

October 11, 2002

Name \_\_\_\_\_

ID number \_\_\_\_\_

## CH461/561 Exam 2

There are **4** short answer questions worth **6** points each.

There are **4** problems worth **24** points each.

CH461 students must answer **3** for full credit.  
(If you do all 4, I will drop the lowest one.)

CH561 students must answer all **4**.

The maximum amount of points is thus:

**96** for CH461 students

**120** for CH561 students

**You are advised to look over the problems first before starting to work them.**

Time should not be a factor, if you are well prepared.

|              |   |     |
|--------------|---|-----|
| Short answer |   | /24 |
| Problems:    | 1 | /24 |
|              | 2 | /24 |
|              | 3 | /24 |
|              | 4 | /24 |
| Total        |   |     |

**No notes or books of any sort may be used during the exam.**

I have neither given nor received aid on this exam.

\_\_\_\_\_  
(signature)



3) Describe one experiment that provided evidence for nucleic acid being the genetic material. What was the basis for the conclusion?

4) Fill in the table describing key parameters of the three different conformational forms of DNA using the choices to the right.

| parameter                             | A form | B form | Z form | choices  |
|---------------------------------------|--------|--------|--------|--|
| Helical sense                         |        |        |        | Right or left handed                                     |
| Base pairs per turn                   |        |        |        | 10.5, 11, or 12  |
| Rise per turn                         |        |        |        | 28 Å, 34 Å, or 45 Å                                      |
| Base pair tilt (normal to helix axis) |        |        |        | 6°, 7°, or 20°   |
| Sugar pucker conformation             |        |        |        | C-2' endo, C-3' endo, or alternating C-2' endo/C-3' endo |
| Glycosyl bond conformation            |        |        |        | Anti, syn, or alternating anti and syn                   |

## Problems

- 1) Nucleic acids:
  - a) Draw the 2 kinds of Watson-Crick base pairs that exist in double-stranded DNA. (You only need to draw the bases and indicate which atom is connected to the deoxyribose.) Indicate which part of the base pair would correspond to the **major groove** and which would correspond to the **minor groove**.

- b) Draw the following oligonucleotide: 5'-pTpApGpC-3'  
(include all atoms; you do not need to indicate H if drawing a "stick figure") What would its complementary sequence be? (Do not draw it.)
- c) DNA can, under special circumstances, form a triple helix. The core is a normal double-helical B-DNA structure with standard Watson-Crick base pairs. The third strand interacts via a nonstandard interaction (called "Hoogsteen" pairing) in the major groove. In this interaction, an A can interact with a T in the complementary strand via Watson-Crick base-pair and via another T in the third strand via Hoogsteen pairing, often symbolized as T•A=T (where • represents Hoogsteen pairing, and = represents Watson-Crick pairing). In a similar way, G can pair with C in the complementary strand and with C<sup>+</sup> (cytosine protonated at N-3) to give a C<sup>+</sup>•G≡C triplet. **Pick one of these triplets and draw it.** Why does cytosine have to be protonated in the third strand?

2) Draw the following molecules:

a)  $\alpha$ -D-glucopyranose

2-deoxyribose (open chain form)

$\alpha$ -D-glucuronate

$\alpha$ -D-galactopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -D-fructofuranosyl-(1 $\rightarrow$ 6)-D-mannopyranose  
also known as Gal(1 $\rightarrow$ 4)Frc(1 $\rightarrow$ 6)Man

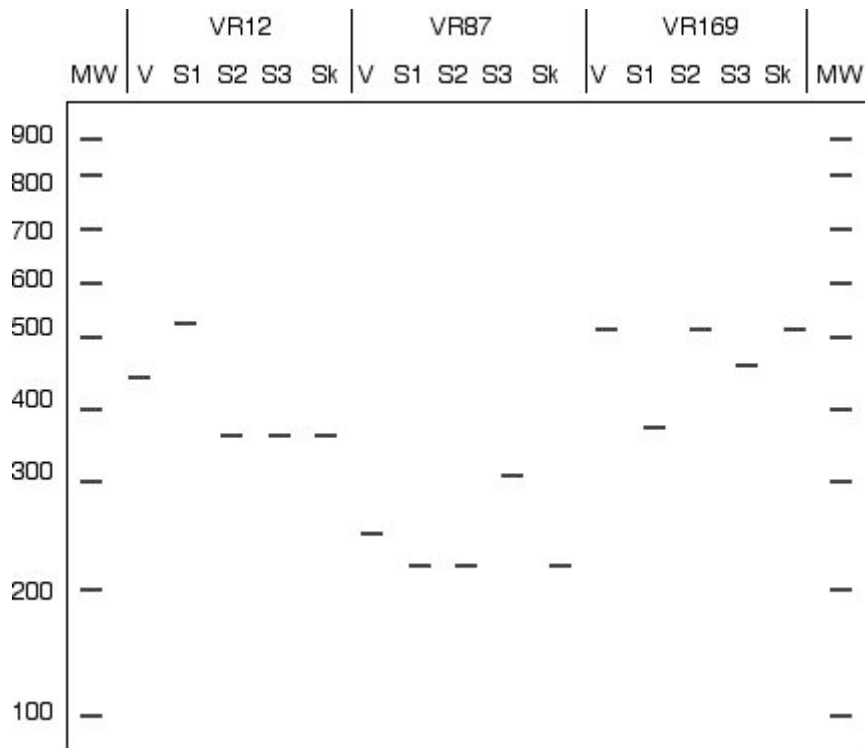
b) 1-palmitoyl, 2-oleoyl phosphatidylserine

1-14:0, 2-18:2<sup>9,12</sup> phosphatidylinositol  
(also known as 1-myristoyl, 2-linoleoyl phosphatidylinositol)

sphingomyelin (sphingosine + stearic acid + phosphocholine)

cholesterol

- 3) You are a crime scene investigator specializing in DNA analysis. You are given a sample of skin found under the fingernails of a murder victim. Given that it was violent death involving a hand-to-hand struggle, it is likely that the murderer was scratched by the victim during the struggle. You are also given tissue samples from the victim and three suspects to see if there is a match.
- a) You start out doing PCR analysis of the skin sample. You only have a limited amount of sample. Since PCR is capable of amplifying DNA from only a few template molecules, in principle, you do not have to purify the template DNA. You can merely put some skin cells in the tube and heat it up to lyse the cells and release the DNA. This is very convenient. You do three different reactions with primers matching sequences just outside of regions that can be quite variable in length in the human population. These regions of variable length (called “VR” for variable region) are where a specific sequence exists in multiple copies. For example, VR87 typically has 10-24 repeats of the sequence ATGCAG near a gene for leucine synthesis. Each specific PCR product will then be a different length depending upon the number of repeats possessed by the specific VR used as template. You run the PCR reactions on an agarose gel stained with ethidium bromide. The result is shown below. (V = victim, S1-S3 = suspects 1-3, Sk = skin sample, MW = molecular weight markers, shown on left side in base pairs)



What you can say about the skin sample and the suspects? Can you rule any of them out? Which ones and why?

- b) You want to develop a new marker to make your identification more unambiguous. Based on the sequence of the human genome, there seems to be another variable region that nobody has used called VR235. It consists of many repeats of CGGTTCGCT. You identify the sequences upstream and downstream of the VR235 region, shown below. Based on this, design primers that can be used for PCR to amplify VR235.

5'-ATCGGGCTACGGTCTGATACAAACTATGCT-(VR235 sequence)<sub>n</sub>-  
TCGAATCTGAGCCTCATGTAGGAGCTAAC-3'

upstream primer: \_\_\_\_\_

downstream primer: \_\_\_\_\_

- c) These variable regions can some times differ not only in the number of repeat sequences, but sometimes in the actual sequence as well. Some of the “repeats” are not perfect and may have a mutation. Such a mutation could be very diagnostic. You decide to clone the VR235 region from the skin sample into a plasmid. Describe briefly how you would go about doing this.

- d) You succeed in making your plasmid, and now you want to analyze it by restriction “digestion”. You treat the plasmid with a few restriction endonucleases and examine the results of digestion using agarose gel electrophoresis. You estimate the size of each fragment you get from each digestion. Using this data, draw a sketch of the plasmid with the distance between each restriction site indicated.

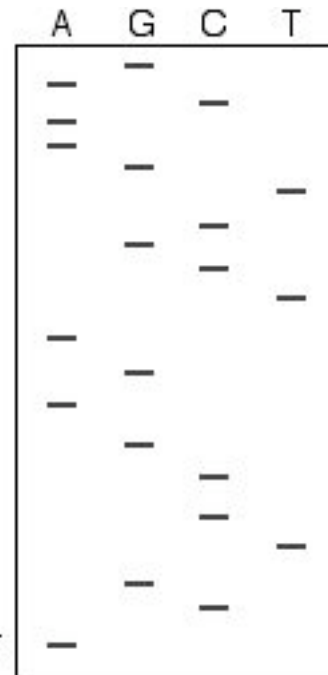
| Restriction enzyme(s)       | Sizes of fragments (bp) |
|-----------------------------|-------------------------|
| <i>EcoRI</i>                | 4000                    |
| <i>PvuII</i>                | 4000                    |
| <i>BamHI</i>                | 2400, 1600              |
| <i>EcoRI</i> + <i>PvuII</i> | 2300, 1700              |
| <i>EcoRI</i> + <i>BamHI</i> | 1600, 1400, 1000        |
| <i>PvuII</i> + <i>BamHI</i> | 2400, 900, 700          |

4. Analysis problems

- a) You are given a polysaccharide to analyze. After methylation and hydrolysis, the only product is 2,3,6-tri-*O*-methyl-D-glucose. If you subject the polymer to limited acid hydrolysis, you get a collection of disaccharides, trisaccharides, tetrasaccharides, *etc.* You find that all of the disaccharide fraction consists of a single kind of molecule: cellobiose. Based on these findings, what is the polysaccharide? (Give common name or draw.) Explain briefly how you know.
- b) You are given a disaccharide to analyze. You subject it to Fehling's reaction (treatment with  $\text{CuCl}_2$  in NaOH), but you get no red  $\text{Cu}_2\text{O}$  deposit. As a control, you treat glucose in the same way, and you get lots of red deposit. If you methylate the disaccharide and hydrolyze it, you get 2,3,4,6-tetra-*O*-methyl-D-glucose and 1,3,4,6-tetra-*O*-methyl-D-fructose. You treat with 4 different enzymes:  $\alpha$ -glucosidase,  $\beta$ -glucosidase,  $\alpha$ -fructosidase, and  $\beta$ -fructosidase. Only  $\alpha$ -glucosidase and  $\beta$ -fructosidase can hydrolyze the disaccharide. Based on these findings, what is the disaccharide? (Give common name or draw.) Explain briefly how you know.

- c) You are given a phospholipid to analyze. You treat it with methanol in base to hydrolyze the fatty acid esters and generate the methyl esters of the fatty acids. You submit these to mass spec analysis and get 2 peaks of similar intensity with masses of 270 and 296 amu. If you treat the phospholipid with phospholipase A1, and then make methyl esters of the hydrolysis product, you only get the 270-amu species. The phospholipid behaves as a charged but neutral molecule, but if you treat it with a phospholipase D specific for either phosphatidylserine or phosphatidylethanolamine, then you convert it to a negatively charged lipid. Identify the phospholipid that you were given to analyze (either name or draw).

- d) You have a plasmid with an insert that you want to sequence, and you have a primer that matches one of the strands. You label this at the 5'-end with [<sup>32</sup>P]-phosphate and use it as a primer in 4 separate reactions with the same template DNA and the addition of a DNA polymerase, the 4 dNTPs and one specific dideoxynucleotide triphosphate (ddNTP). You stop the reactions and denature the products to generate single-stranded DNA, which you run on a polyacrylamide gel. After autoradiography to detect the labeled DNA strands, you get the result shown to the right. The direction of migration is indicated, as well as the 4 lanes corresponding to the 4 different reactions. ("A" = reaction containing ddATP, "G" = reaction containing ddGTP, etc).



Based on this, write out the sequence of the DNA that you have determined here. Write the sequence of the strand corresponding to the primer you used, in the 5' to 3' direction:

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