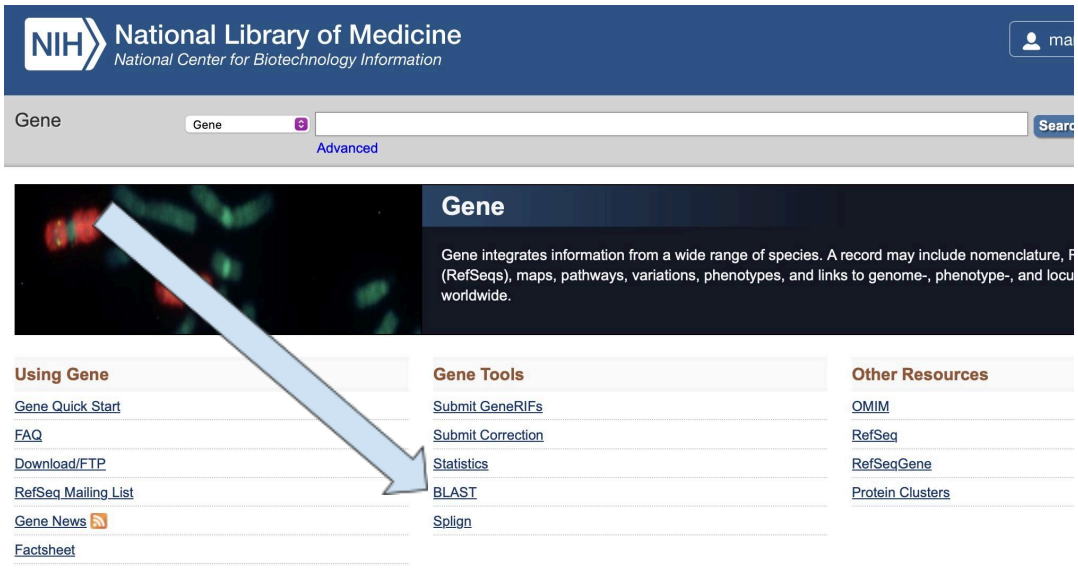
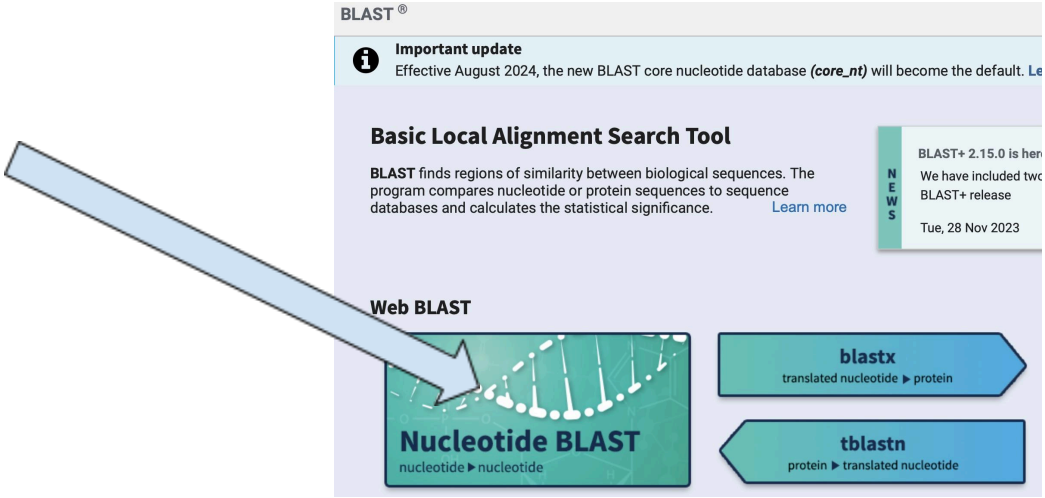
BLAST Analysis Teacher Guide

1. Go to https://www.ncbi.nlm.nih.gov/gene (Gene Database).
2. Click on the link that says BLAST



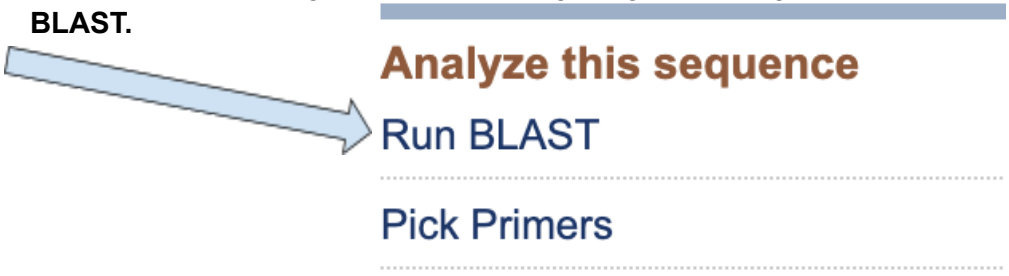
3. Then click on the Nucleotide Blast Button:



4. FOR STEP 4, THERE ARE 2 OPTIONS (A **OR** B):
    A. You can click on the link here (beneath where it says “HBB wild type exon from the link.”

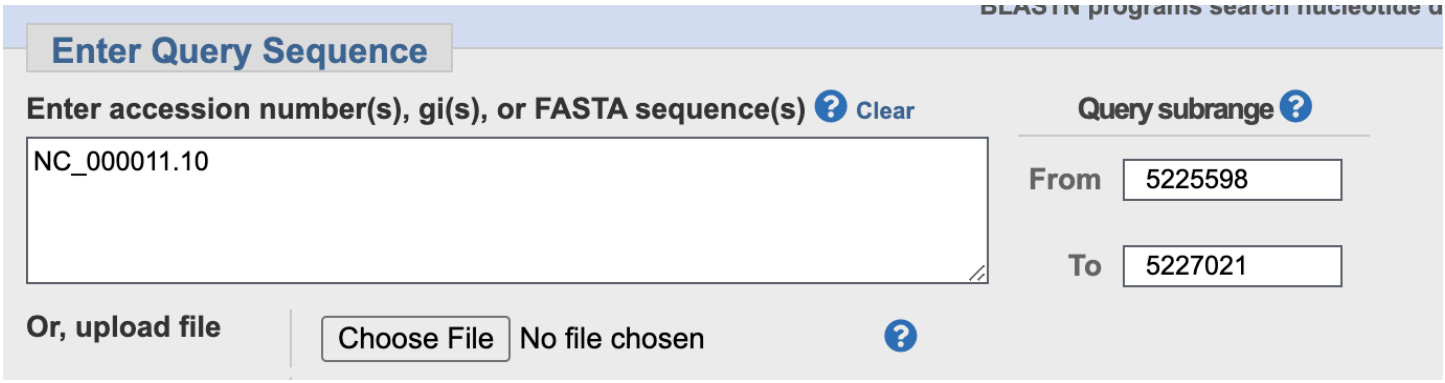
HBB wild type exon from the following link:
https://www.ncbi.nlm.nih.gov/nuccore/NC_000011.10?report=fasta&from=5225598&to=5227021

You will come to a page with the following image on the right-hand side of the page, and you need to click **RUN BLAST.**

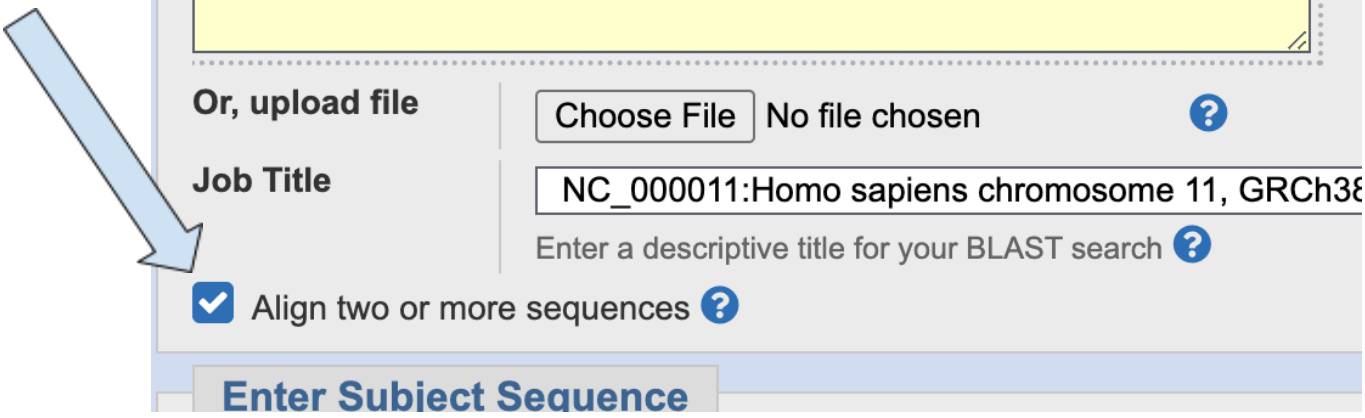


OR

B. This option is INSTEAD of option A. Enter the accession number and query subrange (if you click on the link, it should be there for you, but if you don't, and you get to the below screen through the "clicks," you can enter the numbers that appear below in the screen shot.
C. EITHER WAY - you will end up on this screen below.

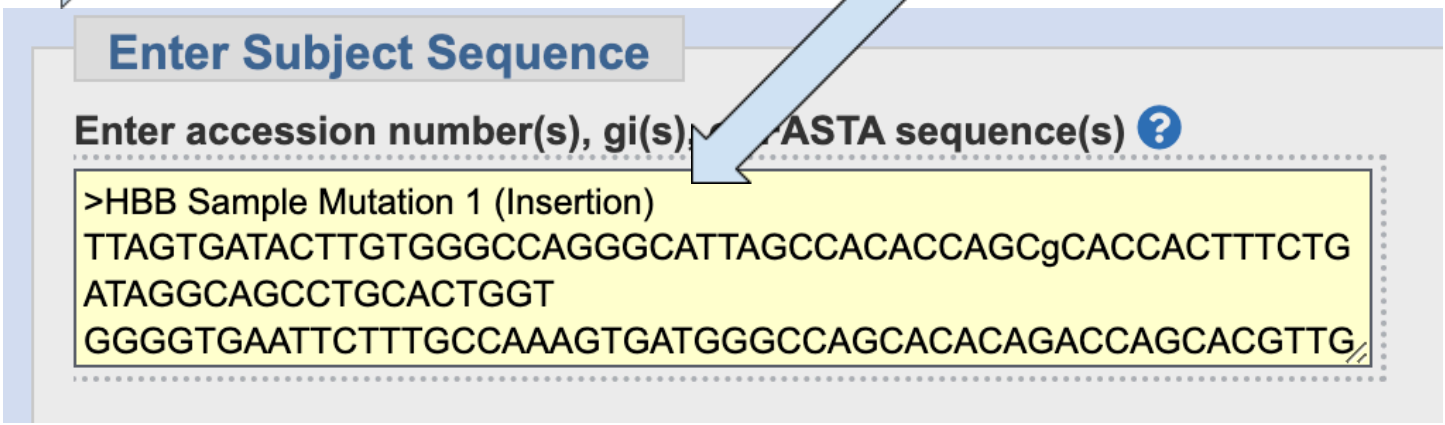


5. Once on this screen, find the "Align two or more sequences" (See picture below) and check the box. Once you do that, you will get another box beneath the check box into which you can enter the sequences you want to compare.



6. In the box shown below, copy & paste in the nucleotide sequence or sequences below. You can do them one at a time, or all at once. In each of the mutation sequences, the location of the mutation is indicated by either a bold-faced red letter or a green lighted letter. The multiple mutations challenge does not show where the mutations are. If you want to make it more difficult, you can remove the color coding that shows where the mutations are located.

7. If you are ONLY entering 1 sequence, you can skip the title. HOWEVER, if you put in all of the sequences at the same time, you should use titles so you can differentiate between the sequences. To enter a title, put a ">" in front of the text that is not part of the nucleic acid sequence.



SAMPLE SEQUENCES FOR COPYING:

HBB Sample Mutation 1 (Insertion)

TTAGTGATACTTGTGGGCCAGGGCATTAGCCACACCAGC**g**CACCACTTTCTGATAGGCAGCCTGCACTGGT
GGGGTGAATTCTTTGCCAAAGTGATGGGCCAGCACACAGACCAGCACGTTGCCCAGGAGCTGTGGGAGGA
AGATAAGAGGTATGAACATGATTAGCAAAAGGGCCTAGCTTGGACTCAGAATAATCCAGCCTTATCCCAA
CCATAAAATAAAAGCAGAATGGTAGCTGGATTGTAGCTGCTATTAGCAATATGAAACCTCTTACATCAGT
TACAATTTATATGCAGAAATATTTATATGCAGAGATATTGCTATTGCCTTAACCCAGAAATTATCACTGT
TATTCTTTAGAATGGTGCAAAGAGGCATGATACATTGTATCATTATTGCCCTGAAAGAAAGAGATTAGGG
AAAGTATTAGAAATAAGATAAACAAAAAAGTATATTAAAAGAAGAAAGCATTTTTTAAAATTACAAATGC
AAAATTACCCTGATTTGGTCAATATGTGTACACATATTAAAACATTACACTTTAACCCATAAATATGTAT
AATGATTATGTATCAATTAAAAATAAAAGAAAATAAAGTAGGGAGATTATGAATATGCAAATAAGCACAC
ATATATTCCAAATAGTAATGTACTAGGCAGACTGTGTAAAGTTTTTTTTTAAGTTACTTAATGTATCTCA
GAGATATTTCCTTTTGTTATACACAATGTTAAGGCATTAAGTATAATAGTAAAAATTGCGGAGAAGAAAA
AAAAAGAAAGCAAGAATTAAACAAAAGAAAACAATTGTTATGAACAGCAAATAAAAGAAACTAAAACGAT
CCTGAGACTTCCACACTGATGCAATCATTCGTCTGTTTCCCATTCTAAACTGTACCCTGTTACTTATCCC
CTTCCTATGACATGAACTTAACCATAGAAAAGAAGGGGAAAGAAAACATCAAGCGTCCCATAGACTCACC
CTGAAGTTCTCAGGATCCACGTGCAGCTTGTCACAGTGCAGCTCACTCAGTGTGGCAAAGGTGCCCTTGA
GGTTGTCCAGGTGAGCCAGGCCATCACTAAAGGCACCGAGCACTTTCTTGCCATGAGCCTTCACCTTAGG
GTTGCCCATAACAGCATCAGGAGTGGACAGATCCCCAAAGGACTCAAAGAACCTCTGGGTCCAAGGGTAG
ACCACCAGCAGCCTAAGGGTGGGAAAATAGACCAATAGGCAGAGAGAGTCAGTGCCTATCAGAAACCCAA
GAGTCTTCTCTGTCTCCACATGCCCAGTTTCTATTGGTCTCCTTAAACCTGTCTTGTAACCTTGATACCA
ACCTGCCCAGGGCCTCACCACCAACTTCATCCACGTTCACCTTGCCCCACAGGGCAGTAACGGCAGACTT
CTCCTCAGGAGTCAGATGCACCAT

HBB Sample Mutation 2 (Deletion - there should be an A in between the two T's - the unhighlighted one and the highlighted one)

TTAGTGATACTTGTGGGCCAGGGCATTAGCCACACCAGCCACCACTTTCTGATAGGCAGCCTGCACTGGT
GGGGTGAATTCTTTGCCAAAGTGATGGGCCAGCACACAGACCAGCACGTTGCCCAGGAGCTGTGGGAGGA
AGATAAGAGGTTGAACATGATTAGCAAAAGGGCCTAGCTTGGACTCAGAATAATCCAGCCTTATCCCAA
CCATAAAATAAAAGCAGAATGGTAGCTGGATTGTAGCTGCTATTAGCAATATGAAACCTCTTACATCAGT
TACAATTTATATGCAGAAATATTTATATGCAGAGATATTGCTATTGCCTTAACCCAGAAATTATCACTGT
TATTCTTTAGAATGGTGCAAAGAGGCATGATACATTGTATCATTATTGCCCTGAAAGAAAGAGATTAGGG
AAAGTATTAGAAATAAGATAAACAAAAAAGTATATTAAAAGAAGAAAGCATTTTTTAAAATTACAAATGC
AAAATTACCCTGATTTGGTCAATATGTGTACACATATTAAAACATTACACTTTAACCCATAAATATGTAT
AATGATTATGTATCAATTAAAAATAAAAGAAAATAAAGTAGGGAGATTATGAATATGCAAATAAGCACAC
ATATATTCCAAATAGTAATGTACTAGGCAGACTGTGTAAAGTTTTTTTTTAAGTTACTTAATGTATCTCA
GAGATATTTCCTTTTGTTATACACAATGTTAAGGCATTAAGTATAATAGTAAAAATTGCGGAGAAGAAAA
AAAAAGAAAGCAAGAATTAAACAAAAGAAAACAATTGTTATGAACAGCAAATAAAAGAAACTAAAACGAT
CCTGAGACTTCCACACTGATGCAATCATTCGTCTGTTTCCCATTCTAAACTGTACCCTGTTACTTATCCC
CTTCCTATGACATGAACTTAACCATAGAAAAGAAGGGGAAAGAAAACATCAAGCGTCCCATAGACTCACC
CTGAAGTTCTCAGGATCCACGTGCAGCTTGTCACAGTGCAGCTCACTCAGTGTGGCAAAGGTGCCCTTGA
GGTTGTCCAGGTGAGCCAGGCCATCACTAAAGGCACCGAGCACTTTCTTGCCATGAGCCTTCACCTTAGG
GTTGCCCATAACAGCATCAGGAGTGGACAGATCCCCAAAGGACTCAAAGAACCTCTGGGTCCAAGGGTAG
ACCACCAGCAGCCTAAGGGTGGGAAAATAGACCAATAGGCAGAGAGAGTCAGTGCCTATCAGAAACCCAA
GAGTCTTCTCTGTCTCCACATGCCCAGTTTCTATTGGTCTCCTTAAACCTGTCTTGTAACCTTGATACCA
ACCTGCCCAGGGCCTCACCACCAACTTCATCCACGTTCACCTTGCCCCACAGGGCAGTAACGGCAGACTT
CTCCTCAGGAGTCAGATGCACCAT

HBB Sample Mutation 3 (Single Nucleotide Polymorphism SNP- Replacement)

TTAGTGATACTTGTGGGCCAGGGCATTAGCCACACCAGCCACCACTTTCTGATAGGCAGCCTGCACTGGT
GGGGTGAATTCTTTGCCAAAGTGATGGGCCAGCACACAGACCAGCACGTTGCCCAGGAGCTGTGGGAGGA
AGATAAGAGGTATGAACATGATTAGCAAAAGGGCCTAGCTTGGACTCAGAATAATCCAGCCTTATCCCAA
CCATAAAATAAAAGCAGAATGGTAGCTGGATTGTAGCTGCTATTAGCAATATGAAACCTCTTACATCAGT
TACAATTTATATGCAGAAATATTTATATGCAGAGATATTGCTATTGCCTTAACCCAGAAATTATCACTGT
TATTCTTTAGAATGGTGCAAAGAGGCATGATACATTGTATCATTATTGCCCTGAAAGAAAGAGATTAGGG
AAAGTATTAGAAATAAGATAAACAAAAAAGTATATTAAAAGAAGAAAGCATTTTTTAAAATTACAAATGC
AAAATTACCCTGATTTGGTCAATATGTGTACACATATTAAAACATTACACTTTAACCCATAAATATGTAT
AATGATTATGTATCAATTAAAAATAAAAGAAAATAAAGTAGGGAGATTATGAATATGCAAATAAGCACAC
ATATATTCCAAATAGTAATGTACTAGGCAGACTGTGTAAAGTTTTTTTTTAAGTTACTTAATGTATCTCA
GAGATATTTCCTTTTGTTATACACAATGTTAAGGCATTAAGTATAATAGTAAAAATTGCGGAGAAGAAAA
AAAAAGAAAGCAAGAATTAAACAAAAGAAAACAATTGTTATGAACAGCAAATAAAAGAAACTAAAACGAT
CCTGAGACTTCCACACTGATGCAATCATTCG**c**CTGTTTCCCATTCTAAACTGTACCCTGTTACTTATCCC
CTTCCTATGACATGAACTTAACCATAGAAAAGAAGGGGAAAGAAAACATCAAGCGTCCCATAGACTCACC
CTGAAGTTCTCAGGATCCACGTGCAGCTTGTCACAGTGCAGCTCACTCAGTGTGGCAAAGGTGCCCTTGA
GGTTGTCCAGGTGAGCCAGGCCATCACTAAAGGCACCGAGCACTTTCTTGCCATGAGCCTTCACCTTAGG
GTTGCCCATAACAGCATCAGGAGTGGACAGATCCCCAAAGGACTCAAAGAACCTCTGGGTCCAAGGGTAG
ACCACCAGCAGCCTAAGGGTGGGAAAATAGACCAATAGGCAGAGAGAGTCAGTGCCTATCAGAAACCCAA
GAGTCTTCTCTGTCTCCACATGCCCAGTTTCTATTGGTCTCCTTAAACCTGTCTTGTAACCTTGATACCA
ACCTGCCCAGGGCCTCACCACCAACTTCATCCACGTTCACCTTGCCCCACAGGGCAGTAACGGCAGACTT
CTCCTCAGGAGTCAGATGCACCAT

HBB Sample Mutation 4 (Challenge- Multiple Mutations - the mutations are not indicated here)

TTAGTGAATACTTGTGGGCCAGGGCATTAGCCACACCAGCCACCACTTTCTGATAGGCAGCCTGCACTGGT
GGGGTGAATTCTTTGCCAAAGTGATGGGCCAGCACACAGACCAGCACGTTGCCCAGAGCTGTGGGACGGAA
GATAAGAGGTATGAACATGATTAGCAAAAGGGCCTAGCTTGGACTCAGAATAATCCAGCCTTATCCCAACC
ATAAAATAAAAGCAGAATGGTAGCTGGATTGTAGCTGCTATTAGCAATATGAAACCTCTCTACATCAGTTA
CAATTTATATGCAGAAATATTTATATGCAGAGATATTGCTATTGCCTTAACTCCAGAAATTATCACTGTTA

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TTCTTTAGAATGTGCAAAGAGGCATGATACATTGTATCATTATTGCCCTGAAAGAAAGAGATTAGGGAAAG
TATTAGAAATAAGATAGACAAAAAAGTATATTAAAAGAAGAAAGCATTTTTTAAAATTACAAATGCAAAAT
TACCCTGATTTGGTCAATATGTGTACACATATTAAAACATTACACTTTAACCCATAAATATGTATAATGAT
CTATGTATCAATTAAAAATAAAAGAAAATAAAGTAGGGAGATTATGAATATGCAAATAAGCACACATATAT
TCCAAATAGTAATGTACTAGGCAGACTGTGTAAAGTTTTTTTTTAAGTTACTTAATGTATCTCAGAGATAT
TTCCTTTTGTTATACACAATGTTAAGGCATTAAGTATAATAGTAAAAATTGCGGAGAAGAAAAAAAAAGAA
AGCAAGAATTAAACAAAAGAAAAACAATTGTTATGAACAGCAATAAAAGAAACTAAAACGATCCTGAGACT
TCCACACTGATGCAATCATTCGCTGTTTCCCATTCTAAACTGTACCCTGTTACTGTATCCCCTTCCTATGA
CATGAACTTAACCATAGAAAAGAAGGGGAAAGAAAACATCAAGCGTCCCATAGACTCACCCTGAAGTTCTC
AGGATCCACGTGCAGCTTGTCACAGTGCAGCTCACTCAGTGTGGCAAAGGTGCCCTTGAGGTTGTCCAGGT
GAGCCAGGCCATCACTAAAGGCACCGAGCACTTTCTTGCCATGAGCCTTCACCTTAGGGTTGCCCATAACA
GCATCAGAGTGGACAGATCCCCAAAGGACTCCATAAAGAACCTCTGGGTCCAAGGGTCAGACCACCAGCAG
AAGGGTGGGAAAATAGACCAATAGGCAGAGAGAGTCAGTGCCTATCAGAAACCCAAGAGTCTTCTCTGTCT
CCACATGCCCAGTTTCTATTGGTCTCCTTAAACCTGTCTTGGTAACCTTGATACCAACCTGCCCAGGGCCT
CACCACCAACTTCATCCACGTTCACCTTGCCCCACAGGGCAGTAACGGCAGACTTCTCCTCAGGAGTCAGA
TGCACCAT
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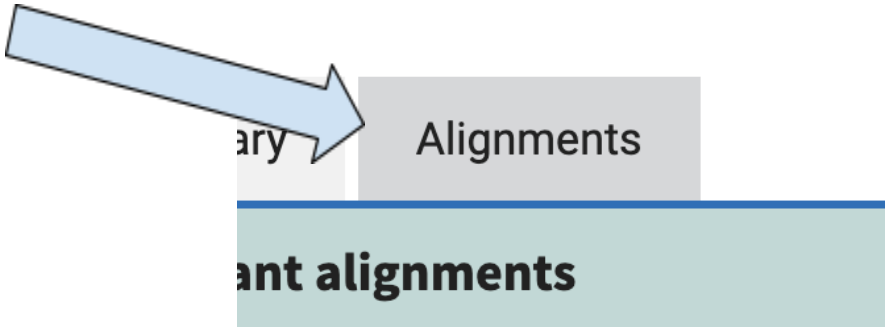
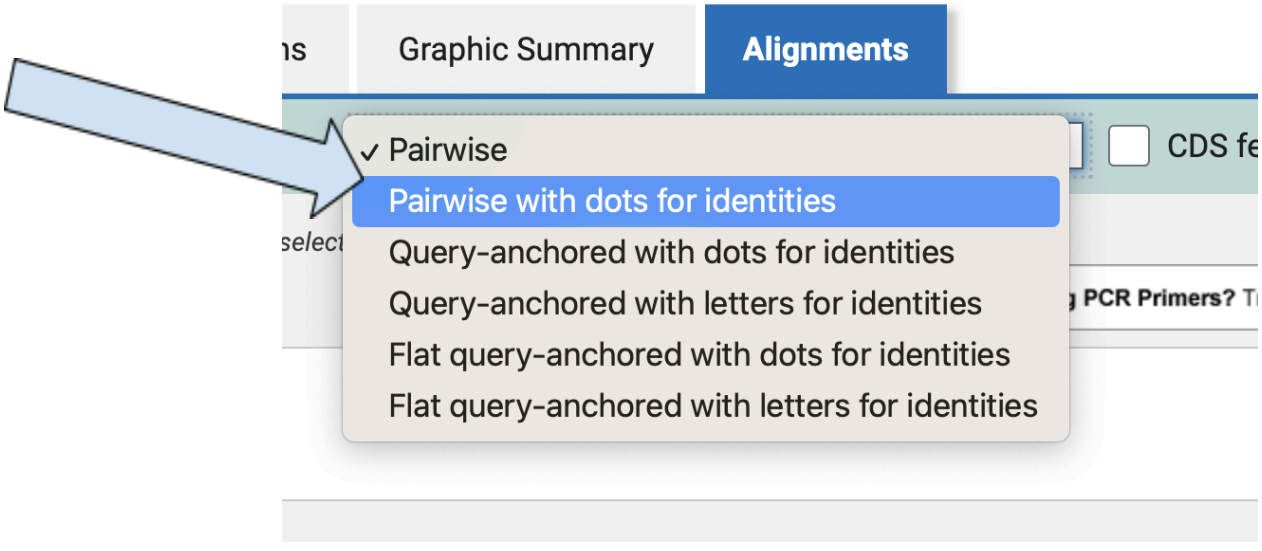
8. Push the BLAST button

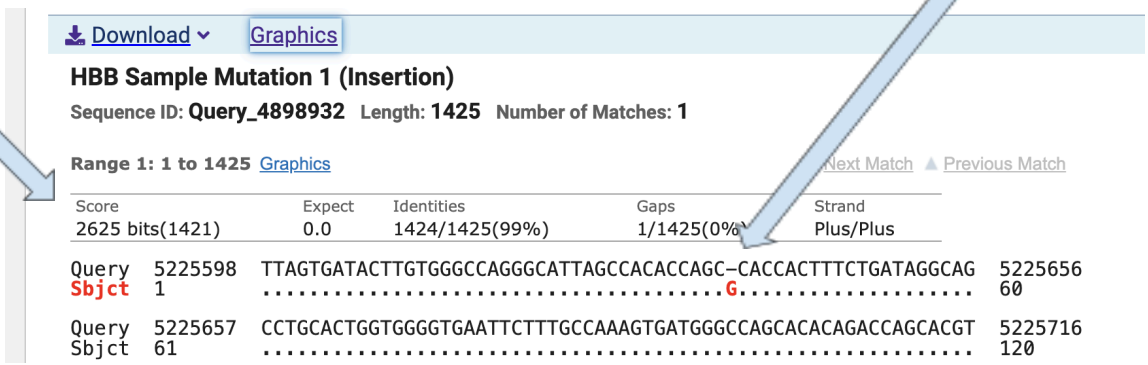
9. Wait for the computation to run, it should only take a few seconds.
10. Once the computations are complete click on the alignment tab down at the bottom of the retrieval



11. Then select “Pairwise with dots for identities.” This will make the differences between the sequences easier to visualize.



12. The difference(s) between the HBB wild type sequence and the mutation sequences should appear as shown below, with the mutation in red.



Resources:

https://www.ncbi.nlm.nih.gov/nuccore/NC_000011.10?report=fasta&from=5225598&to=5227021

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>NC_000011.10:5225598-5227021 Homo sapiens chromosome 11, GRCh38.p14 Primary Assembly
TTAGTGATACTTGTGGGCCAGGGCATTAGCCACACCAGCCACCACTTTCTGATAGGCAGCCTGCACTGGT
GGGGTGAATTCTTTGCCAAAGTGATGGGCCAGCACACAGACCAGCACGTTGCCCAGGAGCTGTGGGAGGA
AGATAAGAGGTATGAACATGATTAGCAAAAGGGCCTAGCTTGGACTCAGAATAATCCAGCCTTATCCCAA
CCATAAAATAAAAGCAGAATGGTAGCTGGATTGTAGCTGCTATTAGCAATATGAAACCTCTTACATCAGT
TACAATTTATATGCAGAAATATTTATATGCAGAGATATTGCTATTGCCTTAACCCAGAAATTATCACTGT
TATTCTTTAGAATGGTGCAAAGAGGCATGATACATTGTATCATTATTGCCCTGAAAGAAAGAGATTAGGG
AAAGTATTAGAAATAAGATAAACAAAAAAGTATATTAAAAGAAGAAAGCATTTTTTAAAATTACAAATGC
AAAATTACCCTGATTTGGTCAATATGTGTACACATATTAAAACATTACACTTTAACCCATAAATATGTAT
AATGATTATGTATCAATTAAAAATAAAAGAAAATAAAGTAGGGAGATTATGAATATGCAAATAAGCACAC
ATATATTCCAAATAGTAATGTACTAGGCAGACTGTGTAAAGTTTTTTTTTAAGTTACTTAATGTATCTCA
GAGATATTTCCTTTTGTTATACACAATGTTAAGGCATTAAGTATAATAGTAAAAATTGCGGAGAAGAAAA
AAAAAGAAAGCAAGAATTAAACAAAAGAAAACAATTGTTATGAACAGCAAATAAAAGAAACTAAAACGAT
CCTGAGACTTCCACACTGATGCAATCATTCGTCTGTTTCCCATTCTAAACTGTACCCTGTTACTTATCCC
CTTCCTATGACATGAACTTAACCATAGAAAAGAAGGGGAAAGAAAACATCAAGCGTCCCATAGACTCACC
CTGAAGTTCTCAGGATCCACGTGCAGCTTGTCACAGTGCAGCTCACTCAGTGTGGCAAAGGTGCCCTTGA
GGTTGTCCAGGTGAGCCAGGCCATCACTAAAGGCACCGAGCACTTTCTTGCCATGAGCCTTCACCTTAGG
GTTGCCCATAACAGCATCAGGAGTGGACAGATCCCCAAAGGACTCAAAGAACCTCTGGGTCCAAGGGTAG
ACCACCAGCAGCCTAAGGGTGGGAAAATAGACCAATAGGCAGAGAGAGTCAGTGCCTATCAGAAACCCAA
GAGTCTTCTCTGTCTCCACATGCCCAGTTTCTATTGGTCTCCTTAAACCTGTCTTGTAACCTTGATACCA
ACCTGCCCAGGGCCTCACCACCAACTTCATCCACGTTCACCTTGCCCCACAGGGCAGTAACGGCAGACTT
CTCCTCAGGAGTCAGATGCACCAT
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