Bio93 Week 7 - Group Worksheet

You are working in a lab that studies diabetes, and your work requires you to have purified human insulin. You decide to use cloning technology to produce human insulin in E. coli. To begin, you decide to isolate mRNA from a human cell, and create a cDNA library from which to isolate the insulin cDNA.

1. Which of the following cell types would you select?
   (a) skin cell  
   (b) liver cell  
   (c) pancreas cell  
   (d) white blood cell

2. Explain why you would use insulin cDNA instead of the insulin gene from the genome.

3. You sequence your cDNA and find that it contains a point mutation that changes the second codon from GCC to GCA. Can you proceed with your experiment? Why or why not?

You've decided to clone the insulin gene (top) into the following pBIO93B plasmid (bottom).

4. Which restriction enzyme(s) do you select for cloning?

5. What type of bonds are cleaved by restriction enzymes?
   (a) ester linkages  
   (b) hydrogen bonds  
   (c) phosphodiester bonds  
   (d) ionic bonds
You want to “transform” *E. coli* with this plasmid so the bacteria transcribes the insulin gene into mRNA.

6. What other sequence must the pBIO93B plasmid have to allow this to happen?

7. Draw a box on your pBIO93B plasmid above indicated where this sequence is likely to be.

Successfully transformed bacteria will produce the human insulin protein by first transcribing the gene into mRNA, and the translating the mRNA into protein.

8. What is it about the genetic code that allows bacteria to produce a human protein?

9. Below is the beginning of exon one of the human insulin gene:

   \[ 5' \text{ATGGCCCTGTGGATGCCTCTGCCC} 3' \]

Write the mRNA sequence: 5’ -

What is the peptide sequence encoded by this mRNA? N-

What anticodons will recognize the first three codons in this mRNA?

\[ 3' - \]

\[ 3' - \]

\[ 3' - \]