

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. I have given you more space than you should need.

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 exam, please write out the following sentence and sign it, or talk to me about it:

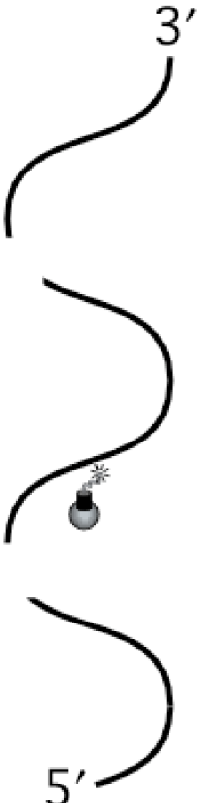
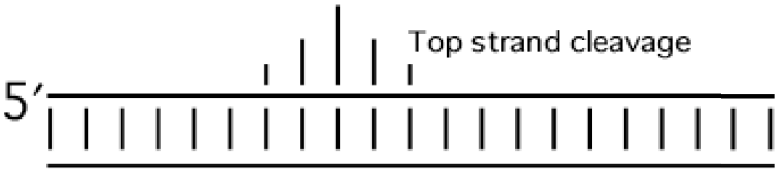
"I pledge on my honor that I have not given or received any unauthorized assistance on this examination."

1. DNA Structure and Base Pairing (25 pts):

- (a; 10 pts) The product of DNA methylation at O-6 of G is O⁶-methylguanosine (O⁶-Me-G), which is a miscoding lesion. Draw the structure of O⁶-Me-G hydrogen-bonded to its most likely W-C base pairing partner. Draw out the sugar only on O⁶-Me-G.

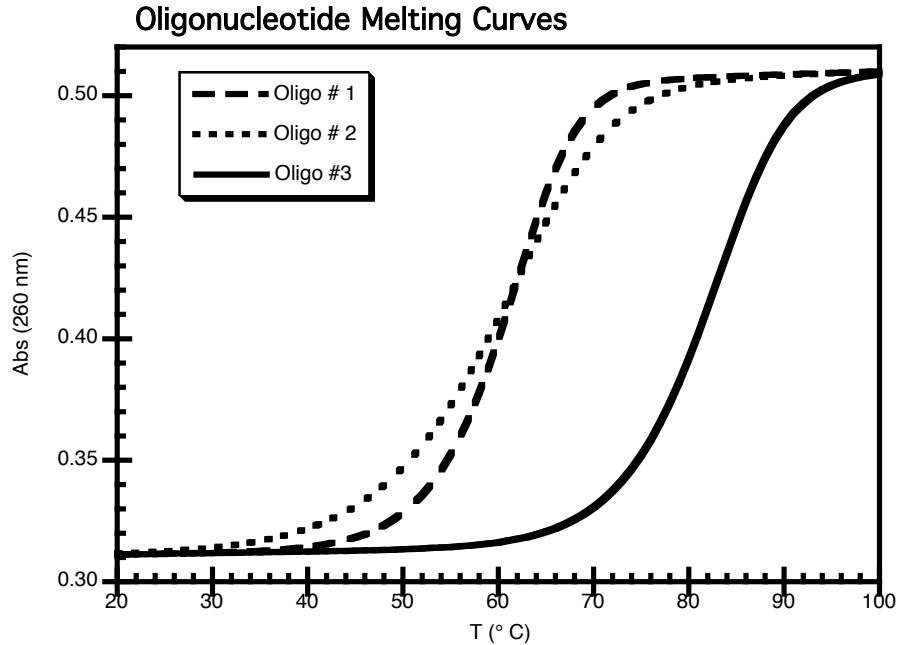
(b; 7 pts) The picture below shows one strand of a B-form duplex DNA. Draw in the other strand and label its ends. Identify the major and minor grooves. Given that the helical repeat is 10.5 bp/turn, calculate how many base pairs are in the helix shown:

(c; 8 pts) The bomb indicates a DNA cleavage agent in one of the grooves. In the figure on the right, the intensity of cleavage along the strand I provided is indicated. Below the figure, draw in the extent of cleavage expected on the other strand (the strand you drew on the left). Explain how the footprinting reveals the groove to which the cleavage agent binds.

<p>b.</p> 	<p>c.</p> 
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2. Hybridization thermodynamics and RNA folding (25 pts):

(a; 5 pts) The absorbance melting curves in the graph at the right correspond to the pairs of (ΔH° , ΔS°) values in the table below (all melts are at the same strand concentration). Identify which is which is which. From the absorbance melt, estimate the T_M for oligonucleotide #3. Remember,
 $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$



ΔH°	ΔS°	Which oligonucleotide?
-84500 cal/mole	-225 cal/mole K	_____
-84500 cal/mole	-210 cal/mole K	_____
-54000 cal/mole	-134 cal/mole K	_____

Estimated T_M for oligo #3: _____

(b; 10 pts) Why is T_M typically measurable with much better precision than ΔH° and ΔS° ? Give a reasonable temperature that you might use for oligo 1 as a hybridization probe, and briefly explain your reasoning.

- (c; 10 pts) In what way are the prediction of the thermodynamics of duplex formation and the prediction of RNA folding inverse problems of each other? Which prediction is more difficult and why? What common set of data do they both rely on?

3. DNA tertiary structure (25 pts):

- (a; 8 pts) Sketch a plectonemic superhelix with $W_r = +3$ and indicate the superhelical nodes. If $\Delta T_w = -6$, then what is ΔL_k ? Name the other form of superhelical writhe.

(b; 10 pts) Sketch the helix axis bending of the following sequences, assuming that: (1) the bend angle due to an A tract is 30° (which is exaggerated) directed into the minor groove, (2) no other sequences contribute to bending, and (3) the DNA helical repeat is 10.5 bp/turn. Only one strand of the duplex is shown.

1. CGGCCAAAAACGGCAAAAAACGGCAAAAAACGGCAAAAAA
 ----- | ----- | ----- | ----- | ----- | (| every 10 bp)
2. CGGCCAAAAACGTCGCTCGAAAAACGGCTGCGCGAAAAAAGTGC
 ----- | ----- | ----- | ----- | ----- |
3. CCAAAAAACGGAAAAAGGAAAAACGCAAAAAACGAAAAAGTGAAAAA
 ----- | ----- | ----- | ----- | ----- |

(c; 7 pts) Which sequence from (b) above would migrate most rapidly in native polyacrylamide gel electrophoresis? Which one would have the largest J factor for cyclization? (One would actually use dimers or trimers of the molecules shown for this experiment). Which one might give an unexpected linking number in the product of cyclization? Give very brief (1-5 word) explanations for your choices.

4. Methods (25 pts):

- (a; 10 pts) Briefly describe the biochemistry, separation, detection, and analysis steps for dye-terminator automated fluorescent sequencing, starting with a template-primer. There is no need to fill the page.

(b; 10 pts) Sketch the major product of the PCR reaction below, writing out the sequence only for the part of the product corresponding to the underlined region of the primers. Then sketch the steps in cloning the product into the expression vector shown below (look at the sequence of the MCS!).

Primer 1

5' -AGTGGATCCTTATACGCAATGT

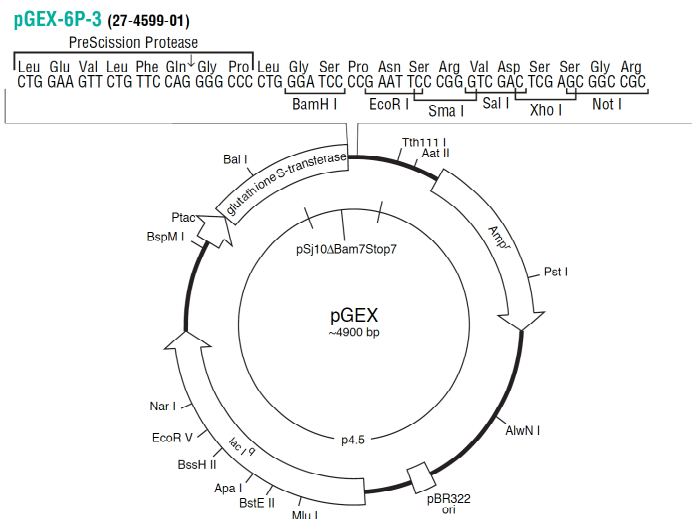
...AGTAGGGTGGTTATACGCAATGT... YFG (not to scale) ...CATGCCTGCAATACATCTCACGTCG...

...TCATCCCACAATATGCGTTACA... GTACGGACGTTATGTAGAGTGCAGC...

Template

GTACGGACGTTATGTAGGAGCTCGC-5'

Primer 2



(c; 6 pts) Expression from the new plasmid will give a GST-YFP fusion protein. In general, why are GST fusion proteins useful? Why is it important that the vector also produces lac repressor?