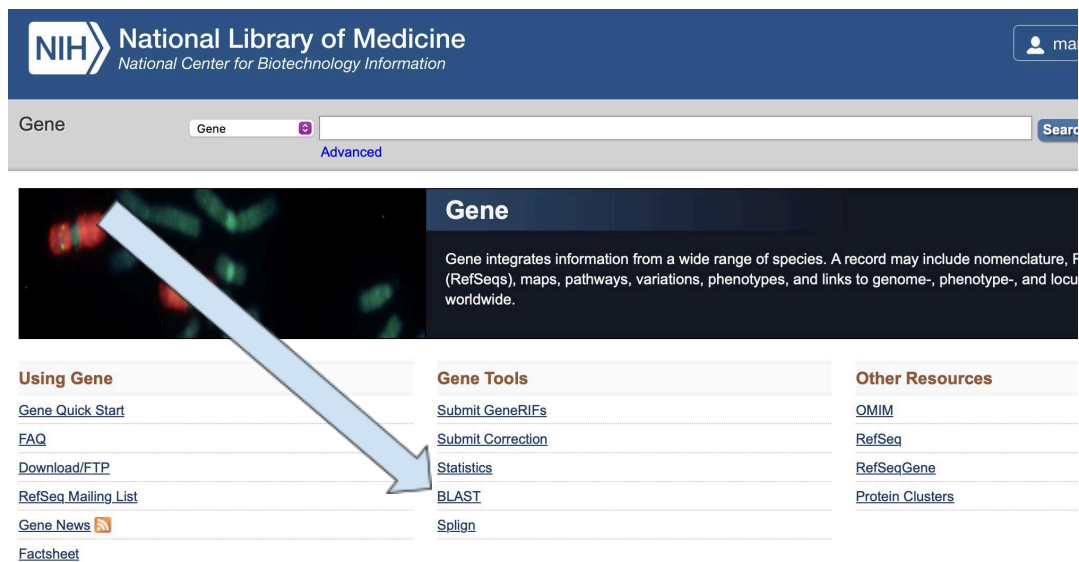


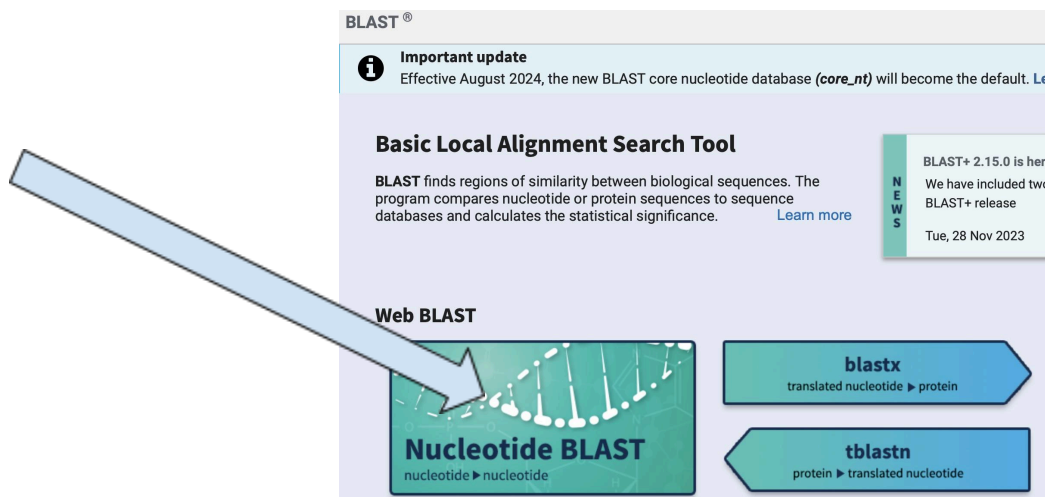
BLAST Analysis Student Handout

Note - types of mutations for the four samples are NOT indicated in this document.

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

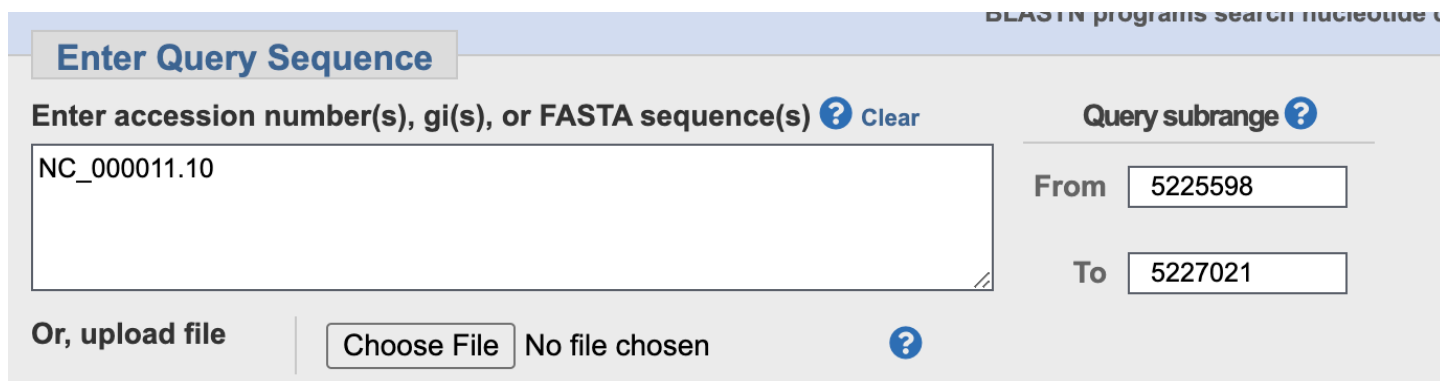
You will come to a page with the following image on the right-hand side of the page, and you need to click **RUN BLAST**.



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.



Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) ? Clear

NC_000011.10

Query subrange ?

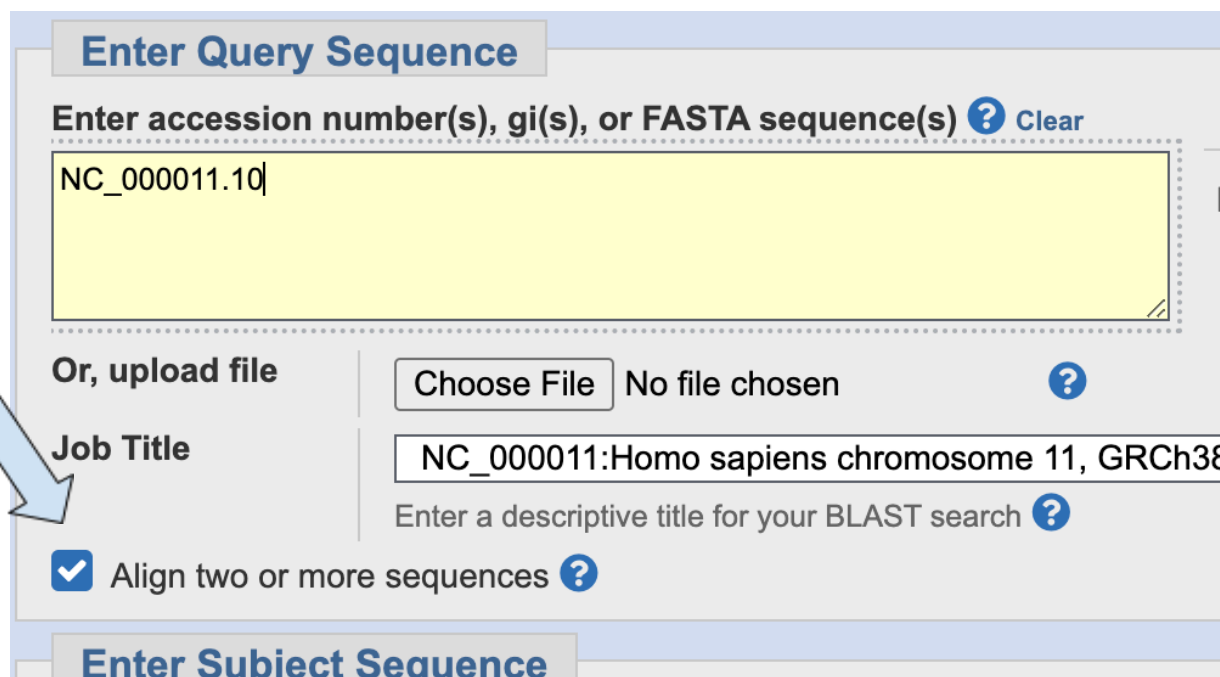
From 5225598

To 5227021

Or, upload file

Choose File No file chosen ?

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.



Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) ? Clear

NC_000011.10

Or, upload file

Choose File No file chosen ?

Job Title

NC_000011:Homo sapiens chromosome 11, GRCh38

Enter a descriptive title for your BLAST search ?

☒ Align two or more sequences ?

Enter Subject Sequence

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.

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.

Enter Subject Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) ?

>HBB Sample Mutation 1 (Insertion)

```
TTAGTGATACTTGTGGGCCAGGGCATTAGCCACACCAGCGCACCACCTTTCTG
ATAGGCAGCCTGCACTGGT
GGGGTGAATTCTTTGCCAAAGTGATGGGCCAGCACACAGACCAGCACGTTG
```

SAMPLE SEQUENCES FOR COPYING:

HBB Sample Mutation 1 TTAGTGATACTTGTGGGCCAGGGCATTAGCCACACCAGCGCACCACCTTTCTGATAGGCAGCCTGCACTGGT
GGGGTGAATTCTTTGCCAAAGTGATGGGCCAGCACACAGACCAGCACGTTGCCCAGGAGCTGTGGGAGGA
AGATAAGAGGTATGAACATGATTAGCAAAAAGGGCTAGCTTGGACTCAGAATAATCCAGCCTTATCCCCAA
CCATAAAATAAAAGCAGAATGGTAGCTGGATTGTAGCTGCTATTAGCAATATGAAACCTCTTACATCAGT
TACAATTTATATGCAGAAATATTTATATGCAGAGATATTGCTATTGCCTTAACCCAGAAATTATCACTGT
TATTCCTTTAGAATGGTGCAAAGAGGCATGATACATTGTATCATTATTGCCCTGAAAGAAAAGAGATTAGGG
AAAGTATTAGAAATAAGATAAACAAAAAGTATATTAAAAGAAGAAAGCATTTTTTTAAATTTACAAATGC
AAAATTACCCTGATTTGGTCAATATGTGTACACATATTAAACATTACACTTTAACCATAAAATATGTAT
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ATATATTCCAAATAGTAATGTACTAGGCAGACTGTGTAAAGTTTTTTTTTAAAGTTACTTAATGTATCTCA
GAGATATTTTCCTTTTGTATACACAATGTTAAGGCATTAAGTATAATAGTAAAAATTGCGGAGAAGAAAA
AAAAAGAAAGCAAGAATTAAACAAAAGAAAACAATTGTTATGAACAGCAAATAAAAGAACTAAAACGAT
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CTGAAGTTCTCAGGATCCACGTGCAGCTTGTTCAGAGTGCAGCTCACTCAGTGTGGCAAAGGTGCCCTTGA
GGTTGTCCAGGTGAGCCAGGCCATCACTAAAGGCACCGAGCACTTTCTTGCCATGAGCCTTCACCTTAGG
GTTGCCCATACAGCATCAGGAGTGGACAGATCCCCAAAGGACTCAAAGAACCTCTGGGTCCAAGGGTAG
ACCACCAGCAGCCTAAGGGTGGGAAAATAGACCAATAGGCAGAGAGAGTCAGTGCCTATCAGAAACCCAA
GAGTCTTCTCTGTCTCCACATGCCCAGTTTCTATTGGTCTCCTTAAACCTGTCTTGTAACCTTGATACCA
ACCTGCCCAGGGCCTCACCACCAACTTCATCCACGTTTCACCTTGCCCCACAGGGCAGTAACGGCAGACTT
CTCCTCAGGAGTCAGATGCACCAT

HBB Sample Mutation 2 TTAGTGATACTTGTGGGCCAGGGCATTAGCCACACCAGCCACCACCTTTCTGATAGGCAGCCTGCACTGGT
GGGGTGAATTCTTTGCCAAAGTGATGGGCCAGCACACAGACCAGCACGTTGCCCAGGAGCTGTGGGAGGA

AGATAAGAGGTTGAACATGATTAGCAAAAGGGCCTAGCTTGGACTCAGAATAATCCAGCCTTATCCCAA
CCATAAAATAAAAGCAGAATGGTAGCTGGATTGTAGCTGCTATTAGCAATATGAAACCTCTTACATCAGT
TACAATTTATATGCAGAAATATTTATATGCAGAGATATTGCTATTGCCTTAACCCAGAAATTATCACTGT
TATTCTTTAGAAATGGTGCAAAGAGGCATGATACATTGTATCATTATTGCCCTGAAAGAAAGAGATTAGGG
AAAGTATTAGAAATAAGATAAAACAAAAAGTATATTAAAAGAAGAAAGCATTTTTTTAAAAATTACAAATGC
AAAATTACCCTGATTTGGTCAATATGTGTACACATATTAAAACATTACACTTTAACCCATAAATATGTAT
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GGTTGTCCAGGTGAGCCAGGCCATCACTAAAGGCACCGAGCACTTTCTTGCCATGAGCCTTCACCTTAGG
GTTGCCCATAACAGCATCAGGAGTGACAGATCCCCAAAGGACTCAAAGAACCTCTGGGTCCAAGGGTAG
ACCACCAGCAGCCTAAGGGTGGGAAAAATAGACCAATAGGCAGAGAGAGTCAGTGCCTATCAGAAACCCAA
GAGTCTTCTCTGTCTCCACATGCCCAGTTTCTATTGGTCTCCTTAAACCTGTCTTGTAACCTTGATACCA
ACCTGCCCAGGGCCTCACCACCAACTTCATCCACGTTACCTTGCCCCACAGGGCAGTAACGGCAGACTT
CTCCTCAGGAGTCAGATGCACCAT

HBB Sample Mutation 3

TTAGTGATACTTGTGGGCCAGGGCATTAGCCACACCAGCCACCACTTTCTGATAGGCAGCCTGCACTGGT
GGGGTGAATTCTTTGCCAAAGTGATGGGCCAGCACACAGACCAGCACGTTGCCCAGGAGCTGTGGGAGGA
AGATAAGAGGTATGAACATGATTAGCAAAAGGGCCTAGCTTGGACTCAGAATAATCCAGCCTTATCCCAA
CCATAAAATAAAAGCAGAATGGTAGCTGGATTGTAGCTGCTATTAGCAATATGAAACCTCTTACATCAGT
TACAATTTATATGCAGAAATATTTATATGCAGAGATATTGCTATTGCCTTAACCCAGAAATTATCACTGT
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AAAAAGAAAGCAAGAATTAAACAAAAAGAAAACAATTGTTATGAACAGCAAATAAAAGAAACTAAAACGAT
CCTGAGACTTCCACACTGATGCAATCATTCGCCTGTTTCCCATTCTAACTGTACCCTGTTACTTATCCC
CTTCCTATGACATGAACTTAACCATAGAAAAGAAGGGGAAAGAAAACATCAAGCGTCCCATAGACTCACC
CTGAAGTTCTCAGGATCCACGTGCAGCTTGTACAGTGCAGCTCACTCAGTGTGGCAAAGGTGCCCTTGA
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GTTGCCCATAACAGCATCAGGAGTGACAGATCCCCAAAGGACTCAAAGAACCTCTGGGTCCAAGGGTAG
ACCACCAGCAGCCTAAGGGTGGGAAAAATAGACCAATAGGCAGAGAGAGTCAGTGCCTATCAGAAACCCAA
GAGTCTTCTCTGTCTCCACATGCCCAGTTTCTATTGGTCTCCTTAAACCTGTCTTGTAACCTTGATACCA
ACCTGCCCAGGGCCTCACCACCAACTTCATCCACGTTACCTTGCCCCACAGGGCAGTAACGGCAGACTT
CTCCTCAGGAGTCAGATGCACCAT

HBB Sample Mutation 4

TTAGTGAATACTTGTGGGCCAGGGCATTAGCCACACCAGCCACCACTTTCTGATAGGCAGCCTGCACTGGT
GGGGTGAATTCTTTGCCAAAGTGATGGGCCAGCACACAGACCAGCACGTTGCCCAGAGCTGTGGGACGGAA
GATAAGAGGTATGAACATGATTAGCAAAAGGGCCTAGCTTGGACTCAGAATAATCCAGCCTTATCCCAACC
ATAAAATAAAAGCAGAATGGTAGCTGGATTGTAGCTGCTATTAGCAATATGAAACCTCTCTACATCAGTTA

CAATTTATATGCAGAAATATTTATATGCAGAGATATTGCTATTGCCTTAACCTCCAGAAATTATCACTGTTA
 TTCTTTAGAAATGTGCAAAGAGGCATGATACATTGTATCATTATTGCCCTGAAAGAAAGAGATTAGGGAAAG
 TATTAGAAATAAGATAGACAAAAAAGTATATTTAAAGAAGAAAGCATT'TTTTAAAAATTACAAATGCAAAAT
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 CTATGTATCAATTTAAAAATAAAAAGAAAATAAAGTAGGGAGATTATGAATATGCAAATAAGCACACATATAT
 TCCAAATAGTAATGTACTAGGCAGACTGTGTAAAGTTTTTTTTTTAAGTTACTTAATGTATCTCAGAGATAT
 TTCCTTTTGTATACACAATGTTAAGGCATTAAGTATAATAGTAAAAATTGCGGAGAAGAAAAAAAAAGAA
 AGCAAGAATTAAACAAAAGAAAAACAATTGTTATGAACAGCAATAAAAGAACTAAACGATCCTGAGACT
 TCCACACTGATGCAATCATTCGCTGTTTCCCATTCTAAACTGTACCCTGTTACTGTATCCCCTTCCTATGA
 CATGAACCTTAACCATAGAAAAGAAGGGGAAAGAAAACATCAAGCGTCCCATAGACTCACCTGAAGTTCTC
 AGGATCCACGTGCAGCTTGTACAGTGCAGCTCACTCAGTGTGGCAAAGGTGCCCTTGAGGTTGTCCAGGT
 GAGCCAGGCCATCACTAAAGGCACCGAGCACTTTCTTGCCATGAGCCTTCACCTTAGGGTTGCCATAACA
 GCATCAGAGTGGACAGATCCCCAAAGGACTCCATAAAGAACCTCTGGGTCCAAGGGTCAGACCACCAGCAG
 AAGGGTGGGAAAATAGACCAATAGGCAGAGAGAGTCAGTGCCTATCAGAAACCCAAGAGTCTTCTCTGTCT
 CCACATGCCAGTTTCTATTGGTCTCCTTAAACCTGTCTTGTAACCTTGATACCAACCTGCCCAGGGCCT
 CACCACCAACTTCATCCACGTTACCTTGCCCCACAGGGCAGTAACGGCAGACTTCTCCTCAGGAGTCAGA
 TGCACCAT

8. Push the BLAST button

BLAST

Search **nucleotide sequence** using **Megablast (Optimize for highly similar sequences)**

☐ Show results in a new window

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

ary **Alignments**

Alignments

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

Graphic Summary **Alignments**

view ☒ Pairwise

Pairwise with dots for identities

Query-anchored with dots for identities

Query-anchored with letters for identities

Flat query-anchored with dots for identities

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

[Download](#)

[Graphics](#)

HBB Sample Mutation 1 (Insertion)

Sequence ID: **Query_4898932** Length: **1425** Number of Matches: **1**

Range 1: 1 to 1425 [Graphics](#)

[Next Match](#) [Previous Match](#)

Score

Expect

Identities

Gaps

Strand

Resources (Wild Type):

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

```
>NC_000011.10:5225598-5227021 Homo sapiens chromosome 11, GRCh38.p14 Primary Assembly
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GGGGTGAATTCTTTGCCAAAGTGATGGGCCAGCACACAGACCAGCACGTTGCCCAGGAGCTGTGGGAGGA
AGATAAGAGGTATGAACATGATTAGCAAAAAGGGCCTAGCTTGGAATCAGAATAATCCAGCCTTATCCCAA
CCATAAAATAAAAGCAGAATGGTAGCTGGATTGTAGCTGCTATTAGCAATATGAAACCTCTTACATCAGT
TACAATTTATATGCAGAAAATATTTATATGCAGAGATATTGCTATTGCCCTTAACCCAGAAAATTATCACTGT
TATTCTTTAGAAATGGTGCAAAGAGGCATGATACATTGTATCATTATTGCCCTGAAAGAAAGAGATTAGGG
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ATATATTCCAAATAGTAATGTACTAGGCAGACTGTGTAAAGTTTTTTTTTTAAGTTACTTAATGTATCTCA
GAGATATTTTCCTTTTGTATACACAATGTTAAGGCATTAAGTATAATAGTAAAAATTGCGGAGAAGAAAA
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CCTGAGACTTCCACACTGATGCAATCATTCGTCTGTTTCCCATTCTAACTGTACCCTGTTACTTATCCC
CTTCCTATGACATGAACTTAACCATAGAAAAGAAGGGGAAAGAAAACATCAAGCGTCCCATAGACTCACC
CTGAAGTTCTCAGGATCCACGTGCAGCTTGTCACAGTGCAGCTCACTCAGTGTGGCAAAGGTGCCCTTGA
GGTTGTCCAGGTGAGCCAGGCCATCACTAAAGGCACCGAGCACTTTCTTGCCATGAGCCTTCACCTTAGG
GTTGCCCATAACAGCATCAGGAGTGGACAGATCCCCAAAGGACTCAAAGAACCTCTGGGTCCAAGGGTAG
ACCACCAGCAGCCTAAGGGTGGGAAAATAGACCAATAGGCAGAGAGAGTCAGTGCCTATCAGAAACCCAA
GAGTCTTCTCTGTCTCCACATGCCCAGTTTCTATTGGTCTCCTTAAACCTGTCTTGTAACCTTGATACCA
ACCTGCCCAGGGCCTCACCACCAACTTCATCCACGTTACCTTGCCCCACAGGGCAGTAACGGCAGACTT
CTCCTCAGGAGTCAGATGCACCAT
```