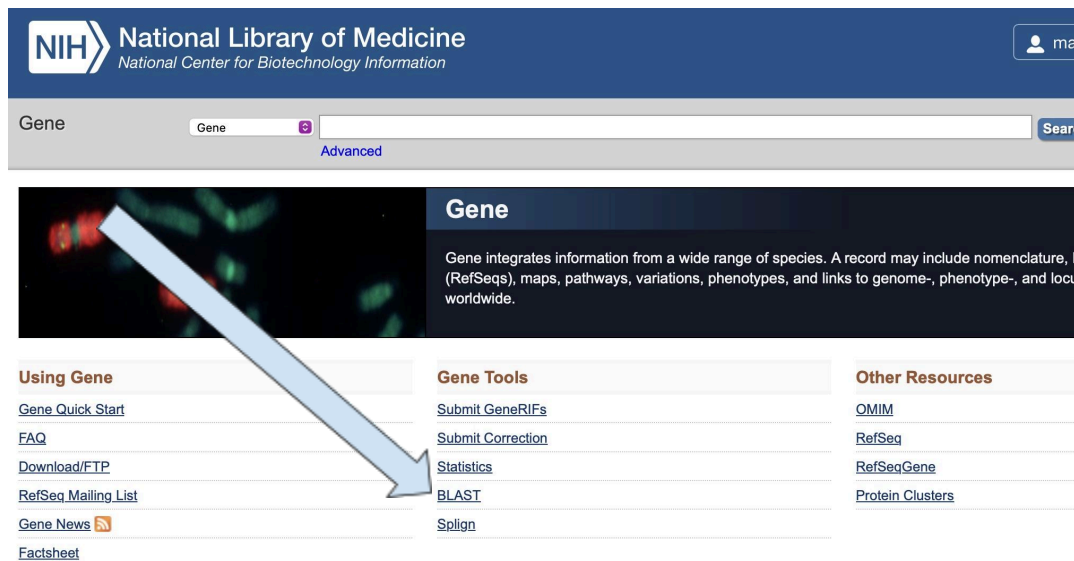
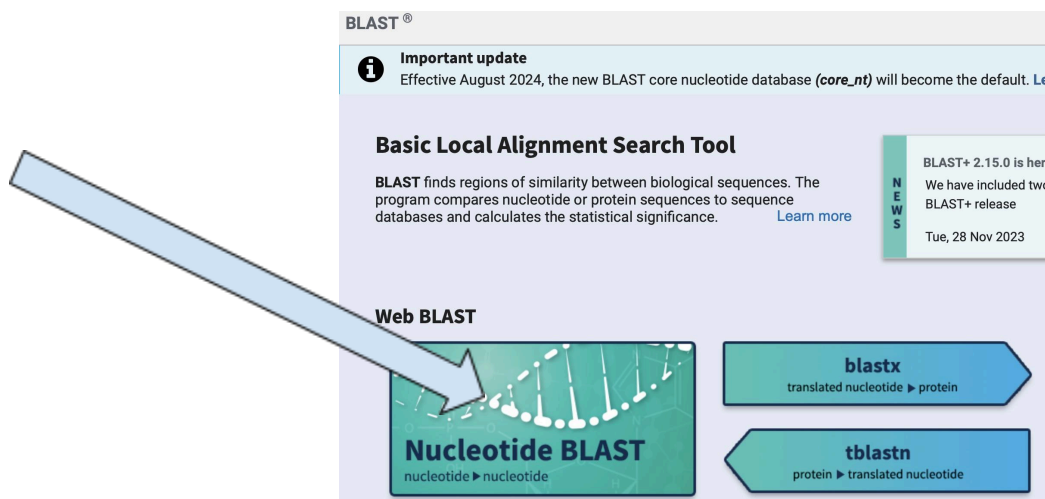


BLAST Analysis Teacher Guide

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:



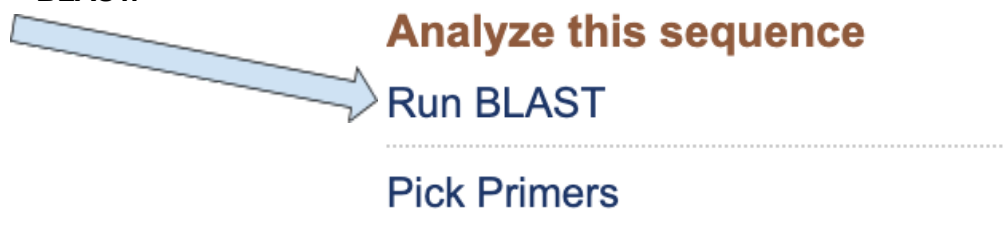
4. FOR STEP 4, THERE ARE 2 OPTIONS (A **OR** B):

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.



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. 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.

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 (Insertion)

```
TTAGTGATACTTGTGGGCCAGGGCATTAGCCACACCAGCgCACCACCTTTCTGATAGGCAGCCTGCACTGGT
GGGGTGAATTCTTTGCCAAAGTGATGGGCCAGCACACAGACCAGCACGTTGCCCAGGAGCTGTGGGAGGA
AGATAAGAGGTATGAACATGATTAGCAAAAGGGCCTAGCTTGGACTCAGAATAATCCAGCCTTATCCCAA
CCATAAAATAAAAGCAGAATGGTAGCTGGATTGTAGCTGCTATTAGCAATATGAAACCTCTTACATCAGT
TACAATTTATATGCAGAAATATTTATATGCAGAGATATTGCTATTGCCTTAACCCAGAAATTATCACTGT
TATTCTTTAGAAATGGTGCAAAGAGGCATGATACATTGTATCATTATTGCCCTGAAAGAAAGAGATTAGGG
AAAGTATTAGAAATAAGATAAAACAAAAAGTATATTTAAAGAAGAAAGCATTTTTTTAAATTTACAAATGC
AAAATTACCCTGATTTGGTCAATATGTGTACACATATTTAAACATTACACTTTAACCATAAAATATGTAT
AATGATTATGTATCAATTAATAAATAAAAGAAAATAAAGTAGGGAGATTATGAATATGCAAATAAGCACAC
ATATATTCCAAATAGTAATGTACTAGGCAGACTGTGTAAAGTTTTTTTTTAAGTTACTTAATGTATCTCA
GAGATATTTTCCTTTTGTATACACAATGTTAAGGCATTAAGTATAATAGTAAAAATTGCGGAGAAGAAAA
AAAAAGAAAGCAAGAATTAAACAAAAGAAAACAATTGTTATGAACAGCAAATAAAAGAAACTAAAACGAT
CCTGAGACTTCCACACTGATGCAATCATTCGTCTGTTTCCCATTCTAACTGTACCCTGTTACTTATCCC
CTTCCTATGACATGAACTTAACCATAGAAAAGAAGGGGAAAGAAAACATCAAGCGTCCCATAGACTCACC
CTGAAGTTCTCAGGATCCACGTGCAGCTTGTCACAGTGCAGCTCACTCAGTGTGGCAAAGGTGCCCTTGA
GGTTGTCCAGGTGAGCCAGGCCATCACTAAAGGCACCGAGCACTTTCTTGCCATGAGCCTTCACCTTAGG
GTTGCCCATAACAGCATCAGGAGTGACAGATCCCCAAAGGACTCAAAGAACCTCTGGGTCCAAGGGTAG
ACCACCAGCAGCCTAAGGGTGGGAAAATAGACCAATAGGCAGAGAGAGTCAGTGCCTATCAGAAACCCAA
GAGTCTTCTCTGTCTCCACATGCCAGTTTCTATTGGTCTCCTTAAACCTGTCTTGTAACCTTGATACCA
ACCTGCCAGGGCCTCACCACCAACTTCATCCACGTTACCTTGCCCCACAGGGCAGTAACGGCAGACTT
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
AGATAAGAGGTGAACATGATTAGCAAAAGGGCCTAGCTTGGACTCAGAATAATCCAGCCTTATCCCAA
CCATAAAATAAAAGCAGAATGGTAGCTGGATTGTAGCTGCTATTAGCAATATGAAACCTCTTACATCAGT
TACAATTTATATGCAGAAATATTTATATGCAGAGATATTGCTATTGCCTTAACCCAGAAATTATCACTGT
TATTCTTTAGAAATGGTGCAAAGAGGCATGATACATTGTATCATTATTGCCCTGAAAGAAAGAGATTAGGG
AAAGTATTAGAAATAAGATAAAACAAAAAGTATATTTAAAGAAGAAAGCATTTTTTTAAAAATTACAAATGC
AAAATTACCCTGATTTGGTCAATATGTGTACACATATTTAAACATTACACTTTAACCATAAAATATGTAT
AATGATTATGTATCAATTAATAAATAAAAGAAAATAAAGTAGGGAGATTATGAATATGCAAATAAGCACAC
ATATATTCCAAATAGTAATGTACTAGGCAGACTGTGTAAAGTTTTTTTTTTAAGTTACTTAATGTATCTCA
GAGATATTTCTTTTGTATACACAATGTTAAGGCATTAAGTATAATAGTAAAAATTGCGGAGAAGAAAA
AAAAAGAAAGCAAGAATTAACAAAAAGAAAACAATTGTTATGAACAGCAAATAAAAGAACTAAAACGAT
CCTGAGACTTCCACACTGATGCAATCATTCGTCTGTTTCCCATTCTAACTGTACCCTGTACTTATCCC
CTTCCTATGACATGAACTTAACCATAGAAAAGAAGGGGAAAGAAAACATCAAGCGTCCCATAGACTCACC
CTGAAGTTCTCAGGATCCACGTGCAGCTTGTGCAGTGCAGCTCACTCAGTGTGGCAAAGGTGCCCTTGA
GGTTGTCCAGGTGAGCCAGGCCATCACTAAAGGCACCGAGCACTTTCTTGCCATGAGCCTTCACCTTAGG
GTTGCCCATACAGCATCAGGAGTGACAGATCCCCAAAGGACTCAAAGAACCCTCTGGGTCCAAGGGTAG
ACCACCAGCAGCCTAAGGGTGGGAAAAATAGACCAATAGGCAGAGAGAGTCAGTGCCTATCAGAAACCCAA
GAGTCTTCTCTGTCTCCACATGCCCAGTTTCTATTGGTCTCCTTAAACCTGTCTTGTAACCTTGATACCA
ACCTGCCCAGGGCCTCACCACCAACTTCATCCACGTTACCTTGCCCCACAGGGCAGTAACGGCAGACTT
CTCCTCAGGAGTCAGATGCACCAT

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

TTAGTGATACTTGTGGGCCAGGGCATTAGCCACACCAGCCACCACTTTCTGATAGGCAGCCTGCACTGGT
GGGGTGAATTCTTTGCCAAAGTGATGGGCCAGCACACAGACCAGCACGTTGCCCAGGAGCTGTGGGAGGA
AGATAAGAGGTATGAACATGATTAGCAAAAGGGCCTAGCTTGGACTCAGAATAATCCAGCCTTATCCCAA
CCATAAAATAAAAGCAGAATGGTAGCTGGATTGTAGCTGCTATTAGCAATATGAAACCTCTTACATCAGT
TACAATTTATATGCAGAAATATTTATATGCAGAGATATTGCTATTGCCTTAACCCAGAAATTATCACTGT
TATTCTTTAGAAATGGTGCAAAGAGGCATGATACATTGTATCATTATTGCCCTGAAAGAAAGAGATTAGGG
AAAGTATTAGAAATAAGATAAAACAAAAAGTATATTTAAAGAAGAAAGCATTTTTTTAAAAATTACAAATGC
AAAATTACCCTGATTTGGTCAATATGTGTACACATATTTAAACATTACACTTTAACCATAAAATATGTAT
AATGATTATGTATCAATTAATAAATAAAAGAAAATAAAGTAGGGAGATTATGAATATGCAAATAAGCACAC
ATATATTCCAAATAGTAATGTACTAGGCAGACTGTGTAAAGTTTTTTTTTTAAGTTACTTAATGTATCTCA
GAGATATTTCTTTTGTATACACAATGTTAAGGCATTAAGTATAATAGTAAAAATTGCGGAGAAGAAAA
AAAAAGAAAGCAAGAATTAACAAAAAGAAAACAATTGTTATGAACAGCAAATAAAAGAACTAAAACGAT
CCTGAGACTTCCACACTGATGCAATCATTCGCTGTTTCCCATTCTAACTGTACCCTGTACTTATCCC
CTTCCTATGACATGAACTTAACCATAGAAAAGAAGGGGAAAGAAAACATCAAGCGTCCCATAGACTCACC
CTGAAGTTCTCAGGATCCACGTGCAGCTTGTGCAGTGCAGCTCACTCAGTGTGGCAAAGGTGCCCTTGA
GGTTGTCCAGGTGAGCCAGGCCATCACTAAAGGCACCGAGCACTTTCTTGCCATGAGCCTTCACCTTAGG
GTTGCCCATACAGCATCAGGAGTGACAGATCCCCAAAGGACTCAAAGAACCCTCTGGGTCCAAGGGTAG
ACCACCAGCAGCCTAAGGGTGGGAAAAATAGACCAATAGGCAGAGAGAGTCAGTGCCTATCAGAAACCCAA
GAGTCTTCTCTGTCTCCACATGCCCAGTTTCTATTGGTCTCCTTAAACCTGTCTTGTAACCTTGATACCA
ACCTGCCCAGGGCCTCACCACCAACTTCATCCACGTTACCTTGCCCCACAGGGCAGTAACGGCAGACTT
CTCCTCAGGAGTCAGATGCACCAT

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

TTAGTGAACTTGTGGGCCAGGGCATTAGCCACACCAGCCACCACTTTCTGATAGGCAGCCTGCACTGGT
GGGGTGAATTCTTTGCCAAAGTGATGGGCCAGCACACAGACCAGCACGTTGCCCAGAGCTGTGGGACGGAA
GATAAGAGGTATGAACATGATTAGCAAAAGGGCCTAGCTTGGACTCAGAATAATCCAGCCTTATCCCAACC
ATAAAATAAAAGCAGAATGGTAGCTGGATTGTAGCTGCTATTAGCAATATGAAACCTCTCTACATCAGTTA
CAATTTATATGCAGAAATATTTATATGCAGAGATATTGCTATTGCCTTAACCTCCAGAAATTATCACTGTTA

TTCTTTAGAAATGTGCAAAGAGGCATGATACATTGTATCATTATTGCCCTGAAAGAAAGAGATTAGGGAAAG
TATTAGAAATAAGATAGACAAAAAGTATATTTAAAGAAGAAAGCATTTTTTTAAATTACAAATGCAAAAT
TACCCTGATTTGGTCAATATGTGTACACATATTTAAACATTACACTTTAACCATAAAATATGTATAATGAT
CTATGTATCAATTAAAAATAAAAAGAAATAAAGTAGGGAGATTATGAATATGCAAATAAGCACACATATAT
TCCAAATAGTAATGTACTAGGCAGACTGTGTAAAGTTTTTTTTTAAAGTTACTTAATGTATCTCAGAGATAT
TTCCTTTTGTATACACAATGTTAAGGCATTAAGTATAATAGTAAAAATTGCGGAGAAGAAAAAAGAA
AGCAAGAATTAACAAAAAGAAAAACAATTGTTATGAACAGCAATAAAAGAACTAAACGATCCTGAGACT
TCCACACTGATGCAATCATTCGCTGTTTCCCATTCTAAACTGTACCCTGTTACTGTATCCCCTTCCTATGA
CATGAACCTAACCATAGAAAAAGAGGGGAAAGAAACATCAAGCGTCCCATAGACTCACCTGAAGTTCTC
AGGATCCACGTGCAGCTTGTCACAGTGCAGCTCACTCAGTGTGGCAAAGGTGCCCTTGAGGTTGTCCAGGT
GAGCCAGGCCATCACTAAAGGCACCGAGCACTTTCTTGCCATGAGCCTTCACCTTAGGGTTGCCATAACA
GCATCAGAGTGGACAGATCCCCAAAGGACTCCATAAAGAACCTCTGGGTCCAAGGGTCAGACCACCAGCAG
AAGGGTGGGAAAATAGACCAATAGGCAGAGAGAGTCAAGTGCCTATCAGAAACCAAGAGTCTTCTCTGTCT
CCACATGCCAGTTTCTATTGGTCTCCTTAAACCTGTCTTGGTAACCTTGATACCAACCTGCCAGGGCCT
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**

ant alignments

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

Graphic Summary **Alignments**

✓ 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

Flat query-anchored with letters for identities

☐ CDS fe

g PCR Primers? T

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 |
|-----------------|--------|----------------|------------|-----------|
| 2625 bits(1421) | 0.0 | 1424/1425(99%) | 1/1425(0%) | Plus/Plus |

| | | | |
|--------------|-----------|---|------------|
| Query | 5225598 | TTAGTGATACTTGTGGGCCAGGGCATTAGCCACACCAGC-CACCACTTTCTGATAGGCAG | 5225656 |
| Sbjct | 1 | G | 60 |
| Query | 5225657 | CCTGCACTGGTGGGGTGAATTCCTTGCCTAAAGTGATGGGCCAGCACACAGACCAGCACGT | 5225716 |
| Sbjct | 61 | | 120 |

Resources:

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
TTAGTGATACTTGTGGGCCAGGGCATTAGCCACACCAGCCACCACTTTCTGATAGGCAGCCTGCACTGGT
GGGGTGAATTCCTTTGCCAAAGTGATGGGCCAGCACACAGACCAGCACGTTGCCAGGAGCTGTGGGAGGA
AGATAAGAGGTATGAACATGATTAGCAAAAGGGCCTAGCTTGGACTCAGAATAATCCAGCCTTATCCCCAA
CCATAAAATAAAAGCAGAATGGTAGCTGGATTGTAGCTGCTATTAGCAATATGAAACCTCTTACATCAGT
TACAATTTATATGCAGAAATATTTATATGCAGAGATATTGCTATTGCCTTAACCCAGAAATTATCACTGT
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AAAATTACCCTGATTTGGTCAATATGTGTACACATATTAAACATTACACTTTAACCATAAAATATGTAT
AATGATTATGTATCAATTAATAAAAGAAAATAAAGTAGGGAGATTATGAATATGCAAATAAGCACAC
ATATATTCCAAATAGTAATGTACTAGGCAGACTGTGTAAAGTTTTTTTTTAAGTTACTTAATGTATCTCA
GAGATATTTCTTTTGTATACACAATGTTAAGGCATTAAGTATAATAGTAAAAATTGCGGAGAAGAAAA
AAAAAGAAAGCAAGAATTAAACAAAAGAAAACAATTGTTATGAACAGCAAATAAAAGAACTAAAACGAT
CCTGAGACTTCCACACTGATGCAATCATTGCTGTGTTCCCATCTAACTGTACCCTGTTACTTATCCC
CTTCCTATGACATGAACTTAACCATAGAAAAGAAGGGGAAAGAAAACATCAAGCGTCCCATAGACTCACC
CTGAAGTTCTCAGGATCCACGTGCAGCTTGTCAAGTGCAGCTCACTCAGTGTGGCAAAGGTGCCCTTGA
GGTTGTCCAGGTGAGCCAGGCCATCACTAAAGGCACCGAGCACTTTCTTGCCATGAGCCTTCACCTTAGG
GTTGCCCATAAACAGCATCAGGAGTGGACAGATCCCCAAAGGACTCAAAGAACCCTCTGGGTCCAAGGGTAG
ACCACCAGCAGCCTAAGGGTGGGAAAATAGACCAATAGGCAGAGAGAGTCAGTGCCTATCAGAAACCCAA
GAGTCTTCTCTGTCTCCACATGCCAGTTTCTATTGGTCTCCTTAAACCTGTCTTGTAACTTGATACCA
ACCTGCCAGGGCCTCACCACCAACTTCATCCACGTTACCTTGCCCCACAGGGCAGTAACGGCAGACTT
CTCCTCAGGAGTCAGATGCACCAT
```