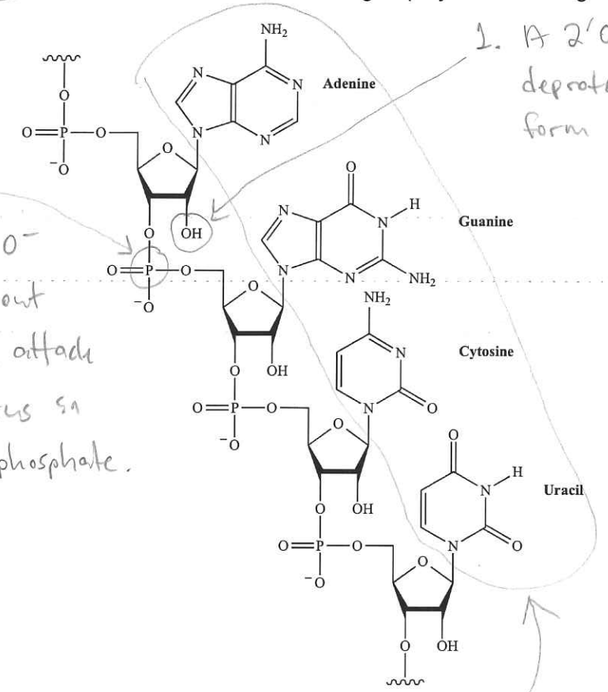
Answer key

BB2950 Molecular Biology, Exam 1, 11-9-17 100 points total
Please write your name on the back of **EACH** page

5. *(6 points)* A strand of RNA is shown below. If this molecule is placed in basic (alkaline) conditions, describe the first two steps that will happen in the process of alkaline hydrolysis. Use circles or arrows to make clear which atoms or groups you are talking about in each step.

3pts each



1. A 2'OH (hydroxyl) will be deprotonated (lose a proton) to form a 2'O⁻

2. The 2'O⁻ will carry out a nucleophilic attack on a phosphorus in the nearest phosphate.

Can circle:
- All bases
- One base
- One or more H-bond forming groups within a base.

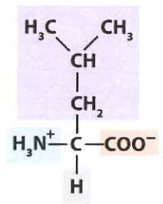
6. *(3 points)* Suppose the RNA shown above is bound by an RNA binding protein that interacts with it in a sequence specific manner. Circle part of the RNA molecule that you predict is likely to make direct contact with the protein.

7. *(4 points)* The following is the chemical structure of the amino acid leucine. The side chain is highlighted in purple.

2pts — A. Is this side chain non-polar, aromatic, polar, or polar charged?

2pts — B. What kind of bond, force, or effect do you predict will mediate interactions between this side chain and other molecules?

Hydrophobic effect



Answer key

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Please write your name on the back of **EACH** page

1. *(4 points)* List the two chemical differences between DNA and RNA.

RNA: uracil and 2'OH
DNA: thymine and no 2'OH

2 pts each

2 pts each — 2. *(4 points)* Describe two ways in which one or both of the chemical differences listed above give DNA and RNA different functional properties.

2'OH:
- Makes RNA more susceptible to hydrolysis (strand breakage)
- Allows RNA to form additional hydrogen bonds and therefore form more complex structures.
- Alters shape of the ribose (sugar), which changes shape of helices.

Uracil: is less bulky than thymines changing shape of helix.

3. *(8 points)* Below is the backbone of two strands of a protein.

A. The nitrogen atoms and some of the R groups are labeled. Label at least two carbon atoms, two hydrogen atoms, and two oxygen atoms. | 0.5 pts each

2 pts. — B. These two strands can interact to form a beta sheet. Draw dashed lines between at least two pairs of atoms that will interact in this structure.

2 pts. — C. What type of bond or force will mediate the interaction between atoms in these pairs? Hydrogen bonds

1 pt. — D. Label a peptide bond.

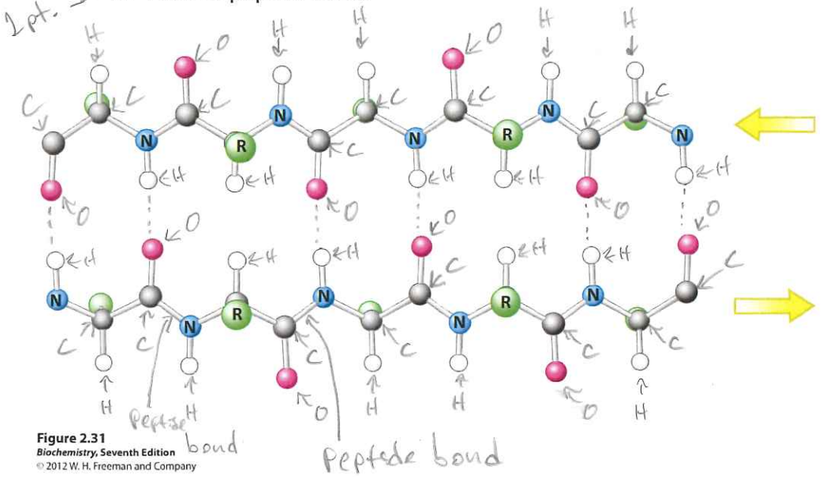

Figure 2.31
Biochemistry, Seventh Edition
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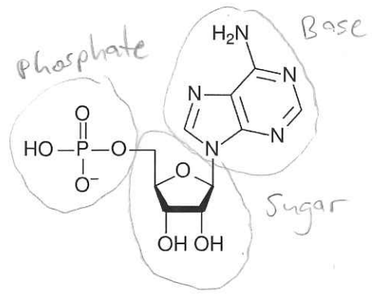
4. *(5 points)* This is a nucleotide that contains the base adenine.

2 pts — A. What is the 3 or 4 letter abbreviation for this nt?

AMP

B. Circle and label the base, the sugar, and the phosphate.

1 pt each



Answer key

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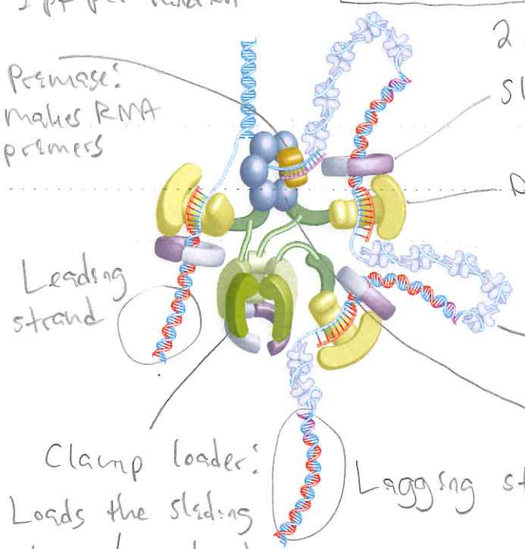
13. *(14 points)* Below is a schematic of a prokaryotic DNA replication fork. The DNA being replicated is blue, and the newly synthesized DNA is red. Six distinct proteins are shown.

A. Label at least five of these proteins, and briefly state the function of each one. *(1 sentence or sentence fragment each).*

B. Label the leading and lagging strands in the figure.

1 pt per ID
1 pt per function



2 pts each

Sliding clamp: holds Pol III in proximity to the DNA (increases processivity)

DNA Polymerase III (Pol III): synthesizes the new DNA for the leading + lagging strands.

Single-stranded DNA binding protein (SSB): Keeps the DNA in a single-strand state until it is replicated (protects ssDNA)

DNA helicase: Separates the strands of the parental DNA (the DNA to be replicated)

1 pt per name, 1 pt per correctly matched function.

14. *(4 points)* Name two additional proteins NOT shown above that are required for DNA replication, and briefly state the function of each one *(1 sentence or sentence fragment each)*.

Topoisomerase: Relaxes positive supercoils ahead of the replication fork.

DNA Polymerase I (Pol I): Removes RNA primers and fills the gaps with DNA.

DNA ligase: Seals nicks between Okazaki fragments (nicks on lagging strand).

Answer key

BB2950 Molecular Biology, Exam 1, 11-9-17 100 points total
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8. *(3 points)* Base pairing and base stacking both contribute to the stability of DNA double helices. Indicate which bonds, forces, or effects underlie each phenomenon:

	Base pairing	Base stacking
Hydrophobic effects		✓
van der Waals forces		✓
Hydrogen bonding	✓	

1 pt. per correct check
-1 pt. per incorrect check

9. *(7.5 points)* Each of the following processes affect DNA supercoiling. Indicate which directly change the linking number and which do not.

Process	Directly causes ΔLk?
Transcription	No
Wrapping around histones	No
Topoisomerase activity	Yes

2.5 pts each

10. *(4 points)* What is each histone octamer composed of?

2 each of the following proteins: H2A, H2B, H3, H4

11. *(4 points)* From a cell's point of view, which mutation in a protein coding sequence is worse: a deletion of one nt or a deletion of three nt? Why?

A deletion of one nt is more likely to cause a problem for the cell, because it will change the reading frame (cause a frameshift) and therefore change the identity of all/most of the subsequent amino acids (residues).

Which is worse: 2pts
Why: 2pts

12. *(2.5 points)* Only 1.5% of the human genome directly codes for proteins. Which of the following does NOT contribute to the remaining 98.5%?

A. Transposons
B. Repetitive sequences
C. Introns
D. Regulatory sequences
E. Plasmid sequences

Answer key

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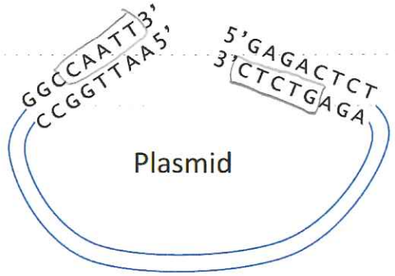
15. *(8 points)* You want to clone the red portion of the DNA below into a plasmid by Gibson Assembly. Only the ends of the plasmid sequence are shown. Real PCR primers need to be longer, but pretend that you only need 5 nt that will anneal to your template and 5 nt that will overlap with the ends of your plasmid. Write the complete 10 nt sequence of each primer from 5' to 3' in the spaces below.

5'-GACCCCGACCCACCGTTCGTCGCCGTCTGGAACATCGTCGCCGCAACGGGGACGT-3'
3'-CTGGGGCTGGGTGGCAAGCAGCGGCAGACCTTGTAGCAGCGGCGTTGCCCCTGCA-5'



- 2 pts per correct annealing seq. at the 3' side of primer
- 2 pts per correct plasmid seq at 5' side of primer

Forward primer: 5'CAATTCCCAC3' Not necessary to label ends if written 5'→3' as directed

Reverse primer: 5'GTCTCTTGCG3'

16. *(2 points)* Which enzyme is NOT a component of your Gibson Assembly enzyme mix?

A. 5' DNA exonuclease
B. DNA polymerase
C. Restriction endonuclease
D. DNA ligase

17. *(4 points)* What other steps will you perform after completing the Gibson Assembly reaction?

2 pts each
1. Transform the reaction products into E. coli
2. Select for drug resistance to obtain clones that contain a plasmid.
3. Isolate plasmid and sequence it to confirm the insert is present.

Optional

Answer key

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18. *(4 points)* Compared to the procedures on the previous page, what would you do differently if you wanted to do restriction enzyme cloning instead?

(- 2 pts per correct answer, -2 for each incorrect statement)

- Add restriction sites to the PCR primers
- Digest the insert (PCR product/DNA to be cloned) and the plasmid (vector) with restriction enzymes (restriction endonucleases)
- Use DNA ligase to ligate the insert and plasmid together (ligate insert into plasmid (vector))

19. *(9 points)* Below are template and primer sequences for an imaginary PCR.

A. After a *single round* of denaturation, annealing, and extension, what are the sequences of the two new molecules that have been synthesized? You may draw them annealed to the denatured template below.

3 pts each

B. Choose one of the two new molecules whose sequence you wrote for part A, and write above or below it the sequence of the new molecule that will be created when it is used as a template in the next cycle of the PCR.

3 pts (one strand)

Template:

5'-TTTTAAAAGGGGCCCCTGACTAGGGAAA-3'
3'-AAAATTTTCCCCGGGGACTGATCCCTTT-5'

Primers:

5'-AAGGG-3'
5'-CCCTA-3'

Denatured template:

5'-TTTTAAAAGGGGCCCCTGACTAGGGAAA-3'
3'AAAATTTTCCCCGGGGACTGATCCC5'
5'AAGGGGCCCCTGACTAGGG3'

From second cycle (B)

3'TTCCCCGGGGACTGATCCC5'
5'AAGGGGCCCCTGACTAGGGAAA3'

From first cycle (A)

3'-AAAATTTTCCCCGGGGACTGATCCCTTT-5'