the products of each of the next two rounds, using a dashed line for the DNA synthesized in each round. Which of the third-round products will be amplified exponentially (i.e. the amount will scale with 2^n, where n is the number of cycles) in subsequent cycles?

(Unrelated to above.) For many applications, it is necessary to make double-stranded DNA which is ^{32}P labeled at or near one and only one end. We often don't really care about what exact DNA sequence or structure is at the end. We will start with the fragment below, with 5′ phosphate ends:



(b; 5 pts) Suggest a method for making a singly-labeled fragment, picking from among the following tools: Pst I, BamH I, phosphatase, T4 polynucleotide kinase, and γ-^{32}P-rATP (there is more than one correct answer). Assume you can separate double-stranded fragments of markedly different sizes using electrophoresis .

(c; 5 pts) Suggest a method for obtaining singly-labeled material using a DNA polymerase instead of polynucleotide kinase, and specify the flavor of radiolabeled triphosphate you would need instead of γ-^{32}P-rATP.

5. Thermodynamics, tertiary structure, hybridization

(a; 4 pts) We know that the hydrolysis of DNA is thermodynamically favorable (nucleases don't require high-energy cosubstrates). Given this fact, what must be true about the mechanism of DNA ligases, even if we don't know the details? Why?

(b; 6 pts) How are RNA folding and protein folding fundamentally different from each other in terms of the relationship between secondary structure and tertiary structure? Why are divalent metals important to RNA tertiary structure?

(c; 5 pts) What common phenomenon underlies both hypochromism and the enthalpic stabilization of DNA secondary structure? What thermodynamic force opposes secondary structure formation, and why?

(d; 5 pts) Think up a possible future application of gene chips (applied to either RNA or DNA) which you think would be a bad idea for our society.

Do Not Write Below This Line

Score:	Question 1:	_____	out of 20: Plasmid cloning
	Question 2:	_____	out of 20: DNA topology and topoisomerases
	Question 3:	_____	out of 20: Base pairing and helical structures
	Question 4:	_____	out of 20: PCR, enzymatic manipulations
	Question 5:	_____	out of 20: Thermodynamics, 3° structure, hybridization
	Total:	_____	**out of 100**