

You will have 1 hour to complete this exam. There are **14** pages containing 5 questions worth 100 points and a bonus question worth 15 points. A page of reference material is provided at the end of the exam. Please answer the questions in the space provided. There is a blank page at the back in case you need additional space. Be sure to indicate that you have completed a problem on this page to insure that you receive credit for the work. Please show all of your work, including units, in order to receive full credit. You may use a calculator but you may NOT share a calculator with your neighbor.

READ THE QUESTIONS CAREFULLY! In several cases, you have the choice of which question you want to answer.

Pace yourself. Many of the questions have multiple parts, so do what you can on one question and then move to the next. The exam will most likely take you the full hour to complete.

Please try to be neat. If we cannot read your writing, we cannot award you credit for your answer.

Question 1 _____/15

Question 2 _____/20

Question 3 _____/25

Question 4 _____/20

Question 5 _____/20

Bonus _____/15

TOTAL: _____/100

1) Define **any 5** the following terms: **(3pts. each/15 pts. total)**

a) cloning vector

b) palindrome

c) restriction endonuclease

d) intercalation

e) propeller twist

f) writhe

g) hyperchromism

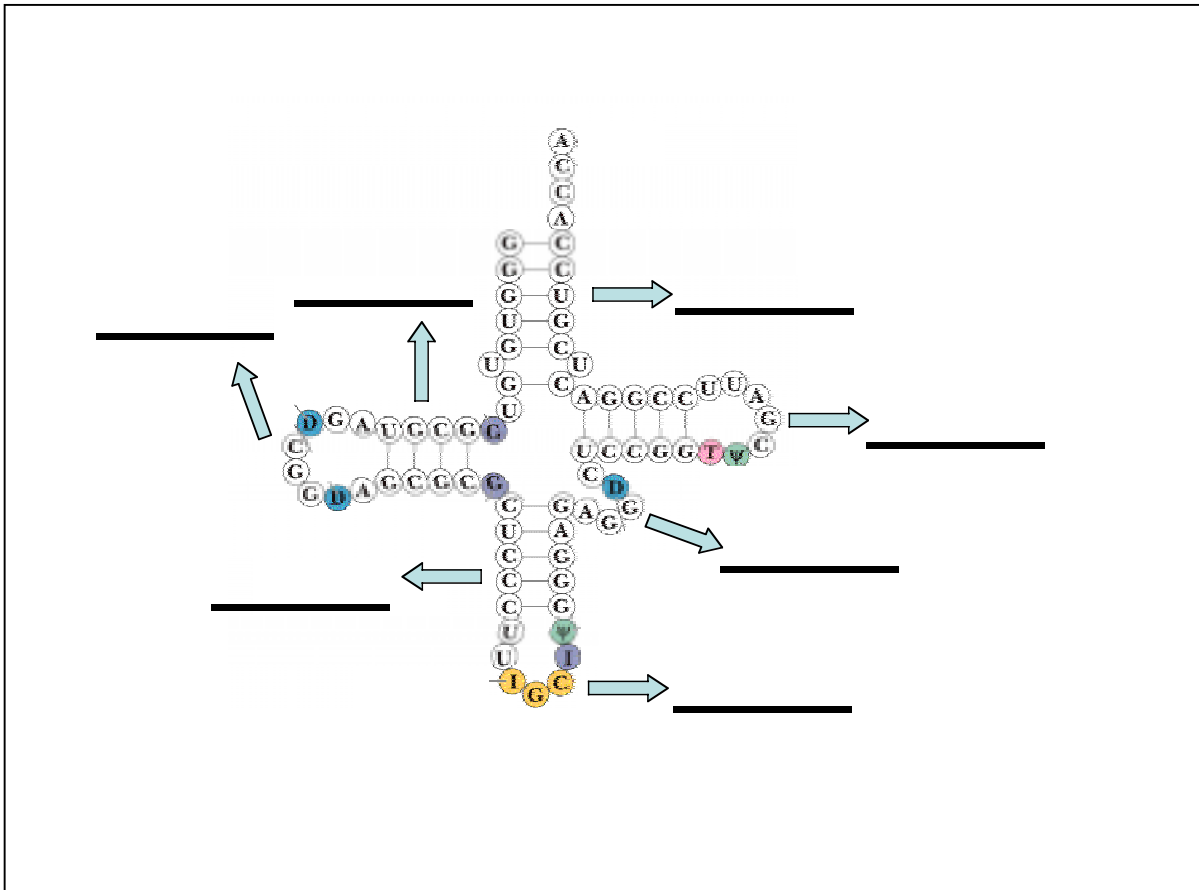
h) nucleosome core particle

2) Nucleobases, nucleosides and nucleotides. **(20pts)**

- a) In RNA, G•U base pairs are quite common and often highly conserved. Draw a G•U base pair. You must indicate the position of the ribose attachment, but you do not need to draw its entire structure (i.e. “---Ribose”). Before you start drawing, read part c of this question and leave room to add this to your sketch. **(6 pts)**

- b) Number the positions on the pyrimidine ring. **(2 pts)**
- c) Select an amino acid that can specifically recognize (discriminate the face of the G•U base pair in part a. Add this amino acid side chain (show the complete side chain) to the drawing above and show the hydrogen bonding interaction(s) it would make. Please provide the 1- and 3-letter codes for this amino acid. **(6 pts)**
- d) Briefly explain why G•U base pairs are acceptable in RNA biochemistry but the equivalent G•T base pair is never observed in genomic or plasmid DNA. **(6 pts)**

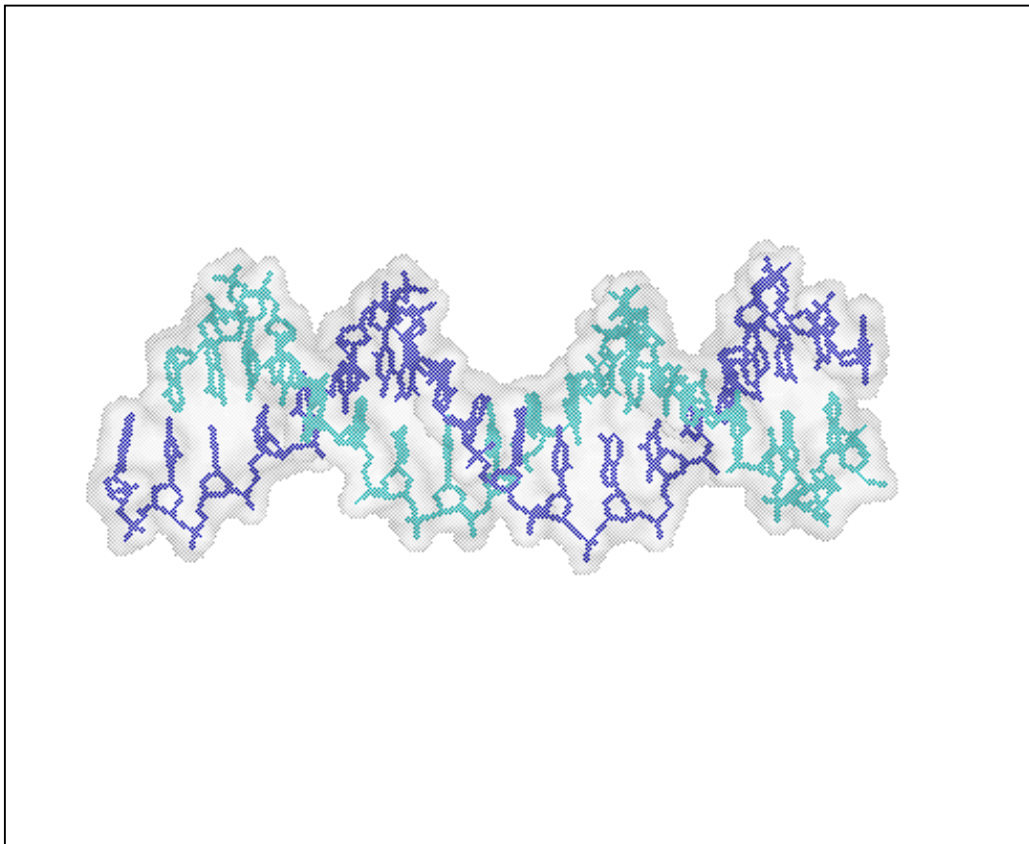
3) tRNA structure and properties (27 pts)



- Add to the diagram the names of the major structural regions of tRNA indicated by the blank lines. (12 pts) (2 pts each, 6 of 7 for full credit)
- Define a pseudoknot and identify one in the tRNA structure above. (4 pts)
- Add a sketch of the mRNA to the tRNA diagram above. Indicate how the mRNA and tRNA interact. Be as specific as possible with respect to the interacting groups and the strand orientation. (2 pts)
- tRNA Aminoacyl synthetases bind to and aminoacylate the 3'-end of tRNAs. Indicate the 3' and 5' ends of the tRNA on the diagram above. (2 pts)

- e) tRNA can also be represented by an L-shaped diagram. Sketch the L-shaped version of a tRNA and indicate which elements from the cruciform representation constitute the vertical, horizontal and joint regions. **(5 pts)**

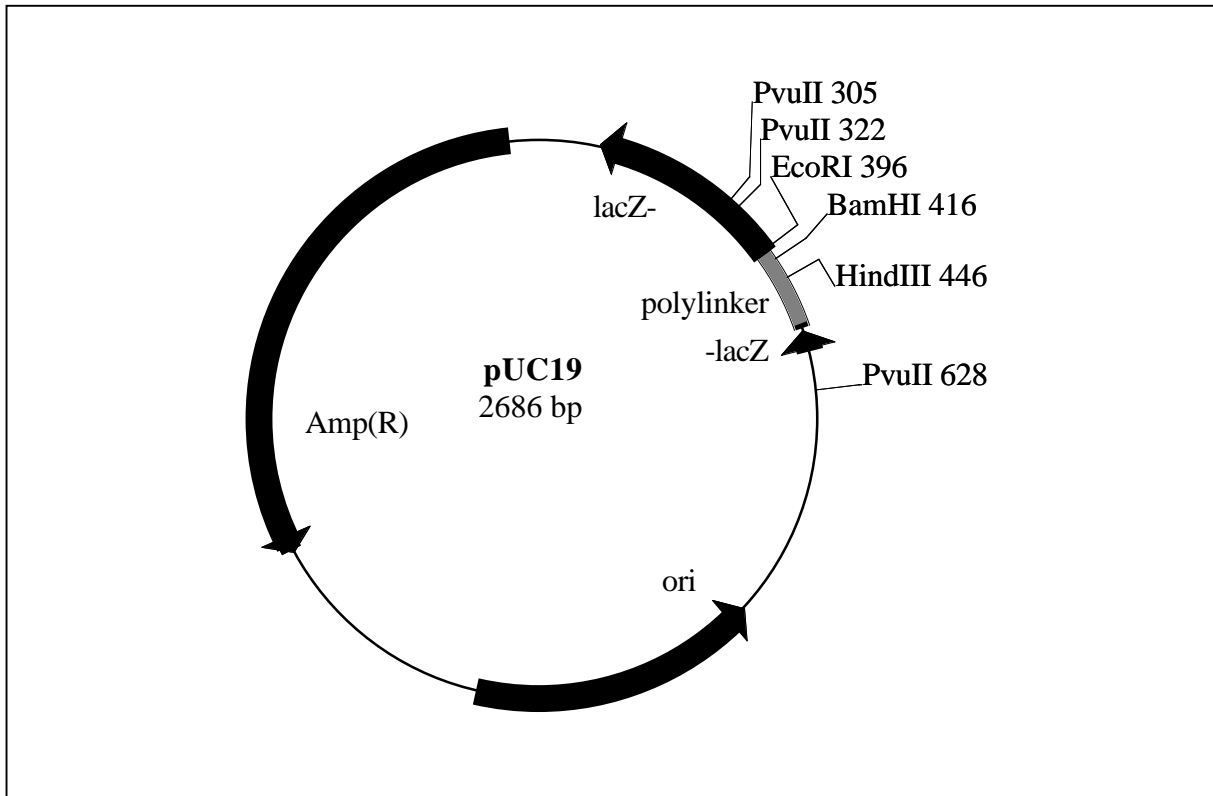
- 4) Below you see schematic diagrams of a B-form DNA double helix. **(20 pts)**



- a) Identify on the diagram the major and minor grooves. **(4 pts)**
- b) Identify two functional groups that you might find in each the major and minor grooves of the DNA. (You will not receive credit for using the same atom on two equivalent bases, i.e. N7 of A and N7 of G.) **(4 pts)**

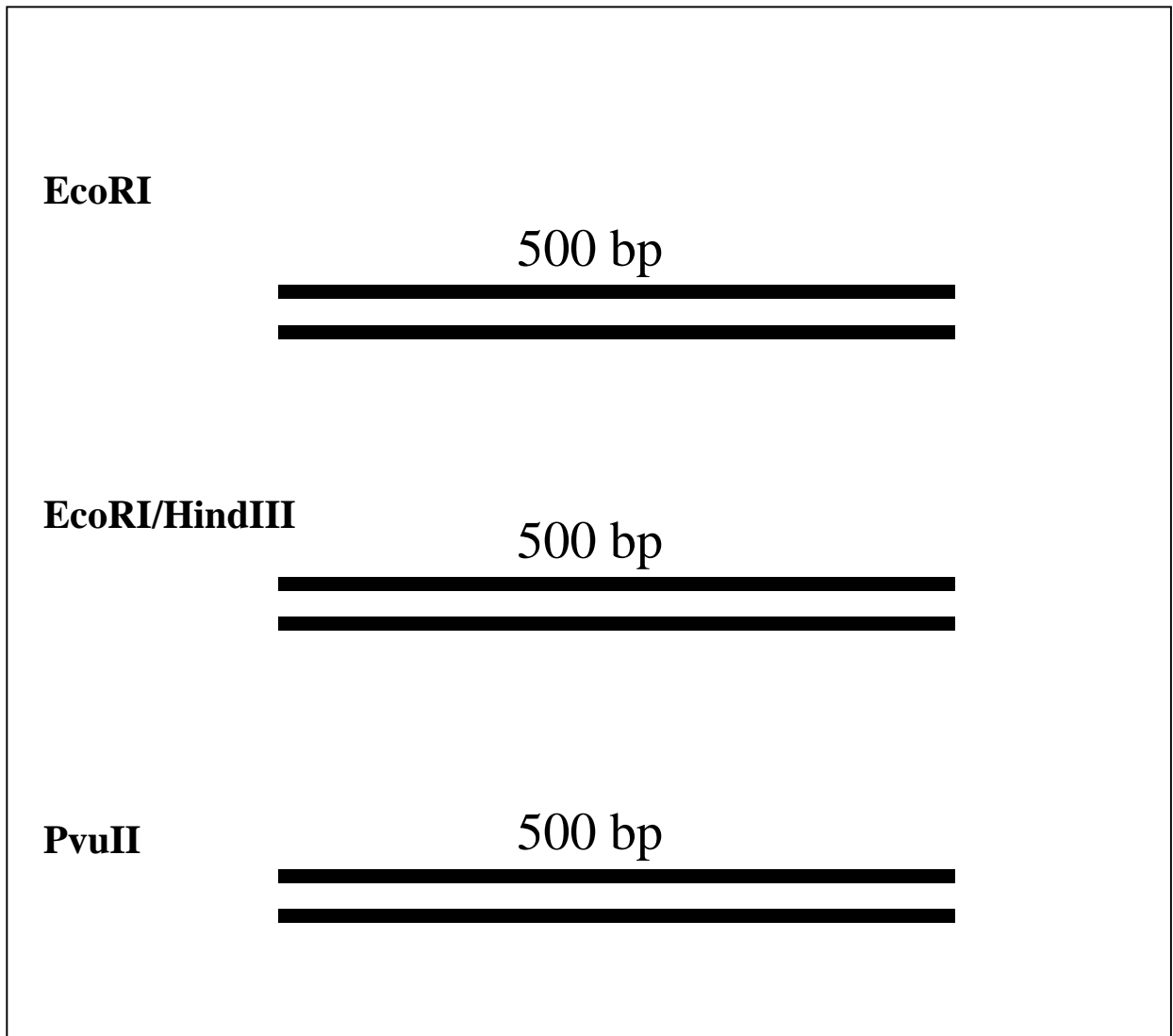
- c) Many transcription factors (i.e. GCN4 and zinc finger proteins) bind to specific DNA sequences. Describe how a protein would most likely interact with this DNA helix and obtain sequence dependent information without disrupting its structure. **(7 pts)**
- d) Explain why many DNA binding proteins recognize palindromic sequences. Use the preceding diagram to assist in your explanation. **(5 pts)**

5) Answer the following questions about the plasmid pUC19 (2686 bp long, map shown below with the relevant restriction sites) and the subsequent manipulations you are making at each step of a hypothetical cloning experiment (next page). The recognition sites for the enzymes you might need are shown below. **(20 pts)**



PvuII	EcoRI	HindIII
↓ CAGCTG GTCGAC ↑	↓ GAATTC CTTAAG ↑	↓ AAGCTT TTCGAA ↑

- a) If you isolate plasmid DNA from *E. coli*, how would you describe this DNA topologically? (2 pts)
- b) You cleave your pUC19 in three different reactions. 1) Digested with EcoRI, 2) Degested with EcoRI and HindIII, and 3) Digested with PvuII. You can clone into any of the three sites you just made so long as your insert DNA has the appropriate ends. Indicate on the schematic diagram below what the DNA insert should look like in order to be ligated into the pUC19 plasmid based on the given restriction digestion. (9 pts)



Briefly describe what is meant by “**directional cloning**” and indicate which method above allows your target DNA to be directionally cloned into pUC19. (5 pts)

- c) On the plasmid map of pUC19 you see a notation for *amp^R*. What is this gene and explain why it has been incorporated into the plasmid? (4 pts)

BONUS QUESTION (15 pts.):

Describe the hierarchical structures starting with a naked DNA double helix and ending with a compact chromosome observed in eukaryotic chromatin. Explain how this structure helps pack DNA into a cell's nucleus.

Physical constant and data that might be required for certain problems on this exam.

Concentrations of metabolites

Metabolite	Conc. μM
1,3-bisphosphoglycerate	1.0
2-phosphoglycerate	30
3-phosphoglycerate	120
ADP	140
ATP	1850
Dihydroxyacetone phosphate	140
Fructose-1,6-P	31
Fructose-6-P	14
Glucose	5000
Glucose-6-P	83
Glyceraldehyde-3-P	19
Glycerol-3-phosphate	25
Lactate	2900
NAD ⁺	540
NADH	50
Phosphoenolpyruvate	23
P _i	1000
Pyruvate	51

Standard Free Energies of hydrolysis for common metabolites

Metabolite	ΔG ^o _{hyd.} kJ mol ⁻¹
Phosphoenolpyruvate	-62
1,3-bisphosphoglycerate	-49
Phosphocreatine	-43
Pyrophosphate	-33
Phosphoarginine	-32
ATP → AMP + PP _i	-32
Acetyl-CoA	-32
ATP → ADP + P _i	-30
Glucose-1-Phosphate	-21
Glucose-6-Phosphate	-14
AMP → Adenosine + P _i	-14
Glycerol-3-phosphate	-9

Amino Acid Hydrophathies

Amino Acid Hydrophathy	KJ/mol
Alanine	1.0
Arginine	-7.5
Asparagine	-2.7
Aspartic Acid	-3.0
Cysteine	0.17
Glutamic Acid	-2.6
Glutamine	-2.9
Glycine	0.67
Histidine	-1.7
Isoleucine	3.1
Leucine	2.2
Lysine	-4.6
Methionine	1.1
Phenylalanine	2.5
Proline	-0.29
Serine	-1.1
Threonine	-0.75
Tryptophan	1.5
Tyrosine	0.08
Valine	2.3

Constants:

$R = 8.315 \text{ J mol}^{-1} \text{ K}^{-1}$

$F = 96.48 \text{ kJ V}^{-1} \text{ mol}^{-1}$

$k = 1.381 \times 10^{-23} \text{ J K}^{-1}$

Conversion Factors:

1 cal = 4.184 J

T in K = °C + 273

Relevant Equations:

$\text{pH} = \text{pK}_a + \log[A^-]/[HA]$

$\Delta G^{\circ'} = -RT \ln(K_{\text{eq}})$

$\Delta G = \Delta G^{\circ'} + RT \ln(Q)$

$\Delta G^{\circ'} = \Delta G^{\circ} \pm RT \ln[H^+]$

