The Genetic Code

Use the following DNA sequence for this exercise:

5'- ATG TTG GAG TTC GAA ACA TGC ATA GAC GGC TTA GCA TCA ATT AAA GTA ATC GGA TAA -3'
3'- TAC AAC CTC AAG CTT TGT ACG TAT CTG CCG AAT CGT AGT TAA TTT CAT TAG CCT ATT- 5'

1. Write the complementary sequence below this sequence. Label the 5' and 3' ends of both chains. Which chain is the sense strand and which is the antisense?

2. What is an ORF?
   a. Can this DNA sequence be an ORF? **Yes. The first codon is a start and the last is a stop**. If so, how many amino acids will the resulting protein be? **19 codons but the last is a stop – so 18 amino acids**
   b. Are there any ORFs that are out of frame (so they start on the 2nd or 3rd position of one of the codons that are grouped here)? If so, how many amino acids will the resulting protein be? Determine the sequence of the protein. **Yes – highlighted in yellow above.**
   c. Is there a start codon on the antisense strand? **Yep – in green.**

3. Below are two sequences with a one nucleotide mutation (underlined and starred). Which of these would most likely not change the protein sequence? Why not? **The 2nd because the mutation is in the wobble position.**

   ATG TTG GAG TTC GAA ACA TGC ATA GAC GGC T**T*** A GCA TCA ATT AAA GTA ATC GGA TAA

   ATG TTG GAG TTC GAA ACA TGC ATA GA**A*** GGC TTA GCA TCA ATT AAA GTA ATC GGA TAA

4. Would this sequence be hydrolyzed by any of the following endonucleases? If yes, clearly show on the sequence where the cut would occur and determine the size of the resulting fragment (report in bp)

NsiI (ATGCA^T)

ATGTTGGAGTTCGAAAC**ATGCA** **T**AGACGGCTTAGCATCAATTAAAGTAATCGGATAA
TACAACCTCAAGCTTTG**T** **ACGTA**TCTGCCGAATCGTAGTTAATTTCATTAGCCTATT

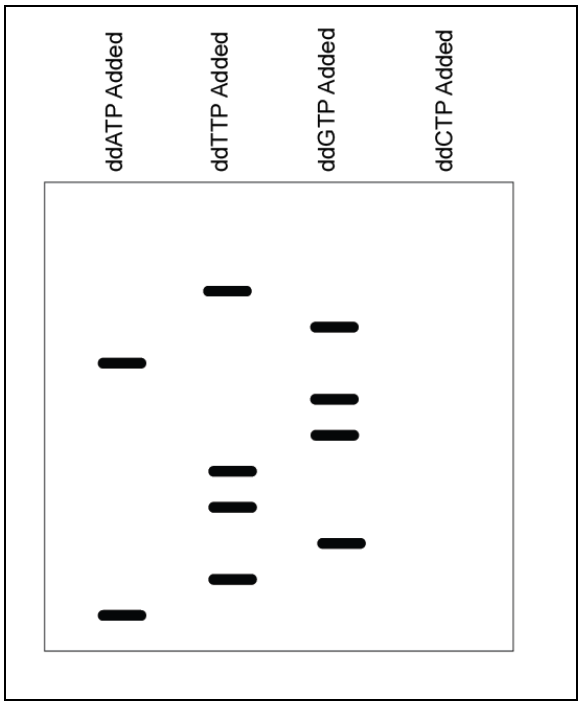
KpnI (GGTAC^C) **None**

NspI (RCATG^Y)

ATGTTGGAGTTCGAA**ACATG** **C**ATAGACGGCTTAGCATCAATTAAAGTAATCGGATAA
TACAACCTCAAGCTT**T** **GTACG**TATCTGCCGAATCGTAGTTAATTTCATTAGCCTATT

Let's focus in on the first 10 nucleic acids: ATGTTGGAGT

5. How could you sequence this using the first generation sequencing strategy? Assume that you have access to standard PCR reaction mixtures, dideoxynucleotides, and everything you need for electrophoresis. Roughly sketch what the gel would look like. 4 separate pcr reactions and evaluate them by electrophoresis. Each reaction has a small amount of one of the ddNTPs. The data would look like the image.



6. You just ran a sequencing reaction using the Sanger Method. What would the data look like? Don't worry about using different colors, just label your sketch appropriately.