NAME: _____

LABORATORY 9; EXERCISE 1. MUTATIONS

Purpose – In this Laboratory we will explore Point Mutations in multiple exercises. First, we will generate Mutations to observe the effects on Amino Acid sequences. Next, we will examine actual sequences to test hypotheses regarding mutational changes. These Exercises will use the BioEdit and BLAST programs. For this exercise, you will insert a mutation into a small gene region (an exon). Changes to the gene will be used to visualize SILENT, MISSENSE, NON SENSE and FRAMESHIHT mutations. After mutating the gene region, you will then Translate the Transcriptional Unit to see if anything changed with the structure of the resulting protein.

SPECIFIC LABORATORY PROTOCOL –

1. Log in to your VM desktop through the COSAM Desktop Portal.

2. OPEN the Windows Start  Icon and OPEN the BioEdit program. **NOTE –** DO NOT close BioEdit through the entire lab, only minimize it if you need to. You will use this information for the whole series of experiments.

3. Click On the FILE tab and then OPEN. Highlight the "This PC"  icon.

4. Select the (J:) Drive labeled "Academic – Class Resources" by double-clicking it.

5. OPEN the "Biol3001 – Genetics Laboratory" subdirectory and be sure at the bottom of the "Open File …" box, that the "Files of Type:" box is showing "ALL FILES (*.*)". You can select this from the drop down menu.

6. OPEN the "LABORATORY 10 – Mutations" subdirectory and then the "Mutation Sequences" subdirectory.

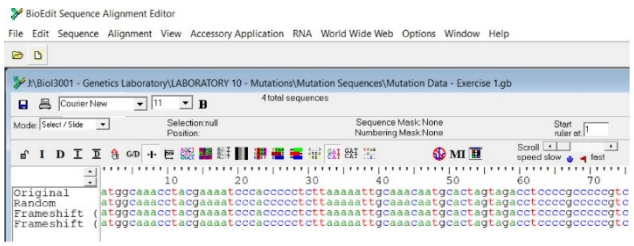
7. Click on and OPEN the labeled "Mutation Data – Exercise 1.gb"

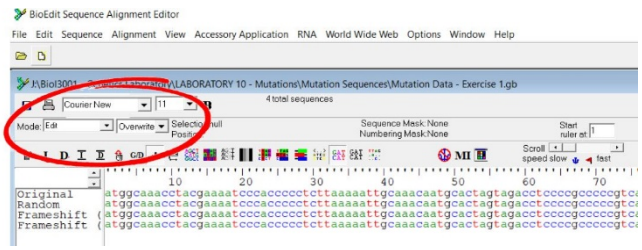
8. In BioEdit you will see that there are FOUR Identical DNA sequences that are present:

   - Original
   - Random
   - Frameshift (Delete)
   - Frameshift (Insert)

   For simplicity, you may assume that the sequences have already gone through Transcriptional Unit editing (all introns have been removed and exons are spliced together). The sequences have been aligned and you will also notice the first three bases of each sequence at "atg". All four seqences can considered to be "in frame" at this point so that Translation can proceed.

9. In the upper left corner of the BioEdit box, change the MODE from "Select/Slide" to "Edit" by using the drop down menu. Also be sure the box that opens to the right of the MODE has "Overwrite" selected as indicated below.





10. Locate the sequence labeled **"Random"** and Click on the name to highlight that sequence as the one you are working on. You are going to play the role of a REACTIVE OXYGEN SPECIES (ROS). These are normal byproducts of your natural metabolism of oxygen and play an important physiological role in your body. However, ROS are electron scavengers and can cause detrimental effects to your DNA and other cell structures. You can learn more about them by using the following link. (https://en.wikipedia.org/wiki/Reactive_oxygen_species). You are going to MUTATE this sequence at 10 different nucleotide locations. (*NOTE: you may find it useful to make the left side of the BioEdit box where the names are located wider by Clicking/Dragging the right side of that box to show the entire names of the sequences, but this is not a requirement*)

11. Find the NUCLEOTIDE LOCATIONS indicated below and change them to ANY OTHER RANDOM NUCLEOTIDE. For instance, the first nucleotide you will locate is #15 which is an ADENINE (a). Change that nucleotide to either a THYMINE (t), CYTOSINE (c) or GUANINE (g).....You choose which one!!

12. Use your cursor (should look like and arrow) and Click on each of the nucleotides indicate. You will see a blinking black line appear in front of that nucleotide. If you are in the Overwrite Mode, simply use your keyboard to change the nucleotide to any other. Indicate in the boxes below each nucleotide which MUTATION you inserted into the sequence.

#15	#26	#34	#68	#100	#134	#141	#233	#255	#260

13. Now, locate the sequence labeled **"Frameshift (Delete)"** and Click on the name to highlight that sequence as the one you are working on. In this part of the Exercise, you are going to play the role of IONIZING RADIATION which carries enough energy to cause electrons to detach from molecules resulting in DNA strand breakage among other deleterious effects to your body and your cells. You can read more about this type of mutagen at https://en.wikipedia.org/wiki/Ionizing_radiation.

14. Move your cursor to ANY NUCLEOTIDE at a position GREATER THAN #70 on this sequence. (Be sure you are working with the correct sequence!!). Click on that nucleotide as above and you will see the blinking black line in front of the nucleotide you selected. Using your keyboard, hit the DELETE key and remove that nucleotide from the sequence. Record which nucleotide you deleted below.

Nucleotide Deleted (type and position): ____

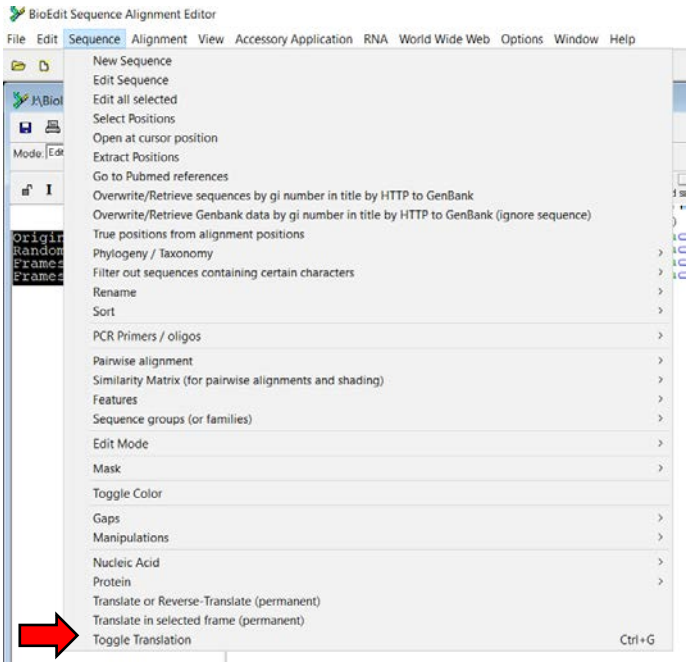
15. Finally, locate the sequence labeled **"Frameshift (Insert)"** and Click on the name to highlight that sequence as the one you are working on. This time you will be playing the role of DNA POLYMERASE and simulating a proofreading mistake during elongation (https://en.wikipedia.org/wiki/DNA_replication).

16. Be sure the MODE is still on "Edit" in the program but change the box to the right to "INSERT". Select the same nucleotide you deleted in the step above and this time, use your keyboard to add ANY NUCLEOTIDE at that same position. Record which nucleotide you inserted below.

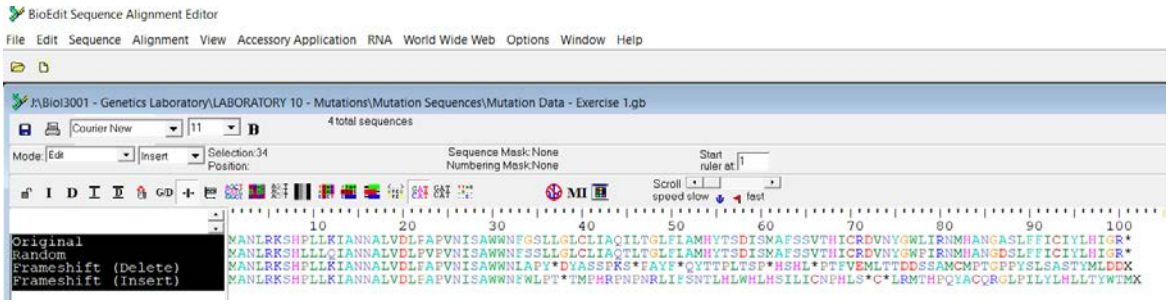
Nucleotide Inserted (type and position): ____

17. You have now been responsible for multiple types of mutations in your DNA sequences by multiple different mechanisms! Lets see what those mutations have caused. You will now TRANSLATE each of the DNA sequences into polypeptide chains. Click on EACH of the four sequence names on the left side of your window while holding down the SHIFT key (this should allow you to select ALL FOUR sequences at one time (i.e they should all be highlighted before proceeding to the next step).

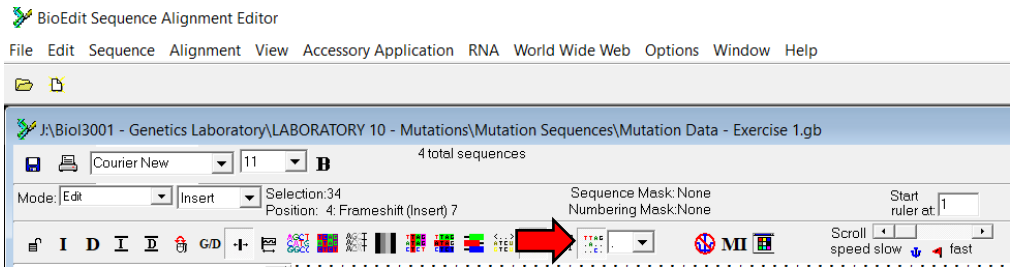
18. With all sequences highlighted, use the toolbar at the top of the BioEdit window and the SEQUENCE tab. Click on SEQUENCE and at the very bottom of the resulting options, Click on the "Toggle Translation" option.



19. You should see the TRANSLATED polypeptide chain of each of your sequences show on the screen.



19. You can SELECT the "DOT DISPLAY" option to help you visualize and identify any differences your mutations caused between the sequences. (NOTE – The asterick symbol "*" indicates a TERMINATION CODON in BioEdit).



Answer the following questions regarding the results of your mutations.

A. Compare the Amino Acid translation sequence for the Original sequence (the one that has not been mutated) to the translation of the Random sequence and provide the following information.

Total Number of Amino Acid Changes – _____

Number of Silent (No Change) Mutations – _____

Number of Missense Mutations – _____

If Missense Mutations occurred, how many were due to changes in each position –

1st Position - _____ 2cd Position - _____ 3rd Position - _____

Number of Nonsense Mutations – _____

B. Compare the Translations for the Frameshift (Delete) to the Original Sequence.

Total Number of Amino Acids in the Original – _____

Total Number of Termination Codons in Frameshift(Delete) – _____

Number of Amino Acids in Frameshirt sequence BEFORE the first Termination Codon – _____

C. Compare the Translations for the Frameshirt (Insert) to the Original Sequence.

Total Number of Amino Acids in the Original – _____

Total Number of Termination Codons in the Frameshift(Insert) – _____

Number of Amino Acids in Frameshirt sequence BEFORE the first Termination Codon – _____

D. Based on the results from this Mutation Experiment, take a little time and hypothesize which type of mutation (Nucleotide Substitution vs. Frameshift (Insertion or Deletion)) is more likely to have the GREATER effect on the Phenotype of an organism. BRIEFLY explain how the data from this experiment support your hypothesis.