Biochemistry 661 **Your Name:** Key

Nucleic Acids, Module I **Prof. Jason Kahn**

Exam I (100 points total) **September 23, 2010**

You have 60 minutes for this exam.

N=32

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. There is a extra space on the last page if you need it.

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

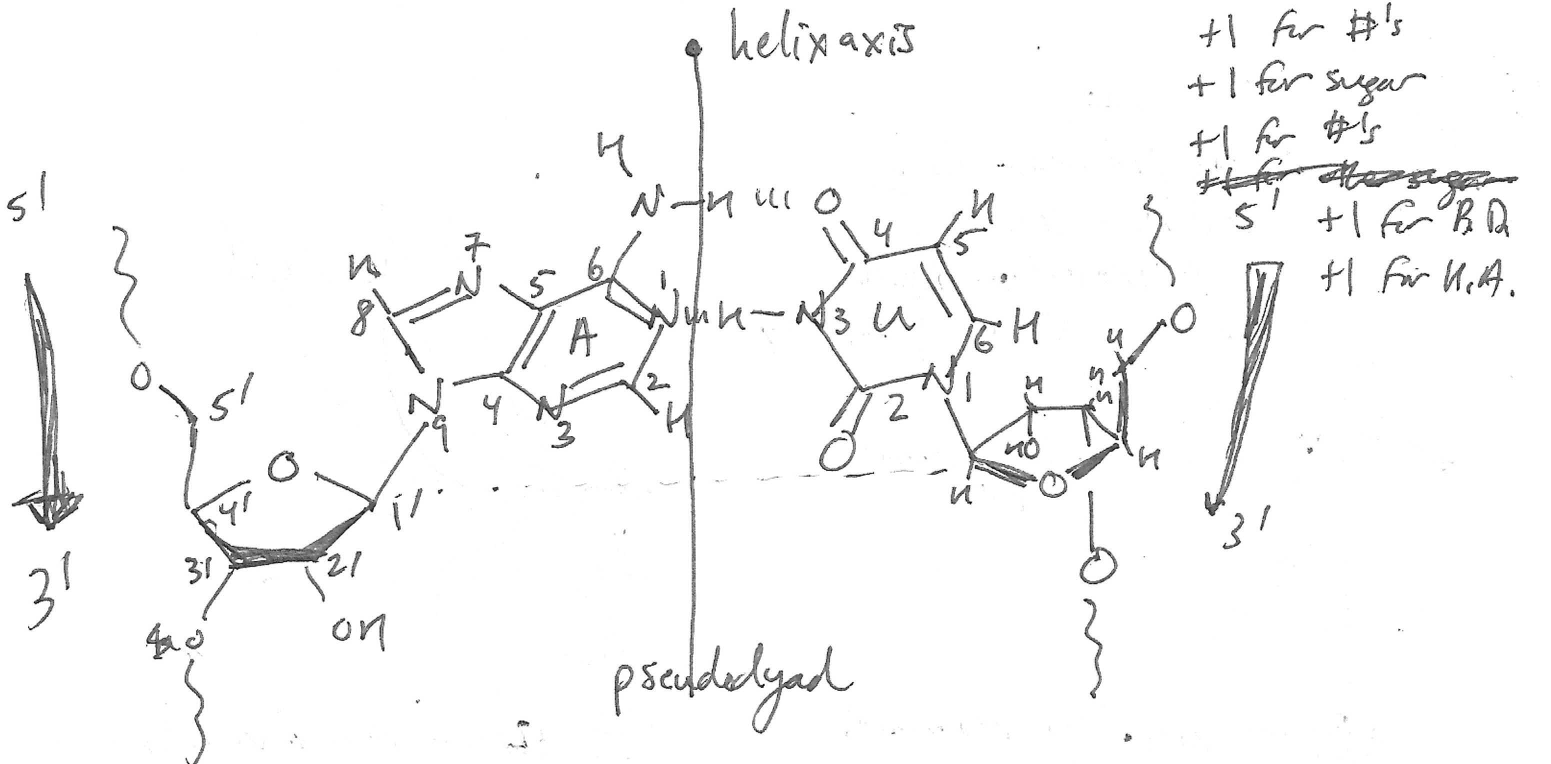
Generous partial credit will be given, *i.e.*, if you don't know, guess.

Honor Pledge: At the end of the examination time , please write out the following sentence 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. DNA Structure and Flexibility (28 pts):

(a; 10 pts) Draw a Watson-Crick A:U pair in RNA. Indicate the pseudodyad axis and the approximate location for the intersection of the A-form helix axis with the base plane. Draw the sugars and include the numbering on one sugar and both bases.



Score for the page_____/10

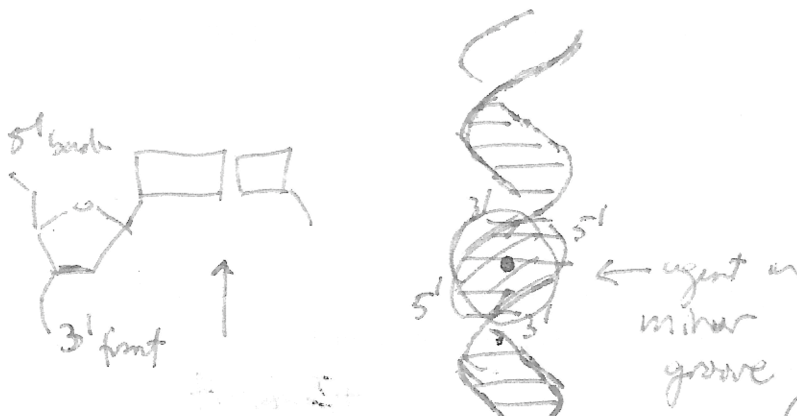
(b; 3 pts) We have emphasized over and over what it is that makes the Watson-Crick base pairs special. What is it?

(+3) They fit into a regular helical geometry

(c; 2 pts) The __α__ phosphate on ATP is incorporated into nucleic acid by polymerases. The __γ__ phosphate is added to the end of a nucleic acid by T4 polynucleotide kinase.

(+1) each

(d; 5 pts) Sketch a DNA helix to explain why the cleavage patterns generated by a groove-bound hydroxyl radical generating reagents in the minor groove would be "staggered" to the 3′ side.

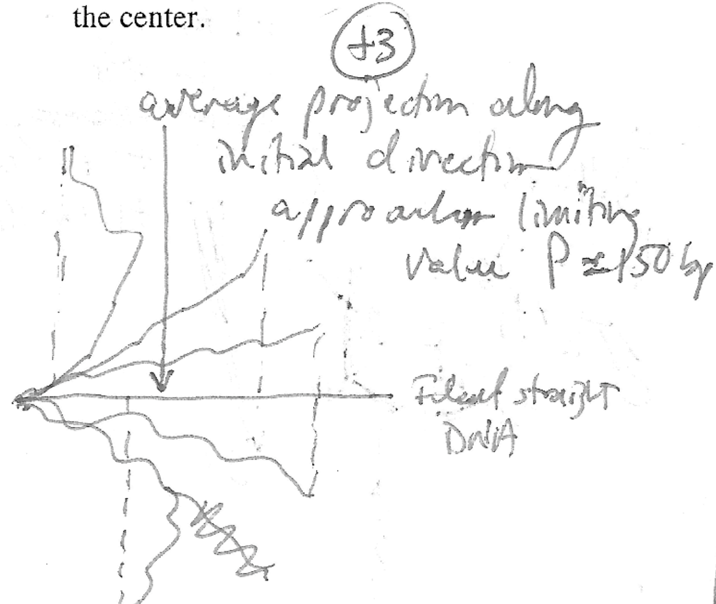


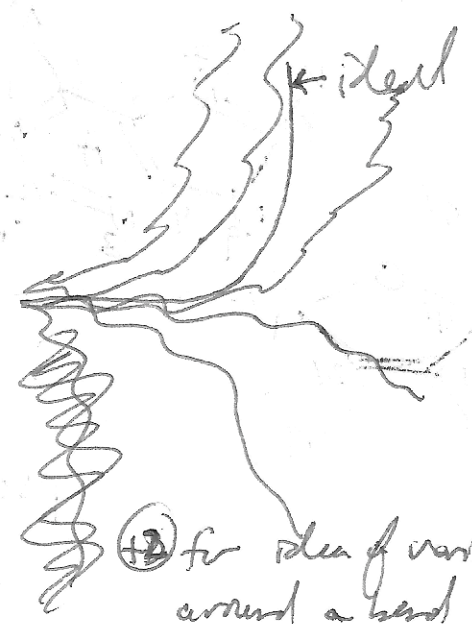
(+2) Diffusion to closest sugar leads to an offset to the 3' side

(+1) for RH DNA sketch

(+2) for sketch that shows the point

(e; 8 pts) Sketch a picture illustrating the idea of persistence length: show five random 300-bp DNA molecules aligned at one end, and indicate the displacement that we talked about in defining the persistence length. Similarly, sketch a set of molecules for a DNA that has an intrinsic 90° bend in the center.





Score for the page ____18____

2. Hybridization (18 pts):

(a; 9 pts) Even though hydrogen bonds are quite strong, to a first approximation they do not contribute very much to the stability of double-stranded nucleic acids. Why not? To a second approximation, they do actually contribute some stabilization. Why? The current nearest-neighbor thermodynamics model includes an H-bond term that is parameterized according to the number of terminal A-T base pairs (as opposed to G-C). Considering the nearest neighbors in the following two sequences, explain why: ATCGA vs. GATCG.

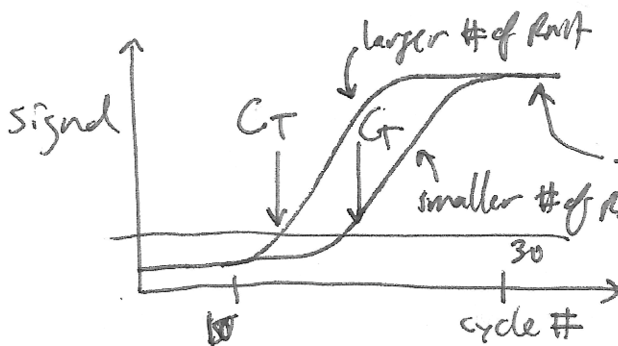
(+3) – H bonds are exchanged with water – ↓ net contribution

(+2) – But H bonds in W-C pairs are formed a larger %age of the time and have closer to ideal geometry

ATCGA → 1 AT, 1 TC, 1 CG, 1 GA
GATCG → " , " , " , "
(+2) for same NN's

(+2) But bottom sequence has 3 GC b/p vs. 2 for top

(b; 9 pts) Briefly describe how quantitative (real-time) PCR can be used to count small numbers of RNA transcripts. You don't need to describe the cycling in detail.



(+2) for general idea of breakthrough followed by exponential growth

C_T = cycle threshold [actually it's all exponential, but early cycles are invisible]

(+2) – Regular PCR is not quantitative because large or small #'s of transcripts can give the same limiting amount of product

(+2) – QPCR measures accumulation at each cycle

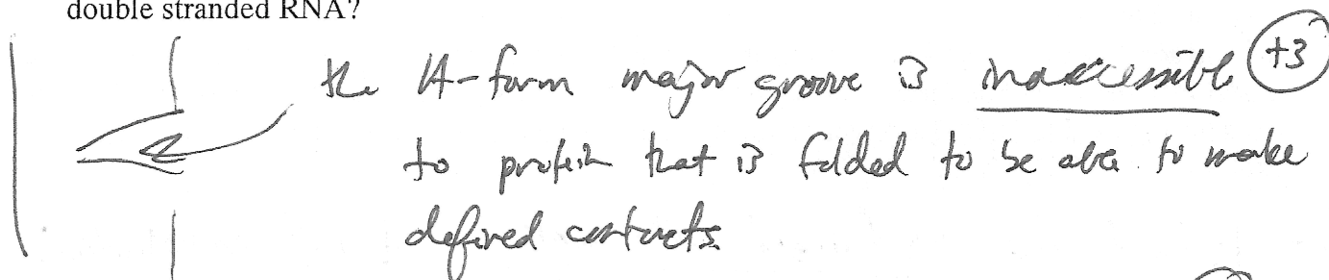
(+3) – The more copies of the RNA, the earlier the signal rises above the noise

– Calibrate vs. a dilution series

Score for the page 18

3. RNA Structure (12 pts):

(a; 3 pts) Why don't sequence-specific RNA binding proteins recognize the major groove of fully double stranded RNA?

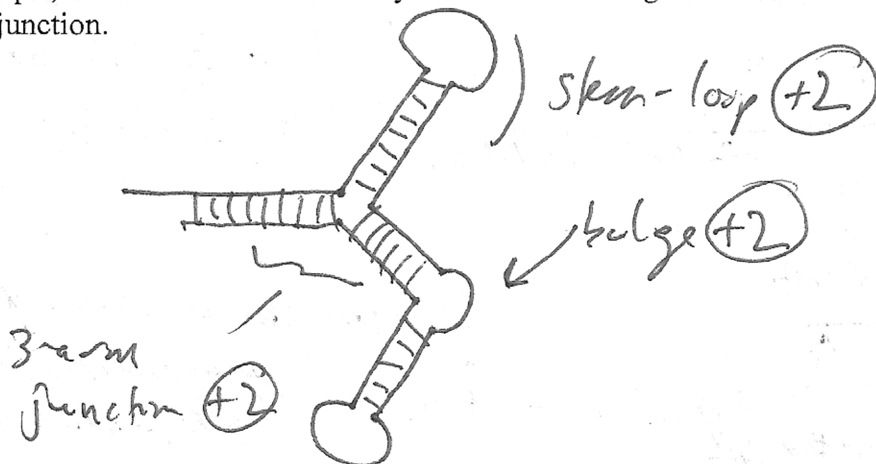


the A-form major groove is inaccessible (+3) to protein that is folded to be able to make defined contacts

(b; 3 pts) Why does tertiary folding of RNA usually require divalent metal ions? (+2)

– tertiary folding brings backbone Ⓟ's close together, and charge neutralization requires high charge density (+1)

(c; 6 pts) Sketch an RNA secondary structure including at least one stem-loop, a bulge, and a three-arm junction.



4. DNA Topology (24 pts):

(a; 9 pts) Many antibiotics (e.g. Ciprofloxacin) are bacterial gyrase (Type II topoisomerase) inhibitors. Why are they specific to bacteria? Why are several topoisomerase poisons used as chemotherapy agents? What is the ideal step in the topoisomerase reaction cycle with which to interfere?

(+3) – Bacterial gyrase is quite different from eukaryotic Type II topoisomerases – gyrase can induce ⊖ supercoiling, euks. only relax

(+3) – Rapidly dividing cells require topoisomerase activity to complete replication, therefore cancer is more vulnerable.

(+3) – After cleavage and before religation – introduces permanent strand breaks, messes with repair

Score for the page 21

(b; 15 pts) Sketch two closed circular DNAs, one with ΔLk = +3 and one with ΔLk = -3, showing toroidal superhelices, one with writhe = +3 and one with writhe = -3 (i.e. assuming no ΔTw relative to relaxed DNA). Then draw two molecules with the same ΔLk's but with each having ΔTw = -3 relative to relaxed DNA. It is not easy to do this conversion for positively supercoiled DNA. Why do you think proteins that create or stabilize positive writhe are found in some thermophiles (heat loving organisms)?

ΔWr = -3
ΔTw = 0
ΔLk = -3
(+3)

(+3)
Wr = +3
ΔTw = 0
ΔLk = +3
(same)

Wr = 0
ΔTw = -3
ΔLk = -3
uniform untwisting
or
relaxes writhe
3 turns melted
(+3)

ΔTw = -3
Wr = +6
(+3)

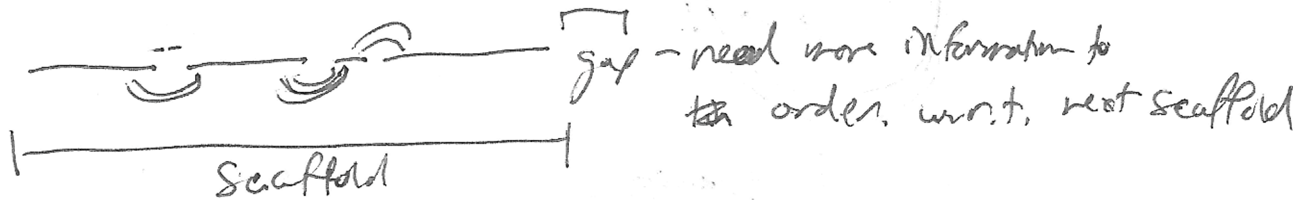
- for ⊕'ly supercoiled DNA, ⊖ ΔTw increases writhe - energetically unfavorable

- i.e. ⊕ supercoiling resists DNA denaturation at high T.
(+3)

5. Sequencing and Methods (18 pts):

(a; 9 pts) Define a scaffold in genome sequencing. What are the "mate pairs" used in shotgun sequencing and how are they used to order contigs?

- A scaffold is a continuous set of ordered contigs - missing some internal sequence (+3) (+2)

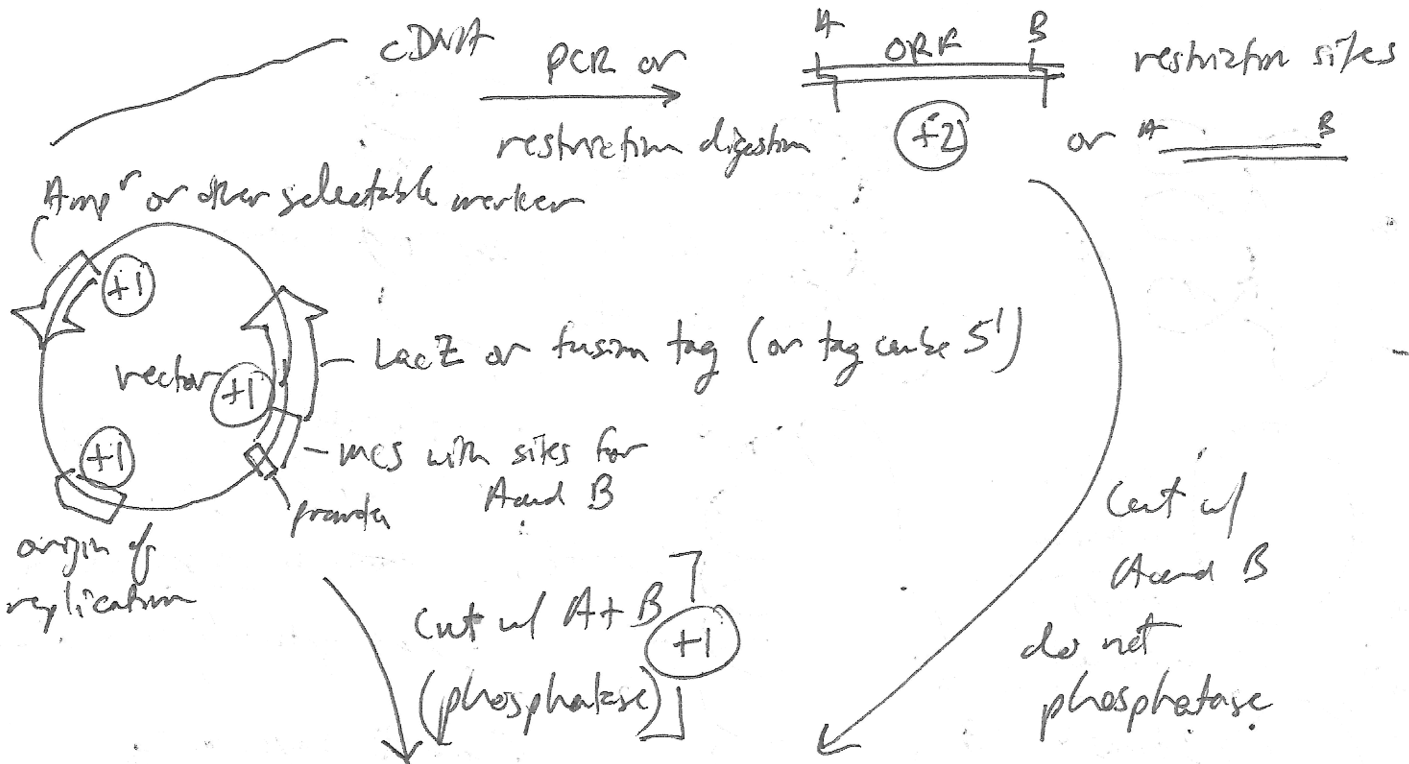


- Mate pairs are sequences obtained from either end of fragments of known size [more efficient than making new primers to sequence through] (+2)

- Mate pairs that end up in neighboring contigs allow ordering even without knowing the sequence in the middle (+2)

Score for the page 24

(b; 9 pts) Outline how to clone and express a eukaryotic gene in *E. coli* using a plasmid expression vector. Assume you know the genome sequence and have access to a cDNA library. Include a sketch of the plasmid with its key features identified.



Mix and ligate (+2)

B P
nick
nick
5' A
P

↓ transform + plate out

↓ liquid culture

↓ induce plasmid promoter

↓ purify protein

↓ publish

(+1)

Page	Score
1	10
2	18
3	18
4	21
5	24
6	9
Total	100

Score for the page____________