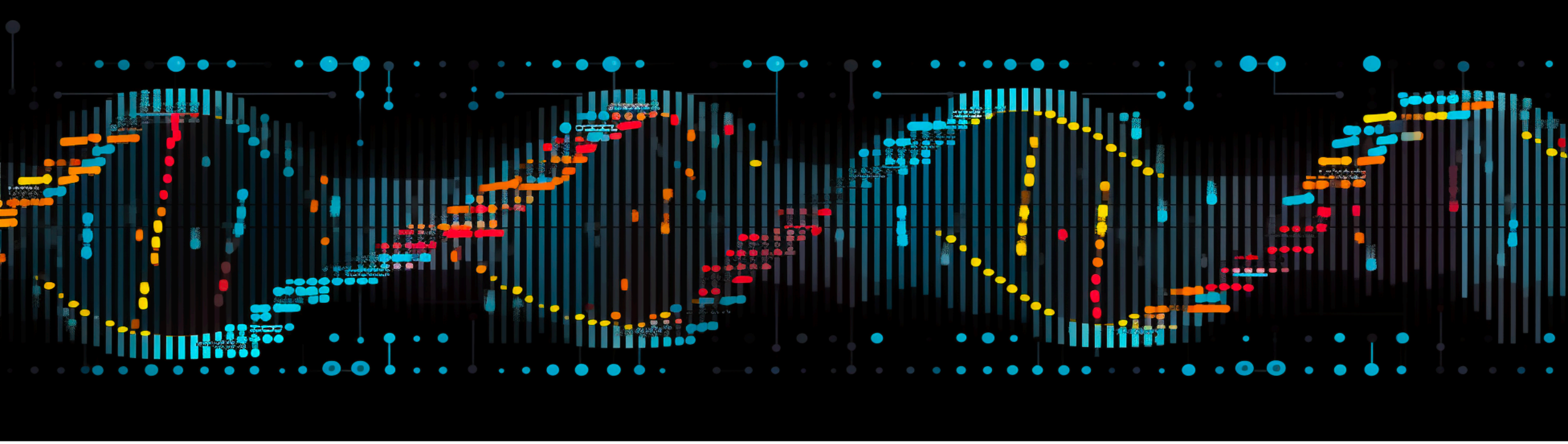


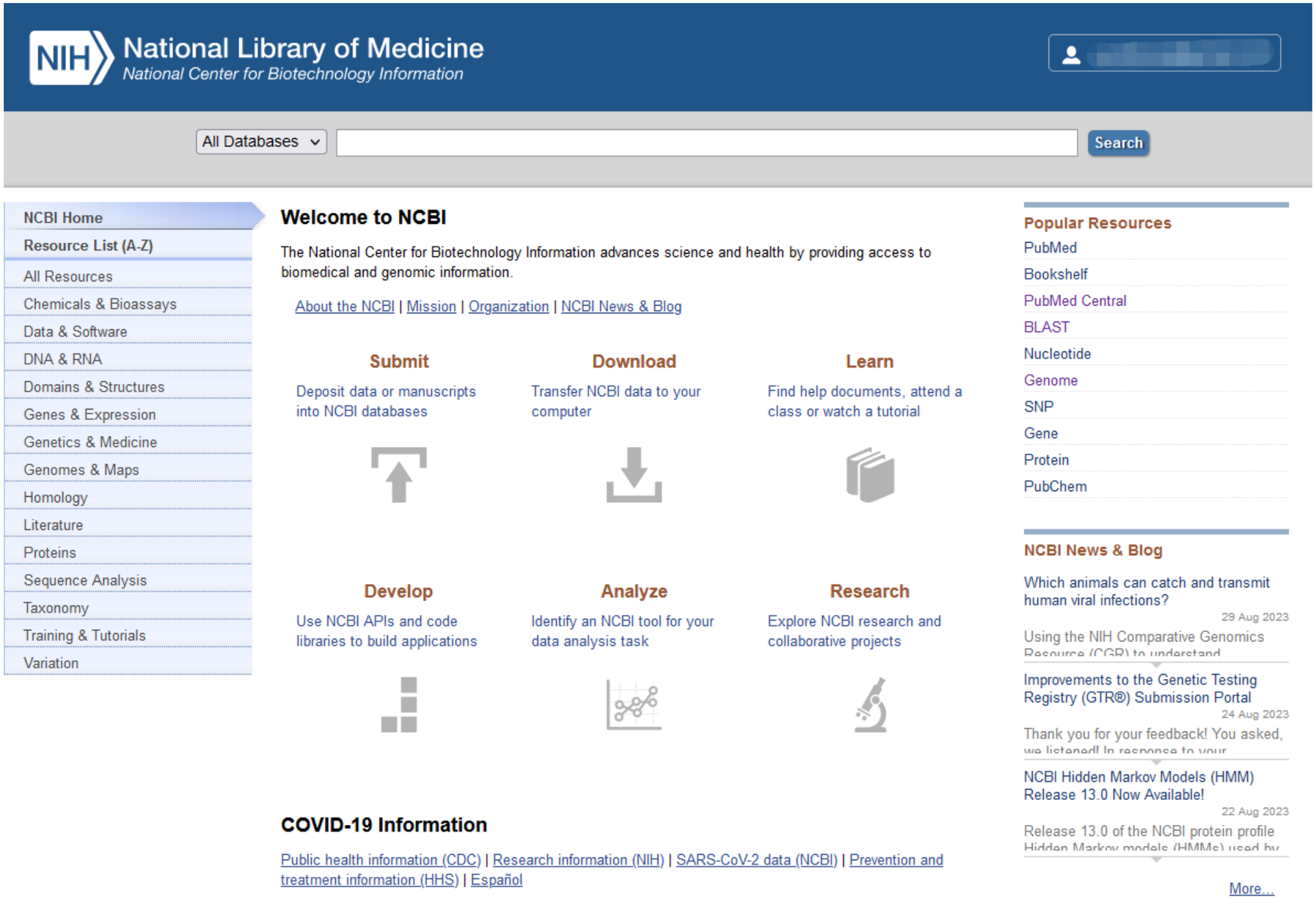
Introduction to bioinformatics

lab1 - Alignment and Phylogenetic tree

Jiaxing Chen

NCBI

The National Center for Biotechnology Information (NCBI), also known as the National Library of Medicine, is one of the most influential bioinformatics databases globally. It develops various types of databases and provides online tools for bioinformatics analysis. All NCBI's databases and software programs can be downloaded from its anonymous FTP server.



1. Sequence query

BLAST (Basic Local Alignment Search Tool)

https://blast.ncbi.nlm.nih.gov/Blast.cgi

Step 1. Access the NCBI BLAST homepage;

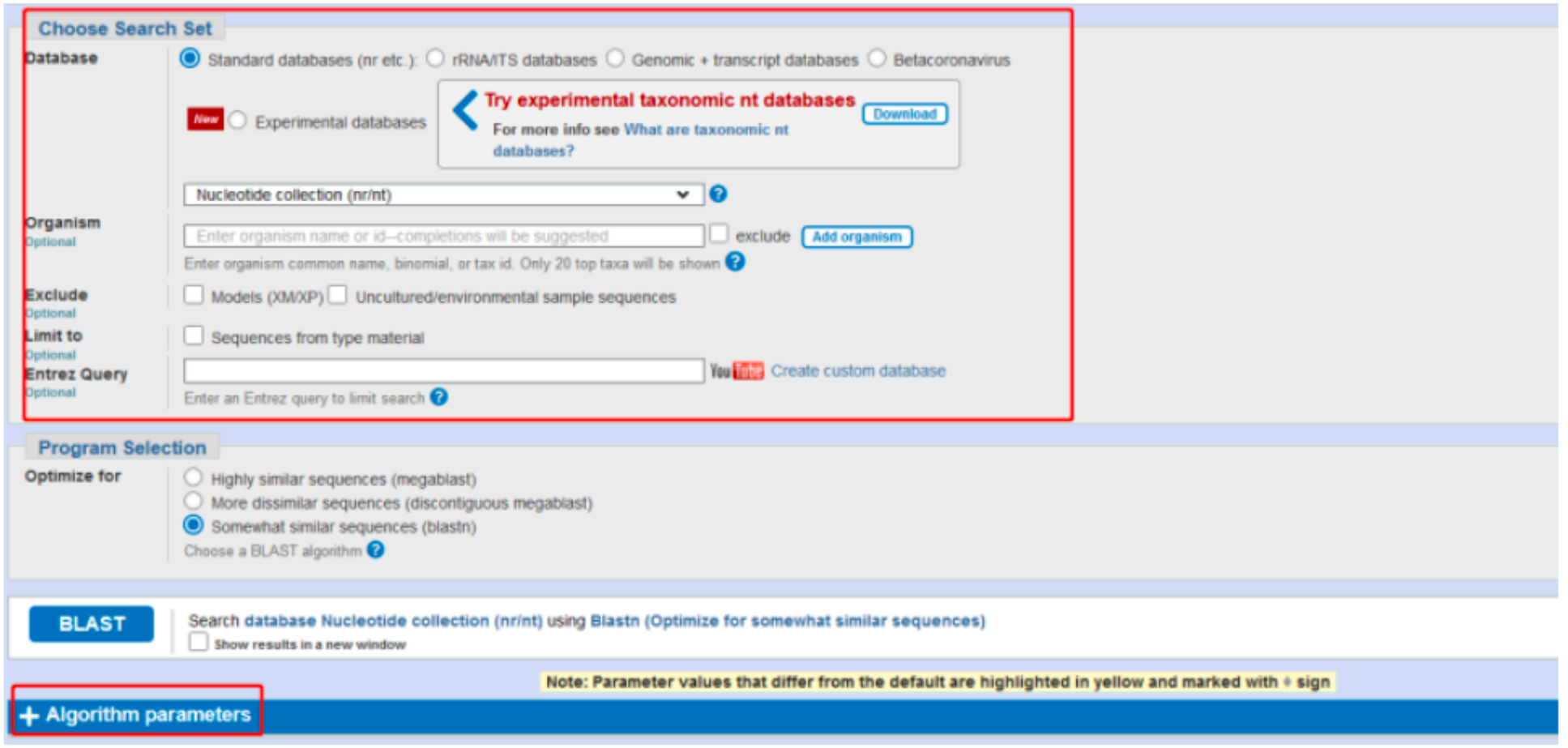
Step 2. Select the corresponding BLAST program based on the input content, selecting BLASTn here.

Step 3. Enter the nucleic acid sequence in the QUERY section.

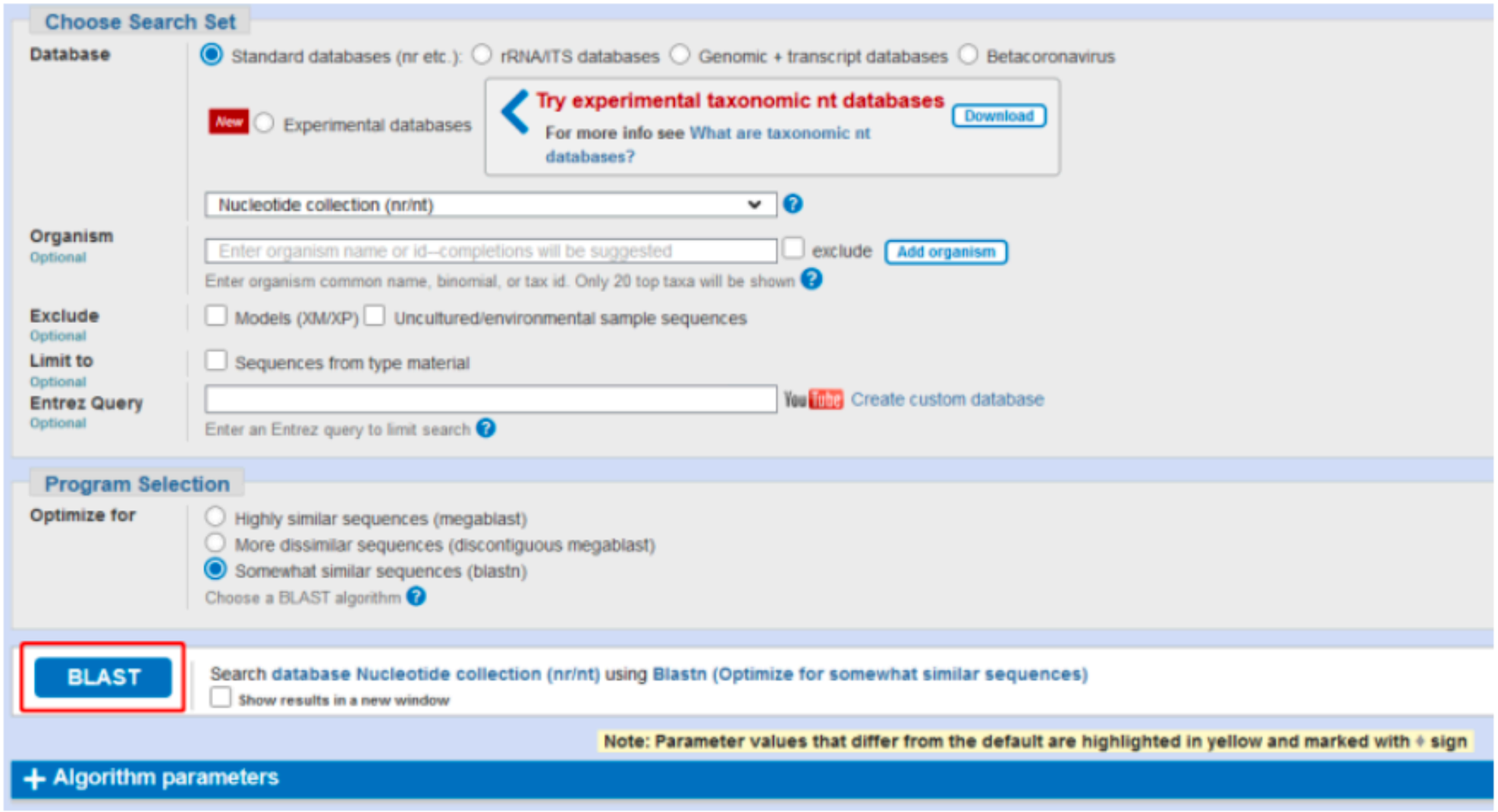
Figure show searching of COVID-19 *Orf1ab* gene as an example
Gene detail in https://www.ncbi.nlm.nih.gov/gene/43740578

Please try searching with sequences in the file “Manual_Nucl.fasta” from ispace

Step 4. Select search set and corresponding parameters;



Step 5. Click “BLAST” to submit the BLASTn query



‹ Edit Search | Save Search | Search Summary | How to read this report? | BLAST Help Videos | Back to Traditional Results Page

Job Title	**Nucleotide Sequence**
RID	FBFD7Y2K016 *Search expires on 09-05 19:21 pm* Download All
Program	BLASTN Citation
Database	nt See details
Query ID	lcl|Query_44933
Description	None
Molecule type	dna
Query Length	490
Other reports	Distance tree of results MSA viewer

Filter Results

Organism *only top 20 will appear* exclude

Type common name, binomial, taxid or group name

+ Add organism

Percent Identity to

E value to

Query Coverage to

Filter Reset

Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments Download Select columns Show 100

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/OKPHL0027690/2023 ORF1ab ...	Severe acute res...	884	884	100%	0.0	100.00%	29822	OR480076.1
Severe acute respiratory syndrome coronavirus 2 genome assembly, complete genome: monopartite	Severe acute res...	884	884	100%	0.0	100.00%	29790	OY639170.1
Severe acute respiratory syndrome coronavirus 2 genome assembly, complete genome: monopartite	Severe acute res...	884	884	100%	0.0	100.00%	29786	OY639141.1
Severe acute respiratory syndrome coronavirus 2 genome assembly, complete genome: monopartite	Severe acute res...	884	884	100%	0.0	100.00%	29786	OY639123.1
Severe acute respiratory syndrome coronavirus 2 genome assembly, complete genome: monopartite	Severe acute res...	884	884	100%	0.0	100.00%	29786	OY639116.1
Severe acute respiratory syndrome coronavirus 2 genome assembly, complete genome: monopartite	Severe acute res...	884	884	100%	0.0	100.00%	29786	OY639070.1

From this page, we can obtain the following main biological information:

RID: Unique identifier assigned to this search.

DESCRIPTIONS: Default display options for BLAST results, where the description of each search result serves as a brief name of the search object.

SCIENTIFIC NAME: Species information corresponding to the search results.

QUERY COVER: The coverage between the target sequence and the query sequence.

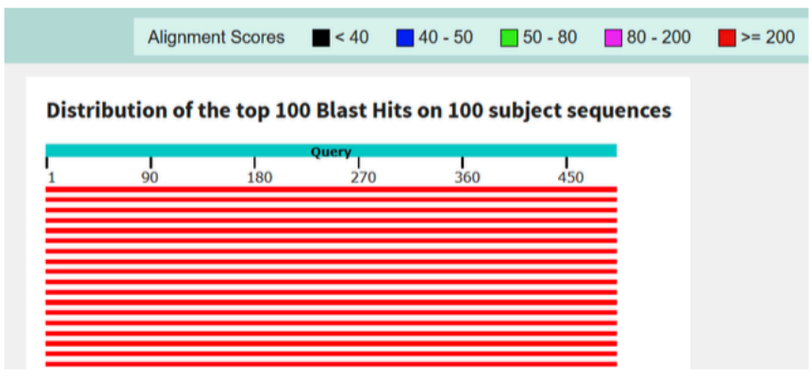
E-VALUE: The reliability of the alignment of the reaction sequence, with smaller E values indicating higher reliability.

PER.IDENT: The value of sequence alignment consistency. (Percentage identity)

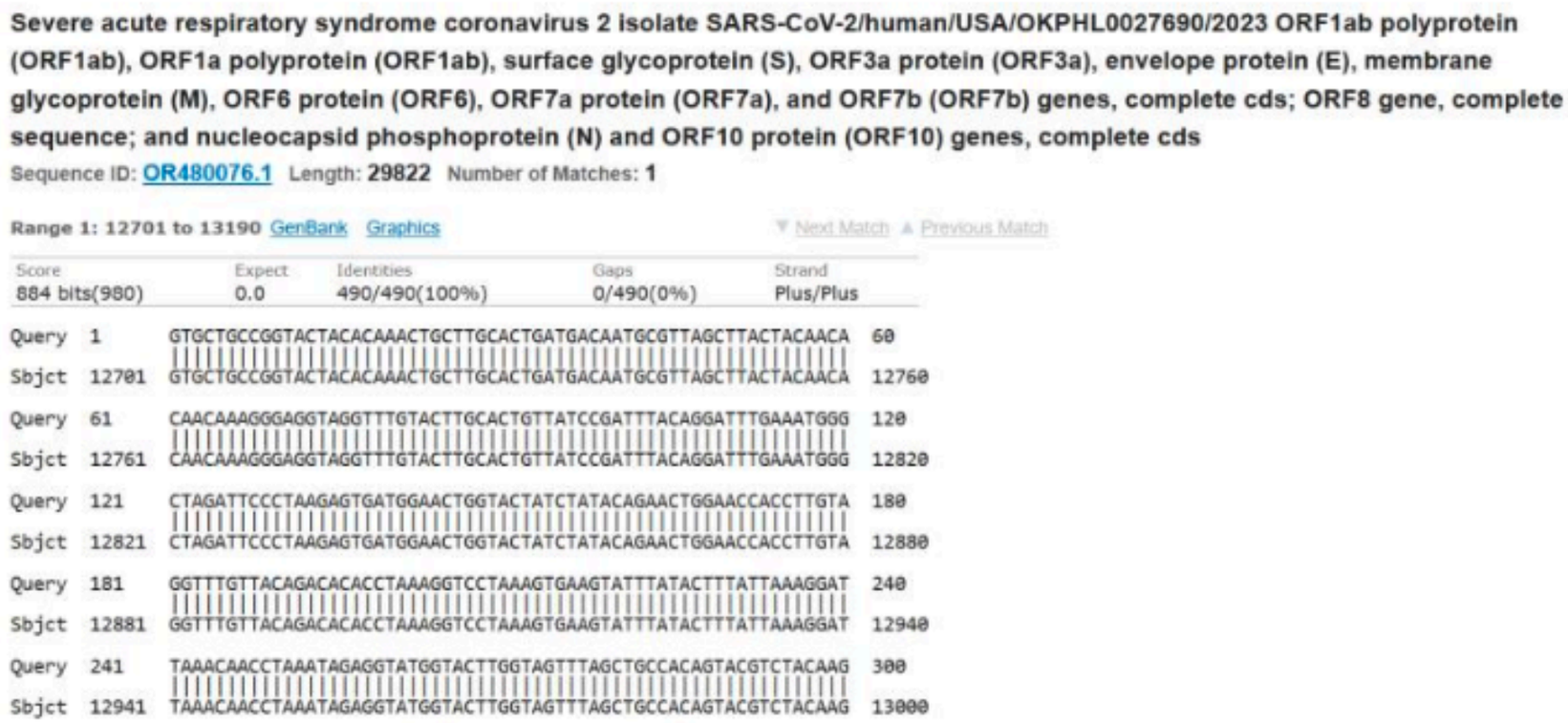
ACC.LEN: Target sequence length.

ACCESSION: Accession ID of target sequence in NCBI database.

GRAPHIC SUMMARY: Visually presenting the alignment results with similar sequences.



ALIGNMENTS: The results/status of sequence alignment, including consistency, similarity, sequence length, etc.



Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/OKPHL0027690/2023 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membrane glycoprotein (M), ORF6 protein (ORF6), ORF7a protein (ORF7a), and ORF7b (ORF7b) genes, complete cds; ORF8 gene, complete sequence; and nucleocapsid phosphoprotein (N) and ORF10 protein (ORF10) genes, complete cds

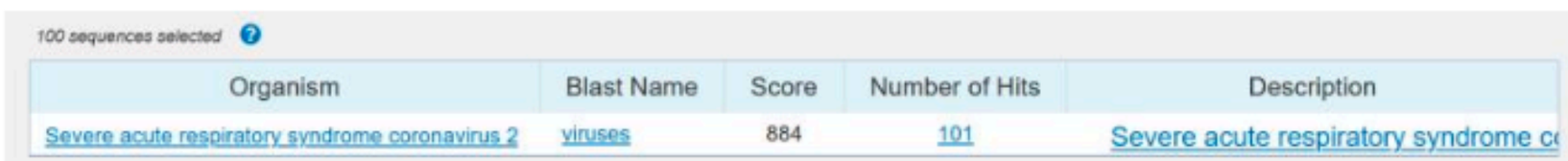
Sequence ID: OR480076.1 Length: 29822 Number of Matches: 1

Range 1: 12701 to 13190 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
884 bits(980)	0.0	490/490(100%)	0/490(0%)	Plus/Plus

```
Query  1      GTGCTGCCGGTACTACACAAACTGCTTGCACTGATGACAATGCGTTAGCTTACTACAACA  60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  12701  GTGCTGCCGGTACTACACAAACTGCTTGCACTGATGACAATGCGTTAGCTTACTACAACA  12760

Query  61     CAACAAAGGGAGGTAGGTTTGTACTTGCACTGTTATCCGATTTACAGGATTTGAAATGGG  120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  12761  CAACAAAGGGAGGTAGGTTTGTACTTGCACTGTTATCCGATTTACAGGATTTGAAATGGG  12820

Query  121    CTAGATTCCCTAAGAGTGATGGAACTGGTACTATCTATACAGAACTGGAACCACCTTGTA  180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  12821  CTAGATTCCCTAAGAGTGATGGAACTGGTACTATCTATACAGAACTGGAACCACCTTGTA  12880

Query  181    GGTTTGTTACAGACACACCTAAAGGTCCTAAAGTGAAGTATTTATACTTTATTAAAGGAT  240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  12881  GGTTTGTTACAGACACACCTAAAGGTCCTAAAGTGAAGTATTTATACTTTATTAAAGGAT  12940

Query  241    TAAACAACCTAAATAGAGGTATGGTACTTGGTAGTTTAGCTGCCACAGTACGTCTACAAG  300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  12941  TAAACAACCTAAATAGAGGTATGGTACTTGGTAGTTTAGCTGCCACAGTACGTCTACAAG  13000
```

TAXONOMY: Classification of similar sequences within species.



100 sequences selected

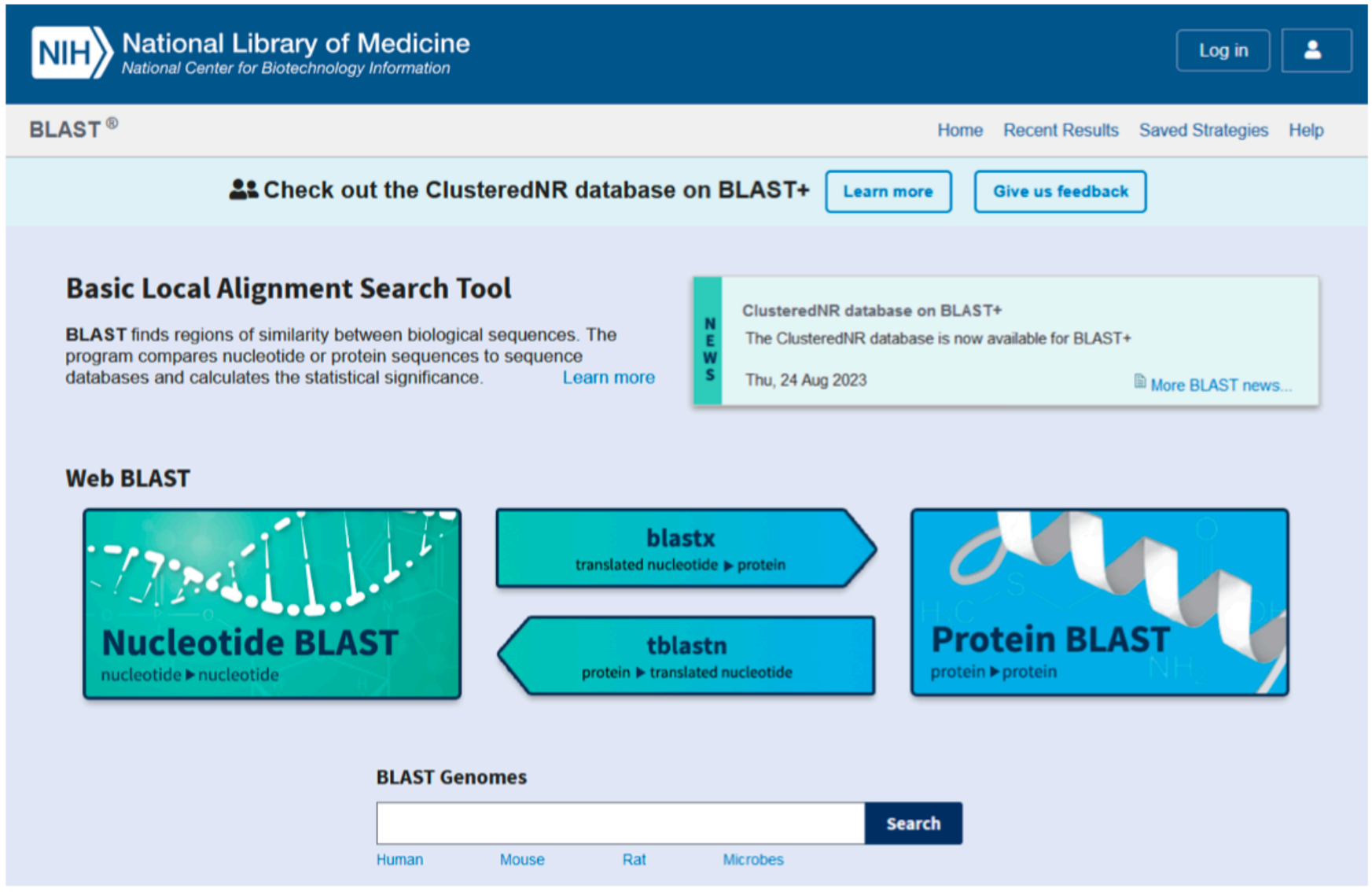
Organism	Blast Name	Score	Number of Hits	Description
Severe acute respiratory syndrome coronavirus 2	viruses	884	101	Severe acute respiratory syndrome c

2. Pairwise Sequence Alignment

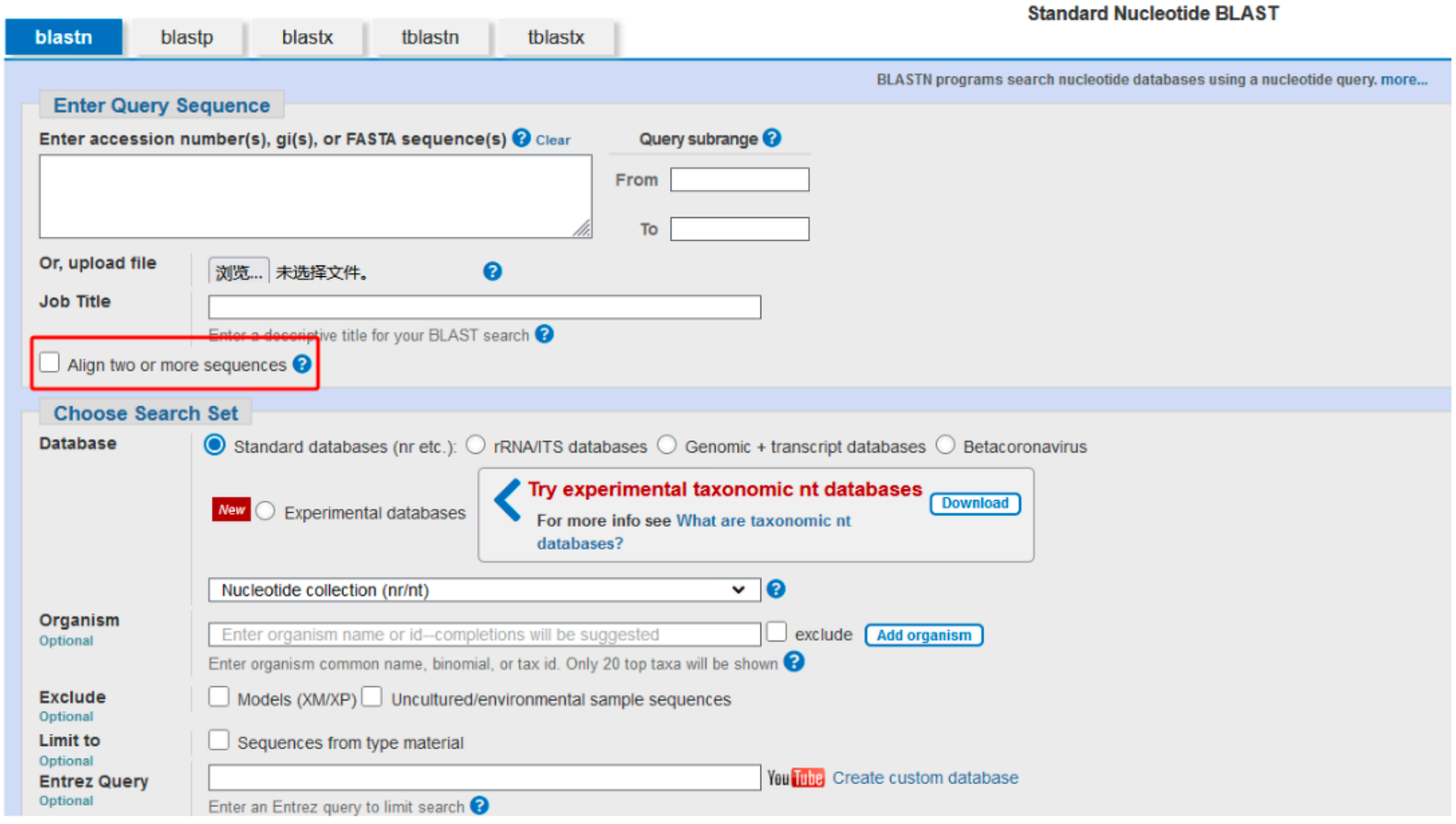
METHOD 1: Use BLAST pairwise alignment

URL: https://blast.ncbi.nlm.nih.gov/Blast.cgi

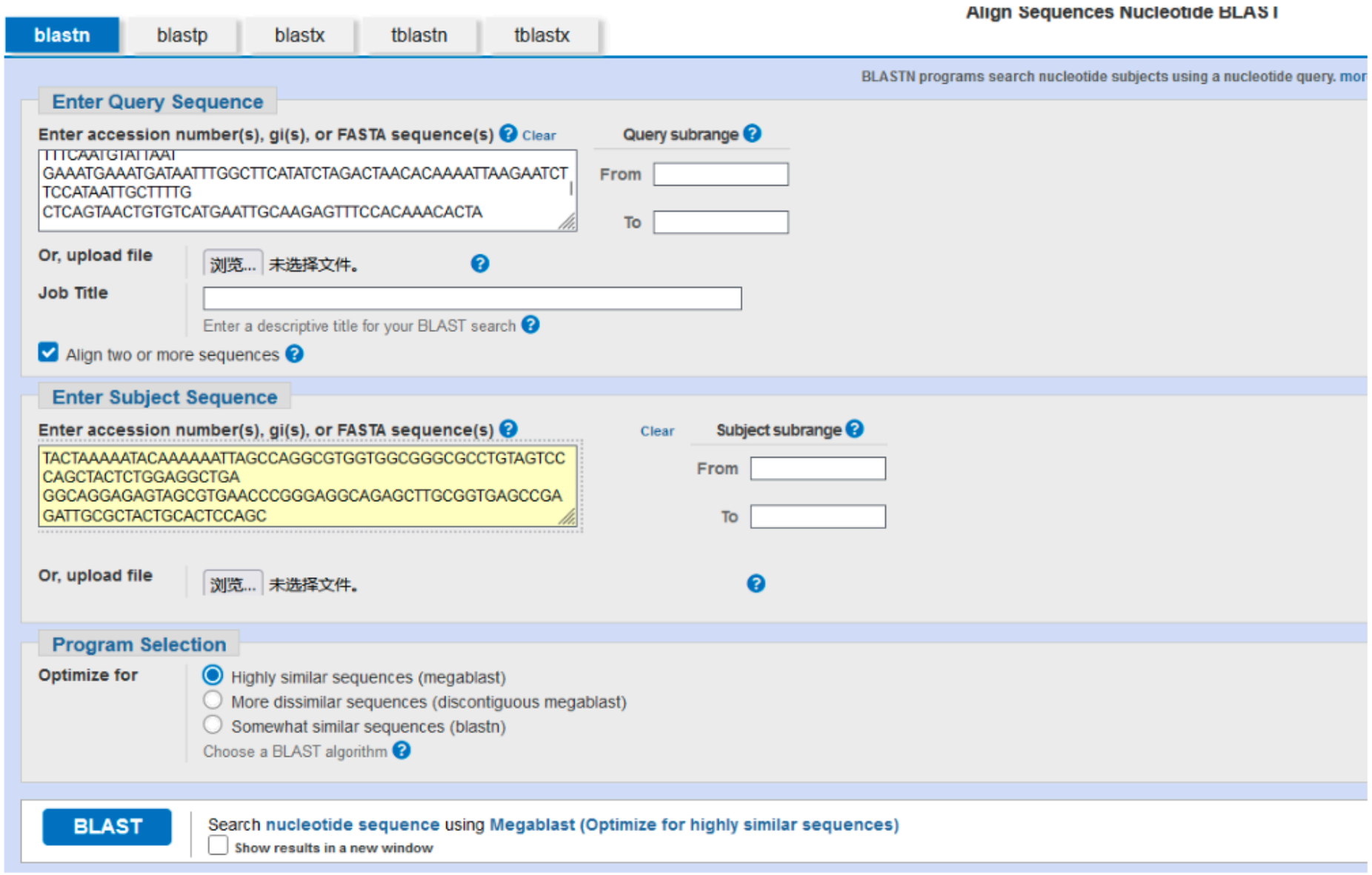
Step 1. Select BLAST mode (Please refer to **Lab1 - Introduction to NCBI Database and BLAST for the content.** Taking BLASTn as an example)



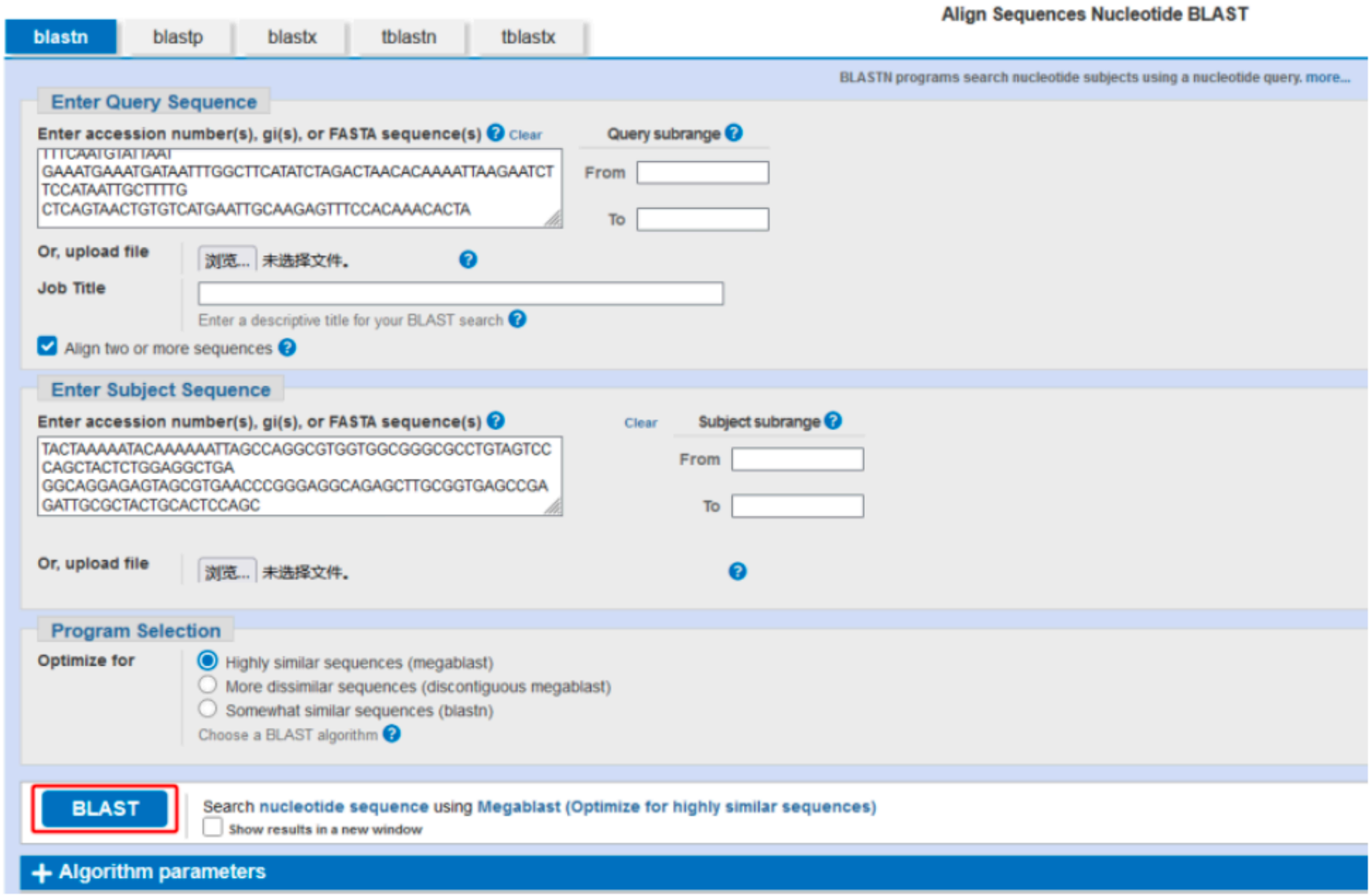
Step 2. Click "**Align two or more sequences**"



Step 3. Input query sequence (or NCBI ID) & target sequence (or NCBI ID) (Taking 2 parts of hAPOBEC3A coding sequence, NCBIID: NM_001270406.2 as example)



Step 4. Click “BLAST”



Step 5. Result

‹ Edit Search | Save Search | Search Summary | How to read this report? | BLAST Help Videos | Back to Traditional Results Page

Job Title	**Nucleotide Sequence**
RID	H3BKW59N114 *Search expires on 09-26 23:59 pm* Download All
Program	Blast 2 sequences Citation
Query ID	lcl|Query_74263 (dna)
Query Descr	None
Query Length	394
Subject ID	lcl|Query_74265 (dna)
Subject Descr	None
Subject Length	210
Other reports	MSA viewer

Filter Results

Percent Identity ___ to ___ E value ___ to ___ Query Coverage ___ to ___ Filter Reset

Descriptions | Graphic Summary | Alignments | Dot Plot

Sequences producing significant alignments Download Select columns Show 100

select all *1 sequences selected* Graphics MSA Viewer

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	None provided		388	388	53%	6e-113	100.00%	210	Query_74265

METHOD 2: Use EMBOSS pairwise alignment toolbox

URL: https://www.ebi.ac.uk/Tools/psa/

Step 1. Select algorithm (Here, take Needleman-Wunsch as example)

Pairwise Sequence Alignment is used to identify regions of similarity that may indicate functional, structural and/or evolutionary relationships between two biological sequences (protein or nucleic acid).

By contrast, **Multiple Sequence Alignment (MSA)** is the alignment of three or more biological sequences of similar length. From the output of MSA applications, homology can be inferred and the evolutionary relationship between the sequences studied.

Global Alignment

Global alignment tools create an end-to-end alignment of the sequences to be aligned.

Needle (EMBOSS)

EMBOSS Needle creates an optimal global alignment of two sequences using the Needleman-Wunsch algorithm.

Launch Needle

Stretcher (EMBOSS)

EMBOSS Stretcher uses a modification of the Needleman-Wunsch algorithm that allows larger sequences to be globally aligned.

Launch Stretcher

GGSEARCH2SEQ

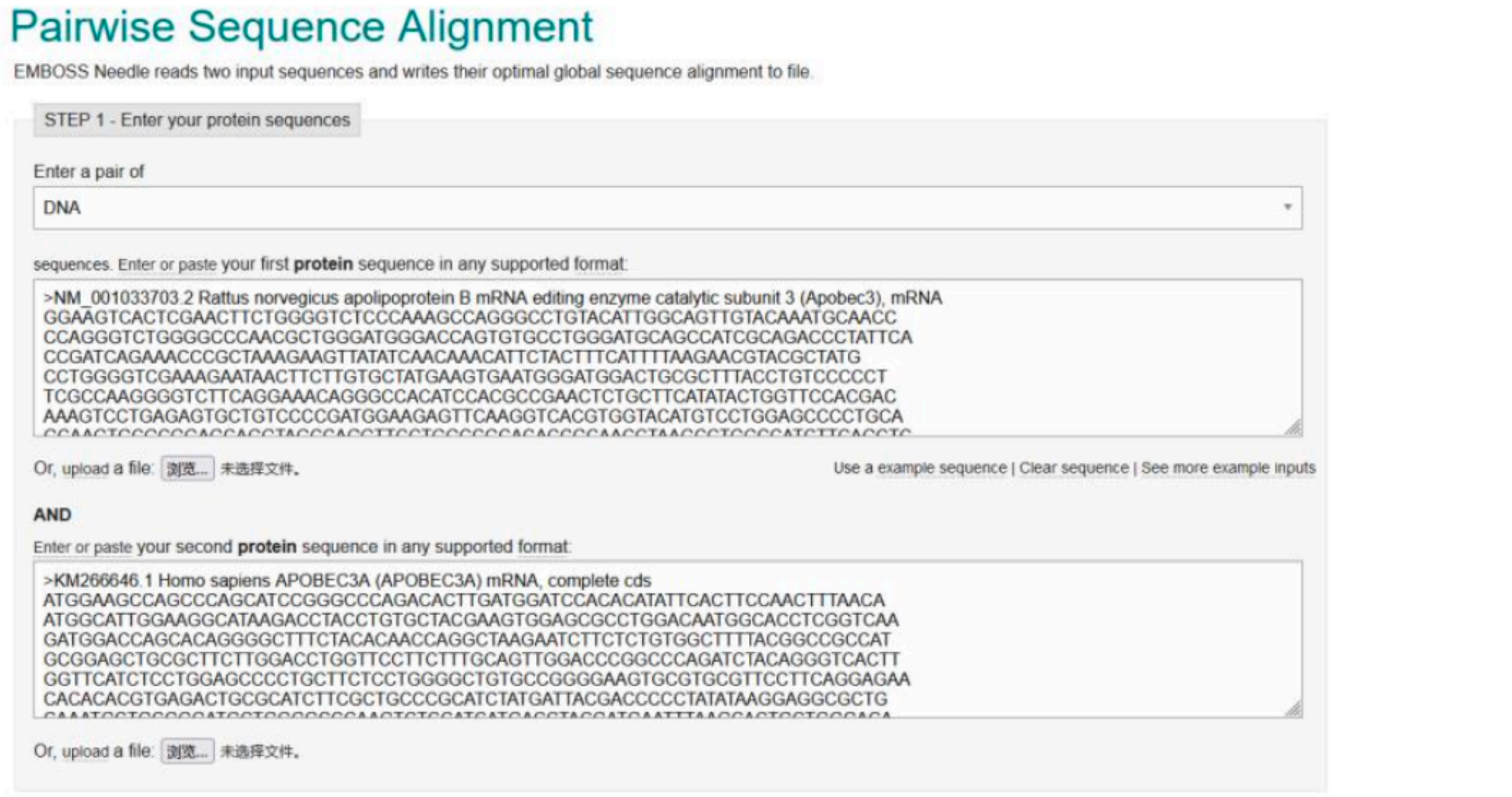
GGSEARCH2SEQ finds an optimal global alignment using the Needleman-Wunsch algorithm.

Launch ggsearch2seq

Step 2. Switch align mode (Enter a pair of: XX) to “DNA”

Step 3. Input query sequence & target sequence (Taking hAPOBEC3A coding sequence, NCBIID: KM266646.1 & rAPOBEC3A coding sequence NCBIID: NM_001033703.2 as example)

(Search in NCBI to get fasta for KM266646.1 and NM_001033703.2
https://www.ncbi.nlm.nih.gov/nuccore/KM266646.1?report=fasta
https://www.ncbi.nlm.nih.gov/nuccore/NM_001033703.2?report=fasta



Step 3. Click “SUBMIT”

STEP 2 - Set your pairwise alignment options

OUTPUT FORMAT

pair

The default settings will fulfill the needs of most users.

More options... *(Click here, if you want to view or change the default settings.)*

STEP 3 - Submit your job

☐ Be notified by email *(Tick this box if you want to be notified by email when the results are available)*

Submit

If you use these services, please consider citing the following publication: **Search and sequence analysis tools services from EMBL-EBI in 2022**

Step 4. Results

Results for job emboss_needle-I20230925-170034-0785-96430502-p1m

Alignment | Submission Details

View Alignment File

```
########################################
# Program: needle
# Rundate: Mon 25 Sep 2023 17:00:36
# Commandline: needle
#    -auto
#    -stdout
#    -asequence emboss_needle-I20230925-170034-0785-96430502-p1m.asequence
#    -bsequence emboss_needle-I20230925-170034-0785-96430502-p1m.bsequence
#    -datafile EDNAFULL
#    -gapopen 10.0
#    -gapextend 0.5
#    -endopen 10.0
#    -endextend 0.5
#    -aformat3 pair
#    -snucleotide1
#    -snucleotide2
# Align_format: pair
# Report_file: stdout
########################################

#=======================================
#
# Aligned_sequences: 2
# 1: NM_001033703.2
# 2: KM266646.1
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 1855
# Identity:     400/1855 (21.6%)
# Similarity:   400/1855 (21.6%)
# Gaps:        1354/1855 (73.0%)
# Score: 857.0
#
#
#=======================================

NM_001033703.      1 GGAAGTCACTCGAACTTCTGGGGTCTCCCAAAGCCAGGGCCTGTACATTG     50

KM266646.1         1 -------------------------------------------------      0

NM_001033703.     51 GCAGTTGTACAAATGCAACCCCAGGGTCTGGGGCCCAACGCTGGGATGGG    100
                                                                 ||||.
KM266646.1         1 ---------------------------------------------ATGGA      5

NM_001033703.    101 A-CCAGTGTGCCTGGGATGCAGCCATCGCAGACCCTATTCACCGATCAGA    149
                     | |||    |||       ||| |||| |.|.||            ||||
```

3. Align multiple sequence

TOOL: EMBOSS MSA toolbox
https://www.ebi.ac.uk/jdispatcher/msa

Step 1. Select algorithm (Here, take Clustal-Omega as example)

Tools > Multiple Sequence Alignment

Service Announcement

The new Job Dispatcher Services beta website is now available at https://wwwdev.ebi.ac.uk/Tools/jdispatcher. We'd love to hear your feedback about the new webpages!

Multiple Sequence Alignment (MSA) is generally the alignment of three or more biological sequences (protein or nucleic acid) of similar length. From the output, homology can be inferred and the evolutionary relationships between the sequences studied.

By contrast, **Pairwise Sequence Alignment** tools are used to identify regions of similarity that may indicate functional, structural and/or evolutionary relationships between two biological sequences.

Clustal Omega

New MSA tool that uses seeded guide trees and HMM profile-profile techniques to generate alignments. Suitable for medium-large alignments.

Launch Clustal Omega

Cons (EMBOSS)

EMBOSS Cons creates a consensus sequence from a protein or nucleotide multiple alignment.

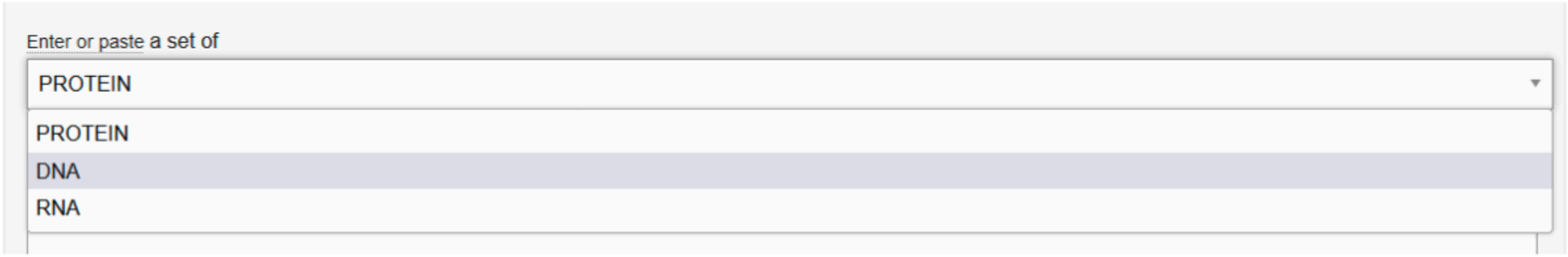
Launch EMBOSS Cons

Kalign

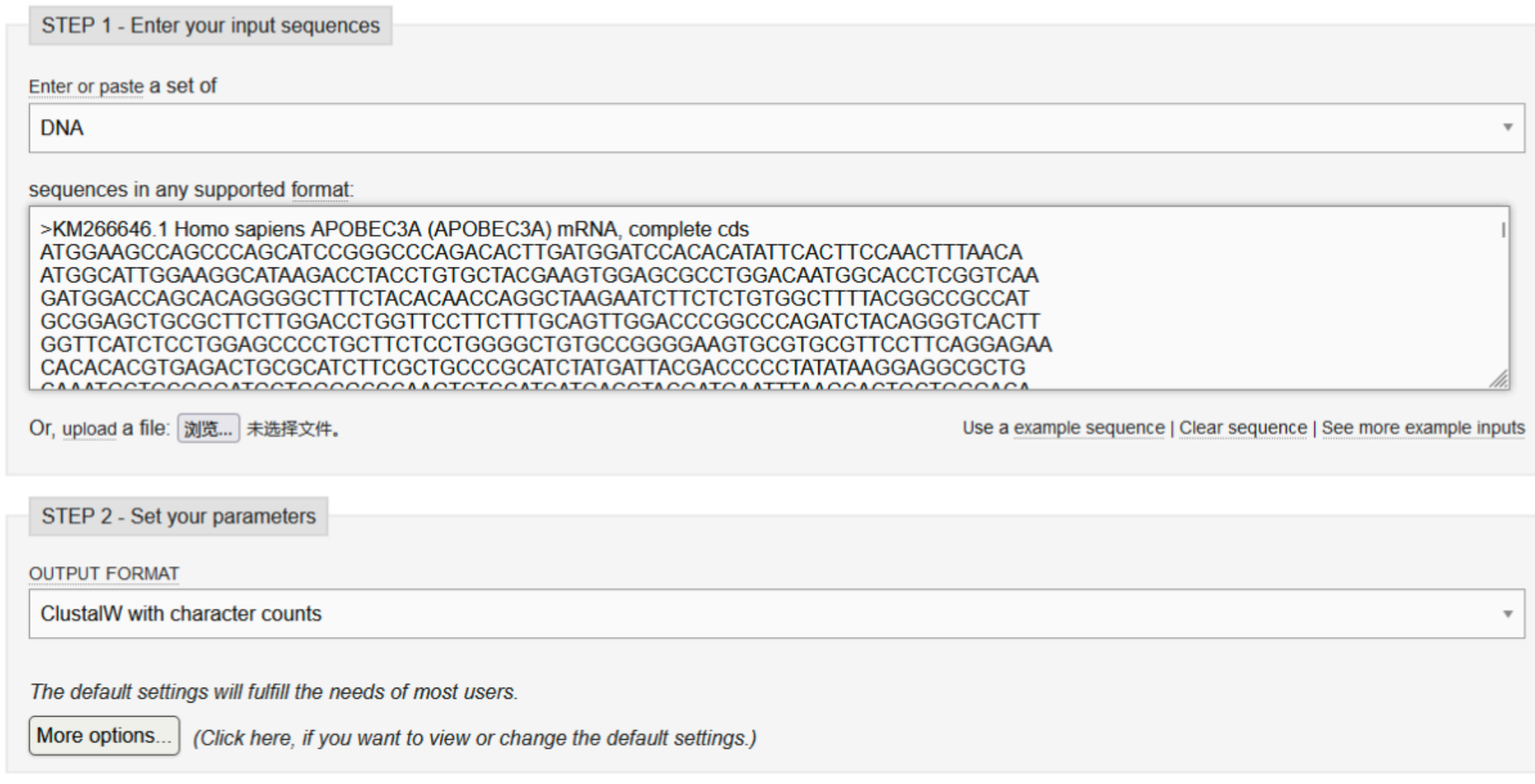
Very fast MSA tool that concentrates on local regions. Suitable for large alignments.

Launch Kalign

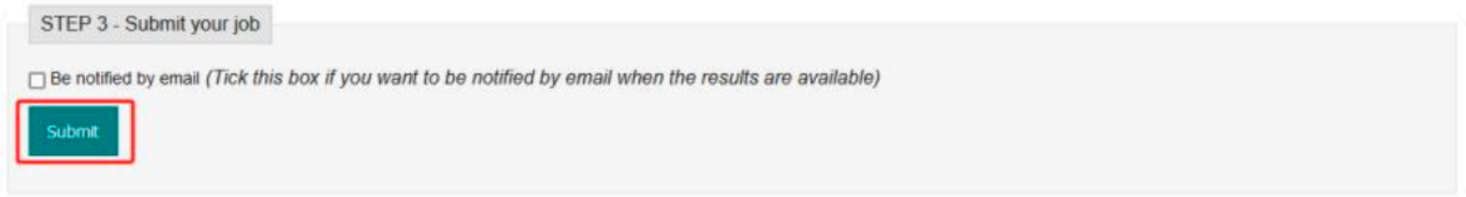
Step 2. Switch align mode (Enter a pair of: XX) to “DNA”



Step 3. Paste multiple sequence or upload files



Step 4. Click “SUBMIT”



Step 5. Result

4. Construct Phylogenetic tree

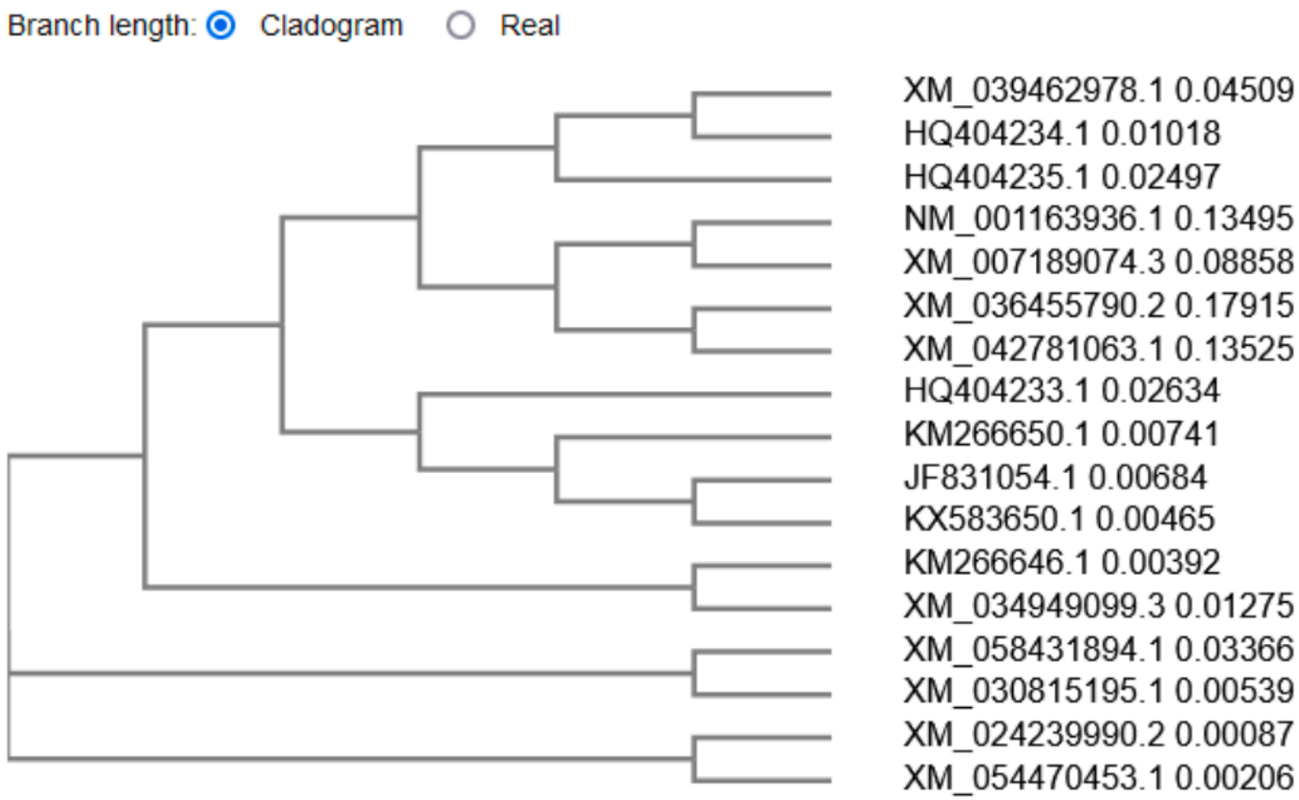
Here, the phylogenetic tree (& nwk text) generated from the MSA will be directly presented on the EMBOSS result page.

Results for job clustalo-I20230926-045902-0090-72510752-p1m

Alignments | Result Summary | Guide Tree | Phylogenetic Tree | Results Viewers | Submission Details

Download Alignment File

```
CLUSTAL O(1.2.4) multiple sequence alignment


XM_039462978.1      ------------------------------------------------------------        0
HQ404235.1          ------------------------------------------------------------        0
HQ404234.1          ------------------------------------------------------------        0
HQ404233.1          ------------------------------------------------------------        0
KM266650.1          ------------------------------------------------------------        0
JF831054.1          ------------------------------------------------------------        0
KX583650.1          ------------------------------------------------------------        0
XM_058431894.1      ------------------------------------------------------------        0
XM_024239990.2      ------------------------------------------------------------        0
XM_054470453.1      ------------------------------------------------------------        0
XM_030815195.1      ------------------------------------------------------------        0
KM266646.1          ------------------------------------------------------------        0
XM_034949099.3      --------------------------CCGATGATATATTAAGGCTCCT------------        22
NM_001163936.1      AAAAAGGAGGAGGACACAGGCTGTGACTGAGCATACCATCAGAGGACTCAGAGCCAGGCC        60
XM_007189074.3      ------------------------------------------------------------        0
XM_036455790.2      ------------------------------------------------------------        0
XM_042781063.1      ------------------------------------------------------------        0


XM_039462978.1      ------------------------------------------------------------        0
HQ404235.1          ------------------------------------------------------------        0
HQ404234.1          ------------------------------------------------------------        0
HQ404233.1          ------------------------------------------------------------        0
KM266650.1          ------------------------------------------------------------        0
JF831054.1          ------------------------------------------------------------        0
KX583650.1          ------------------------------------------------------------        0
XM_058431894.1      ------------------------------------------------------------        0
XM_024239990.2      ------------------------------------------------------------        0
XM_054470453.1      ------------------------------------------------------------        0
XM_030815195.1      ------------------------------------------------------------        0
KM266646.1          ------------------------------------------------------------        0
XM_034949099.3      -GAATCCTAAG--------------------AGAATGTTGGTGAAGATCTTAACACCAC        60
NM_001163936.1      TGCCGCTTGAACAACTTCAAGGAGGAGGCCACAGGCTGTGACTGAGCATACCATCAGAGG        120
XM_007189074.3      ----------------------------------GCTGTGACCAAGAACAGAAACTGAAG        26
XM_036455790.2      ------------------------------------------------------------        0
XM_042781063.1      ------------------------------------------------------------        0
```


Branch length: Cladogram Real
XM_039462978.1 0.04509
HQ404234.1 0.01018
HQ404235.1 0.02497
NM_001163936.1 0.13495
XM_007189074.3 0.08858
XM_036455790.2 0.17915
XM_042781063.1 0.13525
HQ404233.1 0.02634
KM266650.1 0.00741
JF831054.1 0.00684
KX583650.1 0.00465
KM266646.1 0.00392
XM_034949099.3 0.01275
XM_058431894.1 0.03366
XM_030815195.1 0.00539
XM_024239990.2 0.00087
XM_054470453.1 0.00206

5. Task

Background

In the above Manual module, we conducted related exercises utilizing the APOBEC3A mRNA sequences. In the Task module, we will analyze the APOBEC3A coding protein. APOBEC (apolipoprotein B mRNA editing catalytic polypeptide-like) proteins have a characteristic zinc-coordination motif (H-X-E-X23-28-P–C-X-C) within the active site where a water molecule binds Zn^{2+} and the metal ion is coordinated by one histidine and two cysteines. The AID/APOBEC family enzymes convert cytosines in single-stranded DNA to uracil causing base substitutions and strand breaks. Based on this property, relevant research has used human APOBEC3A (hAPOBEC3A) fusion dead Cas9 (dCas9) protein as a base editor for precise genome editing.

Task1. Using the human APOBEC3A (**hAPOBEC3A**) protein sequence (ID: **NP_663745.1**) as query, use BLASTp to search for its homologous protein sequence.

Task2. For the **APOBEC3A.fasta** file (from iSpace), perform multiple sequence alignment and construct phylogenetic tree.

Task3. Attempt to interpret the results of this phylogenetic tree.

Task4. Based on the phylogenetic tree, select the sequences closest to hAPOBEC3A and generate **pairwise sequence alignment**, attempt to interpret the specific differences between the two sequences (hAPOBEC3A and target sequence).
Closest: *Pongo pygmaeus* APOBEC3A protein