

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. Read the question being asked!

You will not 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.

The figures for questions 3 and 4 are reprinted on the last page, which you can just tear off.

1. DNA Polymerase Fidelity (20 pts). [NOTE: This question is a mess]

The extemporaneous analogy I gave in class comparing (slow extension of a mismatch by a polymerase that has a 3'→5' exonuclease activity) to (an instructor trying to prevent students from leaving a lecture) was flawed. Let's try for a better one. The idea is that a biochemistry instructor does not want to let any student out the door of the lecture hall until he/she has correct answers to his weird questions written on a card. Most of the students have the correct answers. The instructor's willing to wait all day, and willing to let someone out with blank answers but not wrong answers (although to pass the course in the end all but 1/10⁸ of the answers must eventually be filled in correctly...). There's a TA in the back of the room erasing answers for any student who presents a card; the TA is only somewhat selective about preferentially erasing wrong answers. The presence of the TA thus saves the instructor's life, and allows him to be so restrictive without getting trampled by students with errors charging the door.

(a; 9 pts) Fill in the biochemistry correlate to each of the following elements of the analogy:

The TA	3'→5' exonuclease activity
The students	
The answers on the card	
The instructor's attitude about not letting mistakes out the door	
What would happen to the instructor and the students if there were no TA	

(b; 8 pts) For the two answers below you have to extend the analogy beyond what is given above, or fill in other details.

The act of writing answers on the card, and where this would occur (you have to dream this up).	
	The irreversibility of phosphodiester hydrolysis

(c; 3 pts) Point out a way in which this analogy is imperfect (that's why they call them analogies...).

2. Chromatin Structure (15 pts)

(a; 5 pts) Sketch (draw) the tuna can model for a mononucleosome.

(b; 6 pts) Briefly describe the higher-order packaging of DNA in chromatin.

Repeat of question(a), for reference: (1) What protein? (2) What is it called? (3) Example of use.
(4) Why the cleavage site for Xa?

(b; 9 pts) If your situation were such that you had to clone a fragment with Xba I sites at both ends (i.e. you're stuck with non-directional cloning), how would you prepare the pMAL-2X vector to be used (in addition to Xba I cleavage)? Why is this necessary? What controls would you run for your cloning?

(c; 6 pts) How would you select for bacteria containing pRS313 (i.e. how would you prevent all the other bacteria from growing)? How would you select for yeast containing the plasmid? Why bother with shuttle vectors at all (why not just maintain the plasmids in the eukaryotic target organism all the time)?

4. Protein-DNA Recognition (25 pts).

The diagram below shows the matrix of major groove hydrogen bond donors and acceptors recognized by one monomer of the dimeric MFDBD (My Favorite DNA Binding Domain), where A = acceptor, D = donor, M = methyl, and the target is good old perfect duplex DNA.

	<p>(a; 6 pts) In the space below, <u>write out as much as we can tell about the sequence to which the monomer unit of the protein binds</u>. Give both the W and C strands.</p>
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(b; 8 pts) If the side chain recognizing the acceptor at the top were moved as in arrow (1), what would the new recognition sequence be? What does this say about the practicality of predicting a binding site based on knowing the structure of MFDBD by itself?

(c; 5 pts) The dashed line through the middle of the site is perpendicular to the symmetry axis of the protein, i.e., the symmetry axis of the protein is perpendicular to the page. Based on your answer to (a) and this information, give the sequence of the entire protein binding site. Assume 10 bp/turn of DNA.

(d; 6 pts) A single side chain could recognize the acceptor and donor linked by the slanted line. What amino acid(s) might provide the side chain? If it were to spin around as indicated by the arrows (2), clearly the recognition site would be different. How do real proteins avoid binding to degenerate sites in this way?

5. DNA Sequencing and Bioinformatics (15 pts)

(a; 6 pts) BLAST and other protein sequence homology search programs rely on “similarity matrices” to quantify the extent to which two sequences are related even if their sequences are not identical. Briefly describe two different sources of similarity matrices.

(b; 4 pts) Genomic sequencing has provided evidence for many examples of lateral gene transfer (LGT) during evolution, in which genes or larger DNA segments have moved from organism to organism. There are suggestions that this is so common that we should be talking about a web of life rather than a tree of life as a metaphor for genealogy. What features of a part of a genome might suggest an LGT event?

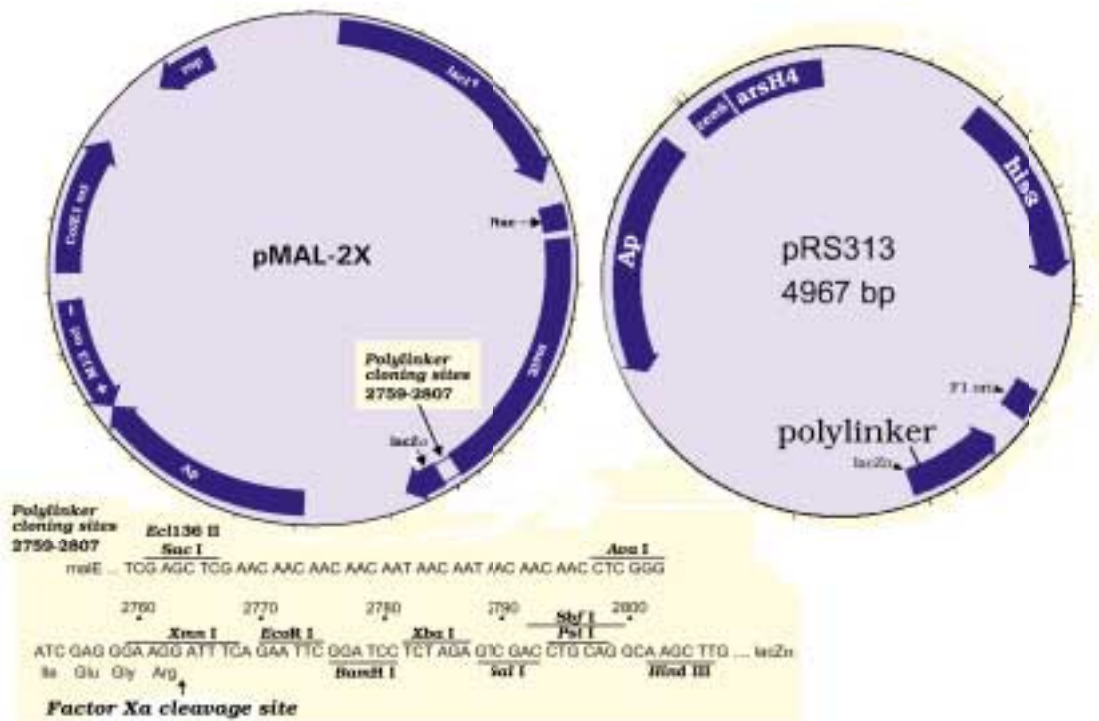
Even though PCR is rather error-prone, DNA can be sequenced directly from a genomic source using PCR. Basically, the sequence is amplified and then the product mixture is subjected to chain termination thermal cycle sequencing with ddNTPs, as follows: In thermal cycle sequencing, a single primer is used and the reaction mix is repeatedly “cycled” in a PCR machine. It is otherwise similar to standard dideoxy sequencing.

(c; 5 pts) What level of signal amplification is obtained in 20 cycles? You might expect it would be better to clone the PCR product and then sequence the cloned DNA. In fact, more accurate sequence is often obtained directly from the mixture. Why is this the case?

Score:	Question 1:	_____	out of 20: DNA Polymerase Fidelity
	Question 2:	_____	out of 15: Chromatin Structure
	Question 3:	_____	out of 25: Cloning and Enzymatic Methods
	Question 4:	_____	out of 25: Protein-DNA Recognition
	Question 5:	_____	<u>out of 15: DNA Sequencing and Bioinformatics</u>
Total:		_____	out of 100

Extra copies of figures -- tear off if you want

Question 3:



Question 4:

