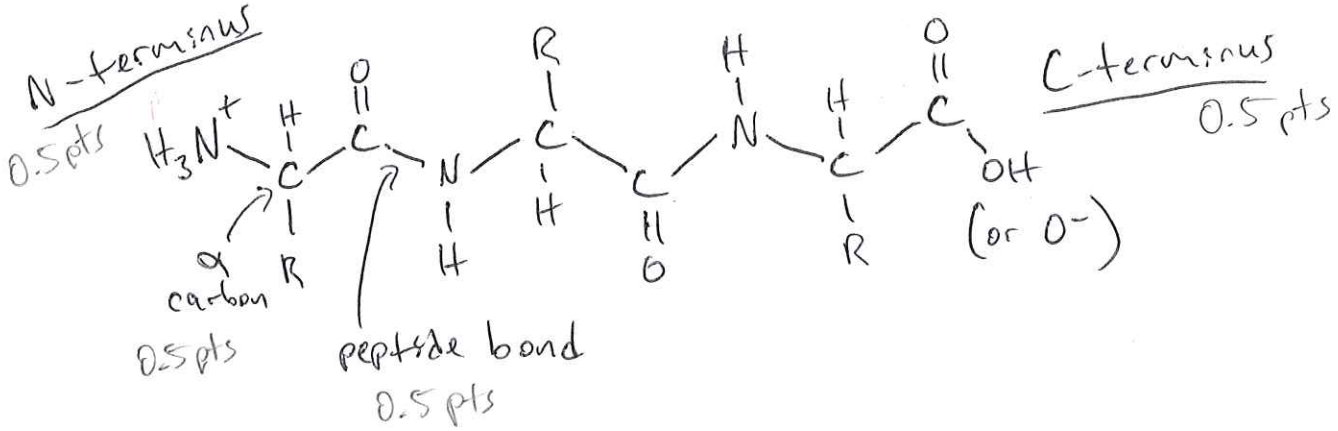
Answer key

BB2950 Molecular Biology, Quiz 2: 11-3-17, 25 points total
PLEASE WRITE YOUR NAME ON THE BACK OF EACH PAGE

1. *(4.5 points)* Draw a peptide that contains three residues (amino acids). Indicate the side-chain with the letter "R" and draw the complete molecular structure of the backbone. Label the N and C termini of the molecule, at least one peptide bond, and at least one alpha carbon.




2. *(6 points)* You want to amplify the **bolded** section of the following DNA molecule in order to clone it. Suppose PCR primers only needed to be 5 nt in length.

5'-CCTAGCAT**GAAACTTCAATGTAGGTTAACCCG**AAAAGCGATG-3'
3'-GGATCGTA**CTTTGAAGTTACATCCAATTGGGC**TTTTCGCTAC-5'

A. Indicate the forward and reverse primers that you will use, with their 5' and 3' ends labeled.

Forward primer: 5'GAAAC 3'
Reverse primer: 5' CGGGT 3'

1 pt | primer sequence
1 pt | correct end labeling

B. You plan to use restriction enzymes and ligation to clone this gene into a plasmid. You therefore need to add sequences to your primers in order to flank your gene with the appropriate restriction enzyme recognition sites. To which end of each primer will you add the extra sequences?

5' ends 2 pts.

3. *(2 points)* Which type of bond, force, or effect is most often responsible for interactions between polar, uncharged side chains and other molecules?

A) Ionic bonds
B) Hydrogen bonds
C) Hydrophobic effects
D) Stacking
E) Van der Waals forces

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4. *(4.5 points)* The schematic below shows the steps in a typical PCR reaction. The upper numbers indicate temperatures and the lower numbers indicate time. Focus on the middle section between the two dashed lines, where 25 cycles of 3 temperatures are indicated. Describe what is happening at each of these three temperature steps (one sentence per step).

1.5 pts each

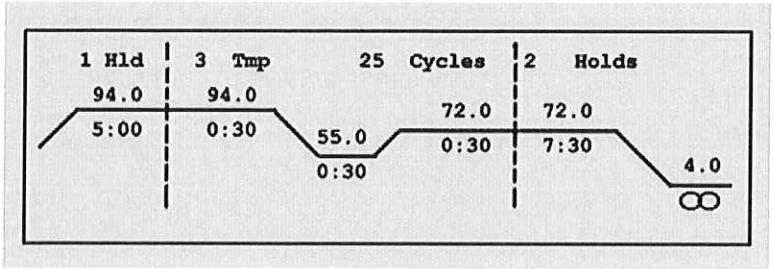


Image from: http://www.bioinformatics.nl/molbi/SimpleCloningLab/PCR.htm

94°: The double-stranded DNA template is denatured to form single-stranded molecules.

55°: The primers anneal to the template DNA at locations where complementary sequence is present.

72°: A thermostable DNA polymerase extends new DNA from the 3' end of the primers (synthesizes a new strand of DNA)

5. *(4 points)* You need to mix at least five different components together in your tube to do a PCR. List at least four of these.

dNTPs (dATP, dCTP, dGTP, dTTP); buffer; thermostable DNA polymerase; template DNA; primers (forward primer, reverse primer).

6. *(4 points)* You want to use Gibson Assembly to clone a gene of interest into a plasmid. Number the following steps in the order in which you will perform them. Two of the steps can be done in parallel, so you can give them the same number.

-1 per out-of-order step

Step 3: Transform DNA into *E. coli*

Step 1: Amplify your gene of interest using primers with extra sequence to create overlaps that match your cloning vector.

Step 4: Plate on drug-containing media to select for *E. coli* clones containing a drug resistance marker.

Step 2: Combine your insert and vector in a tube containing a mix of three enzymes.

Step 1: Amplify your plasmid using outward-facing primers or linearize it with a restriction endonuclease.